Vector competence and filariasis transmission in Mali

Thesis submitted to the University of Liverpool

For the degree of Doctor of Philosophy in Tropical Medicine by

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Declaration

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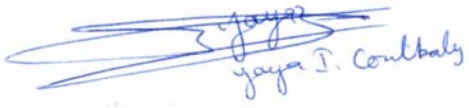

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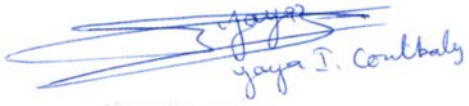

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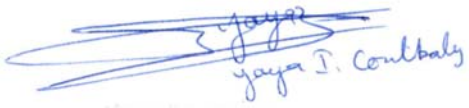

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Dedication

This thesis is dedicated to

My children

Godefroy Ibrahim, Rokiatou Yaya, Assitan Yaya, Maurice Yaya

and Alexandre Makan

to let them know that only work will make them free.

Abstract

Lymphatic filariasis (LF) is a public health problem in 73 countries and is associated with marked morbidity and disability. It is unique because of its transmission by five main genera of mosquitoes, including *Culex*, *Aedes*, *Anopheles*, *Mansonia* and *Ochlerotatus*. In Mali, LF endemicity mapping in 2004 found all eight administrative regions to be endemic for LF. Prior to the National LF Elimination Programme (NPELF), six pilot sentinel villages were selected for baseline research studies to inform the most appropriate strategy for monitoring the impact of the proposed elimination programme based on treatment with ivermectin in combination with albendazole. The following three objectives form the basis of my PhD studies:(i) investigate LF vector population and associated transmission patterns before, during and after the initiation of mass drug administration (MDA) (ii) assess efficacy of new entomological trapping tools for LF post-MDA xenomonitoring and (iii) determine transmission potential in a urban environment in Mali. The overall design is a descriptive study including cross sectional entomological surveys along with longitudinal human surveys to assess the MDA impact. I used standard infection status assessment methods as well as recently developed methods; including the antibody test for Wb123. I conducted these studies in both rural (Sikasso and Kolondieba districts) and urban areas (Bamako, the capital city). My thesis is the first report of the outcome of up to five years post-MDA annual assessment of *W. bancrofti* transmission using both entomological and parasitological data in an *Anopheles* transmission area where albendazole plus ivermectin is the recommended drug regimen and *Anopheles gambiae s.l* the main vector for LF transmission. These features are found mainly in the Western part of Africa. In the pilot sentinel sites in Mali, made of six neighbouring villages, seven MDA rounds with the albendazole plus ivermectin were successful not only at stopping LF transmission (infection rates within 6-7 years old children <2%) in the short term, but also at sustaining it for up to five years after the last MDA. In contrast, impact assessment in another hyper endemic area (two neighbouring villages treated by the NPELF in the district of Kolondieba) did not demonstrate interruption of transmission after the sixth and seventh MDA rounds. The reasons of these different outcomes of MDA implementation in the different areas are discussed. Of note, the failure in the latter villages was detected using only the ICT card, a method that has been found to overestimate the infection rate in children when compared to the circulating filarial antigen test (Og4C3 ELISA) and the Wb123 antibody test in the pilot sentinel area. In *Anopheles* transmission areas, it has been observed that focal low-level transmission can exist without being a real threat for re-emergence of transmission, due to lower capacity of the vector to transmit when parasite density is low. Nevertheless, in areas that fail the Transmission Assessment Survey (TAS), adult populations should be checked in addition to the recommended 6-7 year-old age group. Additionally, this thesis showed very promising results for using the Ifakara tent trap type C (ITTC), a human baited trap alternative to the human landing catch. *Anopheles* yields and infection rates using ITTC were strongly correlated with results using human landing catch (HLC) overall, as well as monthly, in two villages with significantly different *Anopheles* densities. Finally, it appears that the current version of the TAS needs more tools and additional directions for human infection status determination especially when the baseline endemicity level is high. Further evaluation the ITTC after reducing its bulkiness is required to confirm its usefulness for LF entomological studies in *Anopheles* transmission areas. From the 6,174 *Culex spp* and the 16 *Anopheles gambiae s.l* processed and 1,002 volunteers tested, there was no evidence of LF transmission in the urban environment of Bamako in Mali.

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List of key acronyms

ATP: Annual Transmission Potential

ABR: Annual Biting Rate

ADL: Adenolymphangitis

AIBR: Annual Infective Biting Rate

AFR: African Region

AIBR: Annual Infective Biting Rate

ALB/IVER: Albendazoleplus Ivermectin

ATP: Annual Transmission Potential

BGST: Biogents Sentinel Trap

CDC: Center For Diseases Control And Prevention

CELISA: Celllabs Enzyme Linked Immunosorbent Assay

CFA: Circulating Filarial Antigen

CNTD: Centre For Neglected Tropical Diseases

Cx. quinquefasciatus: *Culex quinquefasciatus*

DALY: Disease Adjusted Life Years

DDT: dichloro-diphenyl-trichloro-ethane

DEC: Diethylcarbamazine

DNA: Deoxyribonuleic acid

DRC: Democratic Republic of Congo

EDSM: Demographic and Health Survey in Mali

EIR: Entomological Inoculation Rate

ELISA: Enzyme Linked Immunosorbent Assay

EU: Evaluation Unit

FMPOS: Faculty of Medicine Pharmacy and Odontostomatology

FPSUP: Filariasis Programmes Support Unit

FRU: Filariasis Research Unit

FTS: Filariasis Test Strip

GIS: Geographical Information System

GPELF: Global Programme to Eliminate Lymphatic Filariasis

GPS: Global Positioning System

HBR: Human Biting Rate

HLC: Human Landing Catch

ICT: Immunochromatographic Card Test

INRSP: National Institute for Research in Public health

IRB: Institutional Review Board

IRS: Indoor Residual Spraying

ITN: Insecticide Treated Nets

ITTC: Ifakara Tent Trap Type C

IU: Implementation Unit

IVC: Integrated Vector Control

IVER: Ivermectin

LAMP: Loop-mediated isothermal amplification

LF: Lymphatic filariasis

LLIN: Long Lasting Insecticidal Nets

LSTM: Liverpool School of Tropical Medicine

MBR: Monthly Biting Rate

MBR: Monthly Biting Rate

MDA: Mass Drug Administration

MEWG: Monitoring and Evaluation Group

MIBR: Monthly Infective Biting Rate

MIPL: Maximum infection prevalence likelihood

MMDP: Morbidity Management and Disability Prevention

MRTC: Malaria Research and Training Center

MTP: Monthly Transmission Potential

MVBR: Monthly Vector Biting Rate

ND: Not Done

Neg.: Negative

NIH: National Institutes of Health

NP: Not Planned

NPELF: National Programme For Elimination Of Lymphatic Filariasis

NTD: Neglected Tropical Diseases

PBS: Phosphate Buffering Saline

PCR: Polymerase Chain Reaction

PNG: Papua New Guinea

Pos: Positive

PSC: Pyrethrum Spray Catch

RNA: Ribonucleic Acid

RPRG: the Regional Programme Review Group

RT PCR: Reverse Transcriptase Polymerase Chain Reaction

s.l: *sensu lato*

SPSS: Statistical Package for Social Sciences

s.s: *sensu stricto*

STAGNTD: Strategic And Technical Advisory Group On Neglected Tropical Diseases

TAS: Transmission Assessment Survey

TDR: Rapid Diagnotic Test

TFGH: Task Force for Global Health

WHO: World Health Organization

Chapter 1

Literature review

1.1 Introduction

Lymphatic filariasis (LF) is a chronic debilitating infection caused by the mosquito-borne filarial parasites, *Wuchereria bancrofti*, *Brugia malayi* and *Brugia timori.* Belonging to the class of *Secernentea* and the order of *Spirurida*, these filarial worms are nematode parasites. The adult worms live in tissues and body cavities of their vertebrate hosts. *Filariidae* and *Onchocercidae* are families of the superfamily *Filarioidea*. The filarial parasites capable of human infection belong to the family of *Onchocercidae* (Anderson and Bain 2009; Chabaud 1974).

LF is a public health problem in 73 countries and is associated with marked morbidity and disability (Ramaiah and Ottesen 2014; WHO 2015). It is unique because of its transmission by five genera of mosquitoes including *Culex*, *Aedes, Anopheles (An.)*, *Mansonia* and *Ochlerotatus* (Bockarie, Taylor, and Gyapong 2009; WHO 2013a). LF infection is found in the tropical areas of all continents, albeit with different endemicity levels. These regional variations are linked with the local vector species distribution worldwide (Figure 1.1).

LF is one of the Neglected Tropical Diseases (NTDs) (WHO 2016b). The NTDs do not frequently kill patients but rather cause disabilities. The Disease-Adjusted Life Years (DALY) estimates are the metrics commonly used to assess the chronic impact of these infections, although they do not encompass all of the detrimental consequences of infection (Hotez et al. 2014). When assessing the burden of mental health in LF, Thanh et al in 2015 reported 5.09 million DALYs (Ton, Mackenzie, and Molyneux 2015).

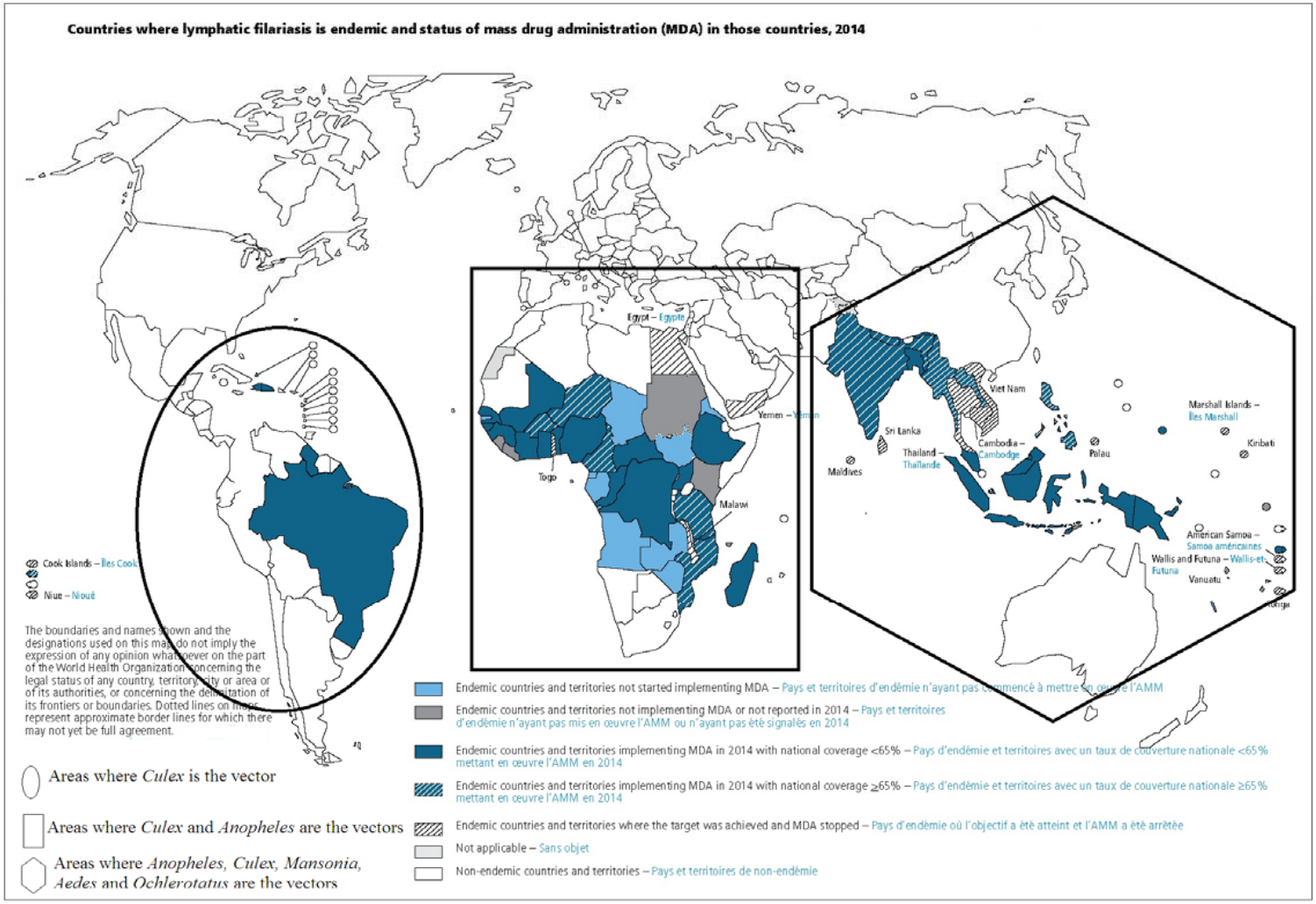


Figure 1.1 Map of lymphatic filariasis endemicity, elimination programme status and vector species distribution worldwide (Modified from WHO 2015).

1.2 Burden and distribution of LF and the GPELF (Global Programme to Eliminate Lymphatic Filariasis)

1.2.1 Global burden

After malaria, LF is the second most common vector transmitted parasitic infection (Wynd et al. 2007). As of 1996, before the current global effort to eliminate LF as a public health problem, an estimated 119 million people were infected including 43 million already affected by at least one of the chronic consequences (lymphedema and hydrocele) (Michael, Bundy, and Grenfell 1996). Additionally, LF was ranked the second most important cause of permanent and long-term disability because of its aesthetic and functional impacts according to the 1995 World Health Report (WHO 1995).

The Global Programme to Eliminate Lymphatic Filariasis (GPELF) was launched in 2000 and considerably scaled up its efforts in MDA (Mass Drug Administration) implementation and as a consequence have significantly reduced LF endemicity to 36.45 million, 19.43 million and 16.68 million cases respectively for microfilaraemia, hydrocele and lymphedema by 2013 (Ramaiah and Ottesen 2014). In 2000, the microfilaraemia, hydrocele and lymphedema were estimated to respectively 91.14, 29.94 and 17.66 cases in the endemic countries (Ramaiah and Ottesen 2014). Nevertheless, in 2014, a total of 73 countries were still considered to be endemic for LF. Among them, 18 had reached the surveillance phase, while 55 still required MDA. Eleven countries did not provide information about their endemicity and had not initiated MDA (WHO 2015). Full country geographical coverage for MDA, as defined by at least one MDA conducted in each endemic implementation unit (IU), was achieved in 21 endemic countries. In 23 additional countries, only a few IUs have

received one or more MDA rounds (WHO 2015). An IU is the smallest geographical area where LF elimination activities are undertaken, usually it is a health district (WHO 2011b). In Mali, the 65 health districts represent the operational level of the health system organization. The 8 administrative regions are made of 4 to 10 health disctritcts. Each district is made of 10 to 265 villages with a total population ranging from 9,788 to 710, 216 inhabitants (Institut national de la statistique Mali 2017). By 2014, about 314.7 million people less were considered to be at risk and in need of MDA. Thus, the highest population initially estimated to be at risk of LF went from 1.4 billion to 1.3 billion from 2011 to 2014 (WHO 2015).

1.2.2 LF burden in the African Region

This section is mainly based on information provided by the last Weekly epidemiological record from WHO published in September 2016 (WHO 2016a). Some of the information was out of date due to the fact that the country did not send the last information to WHO before the report was published. We report here the data made available by WHO.

The last intensified mapping activities allowed shrinking the LF distribution areas in Africa (WHO 2016b). The Gambia is not classified as a non-endemic country because required assessments and the resulting evidence of absence of LF transmission have not been submitted to WHO yet. Similar evidence is also awaited for other countries such as Eritrea, Gabon, Botswana, Mauritania and some parts of Zimbabwe. The regional MDA coverage of 44.7% (based on 20 countries among the originally 35 of the region) in 2015 showed an 18% improvement as compared to 2014. Togo and Malawi are still in the post-MDA surveillance period and continue making progress in

term of Morbidity Management and Disability Prevention (MMDP).

Several countries are significantly increasing their MDA coverage like Cameroon, Democratic Republic of Congo, Ethiopia, Kenya, Nigeria, Senegal, and Zambia. That is contrasting with about 22 countries that are having difficulties in initiating, increasing the geographical coverage rate or maintaining a regular annual MDA.

There is an urgent need of 100% geographical coverage of the IUs in Central African Republic, Congo, Democratic Republic of Congo, Ethiopia, Guinea-Bissau, Madagascar, Nigeria and Zimbabwe. The following countries need to start the MDA as soon as possible in order to get on track for LF elimination- Angola, Chad, Equatorial Guinea, Sao Tome and Principe and South Sudan.

By 2020, a total of eight countries are expected to have completed MDA, namely Benin, Burkina Faso, Ghana, Mali, Niger, Sierra Leone, Uganda and United Republic of Tanzania). Notwithstanding the Ebola outbreak, Sierra Leone (5.4 million) and Guinea (1.5 million) were able to implement the MDA in 2015.

1.2.3 Global Programme for the Elimination of lymphatic filariasis

Given the high burden of LF in endemic countries (120 million people affected including 40 million with incapacities) in 1996 and the availability of diagnosis tools and drug combinations that were proven effective on microfilariae, the World Health Assembly committed in 1997 to eliminate the disease as a public health problem by 2020 (WHA resolution 50.29). The GPELF was launched in 2000 in order to eliminate LF globally by 2020. It has two aims: to interrupt its transmission and to manage morbidity and prevent disability. LF elimination is defined by the World Health Organization as the transmission interruption in an endemic evaluation unit. Children aged 6 to 7 years are the targeted group for the transmission assessment with a prevalence within this group indicating the interruption when it is less than 2% in areas where *Anopheles* and *Culex* are the main vectors (WHO 2011b).

The first and major pillar of transmission interruption is targeted by two means: annual community-directed MDA and vector control. To achieve these aims, the GPELF established a group of partners, including the endemic countries' ministries of health, funding agencies, the drug manufacturers, academic and research institutions, non-governmental organizations and the WHO. This is called the Global Alliance to Eliminate Lymphatic Filariasis (GAELF). Its mission is to eliminate LF by bringing together diverse groups of public-private health partners to support the Global Programme to Eliminate Lymphatic Filariasis by mobilising political, financial and technical resources to ensure success (GAELF 2017).

1.2.4 Programmatic steps for interrupting transmission

The GPELF recommend four main steps to stop LF transmission: disease geographical mapping; MDA; post-MDA surveillance; verification of disease elimination (Figure 1.2).

1.2.4.1 Disease geographical mapping

Mapping is required to determine the level of endemicity and to identify areas with active transmission of LF. It is performed according to IUs- the country-defined administrative units that are used for MDA. The process is comprised of a mapping survey and review of existing data related to LF. The survey determines circulating filarial antigen (CFA) prevalence using immunochromatographic card test (ICT) or microfilaraemia prevalence using blood film in older school-aged or adult populations. If the prevalence is ≥1% of the sample tested, the IU is considered endemic for LF (WHO 2011b).

1.2.4.2 Mass Drug Administration

The total population of the different IUs of a country is considered at risk of LF until MDA is implemented for at least five years with epidemiological coverage ≥ 65% and a Transmission Assessment Survey (TAS) confirms that transmission has been stopped. As soon as transmission interruption is reached, the population of the concerned IUs is considered to no longer be at risk of LF (WHO 2011b). The MDA recommended by the GPELF is based on co-administration of two of the three dedicated drugs. This is due to the fact that two drugs have been found to be more effective at clearing microfilaraemia than any single drug. Albendazole plus ivermectin is used in countries where onchocerciasis is co-endemic with LF, and

diethylcarbamazine (DEC) plus albendazole is used in countries free of onchocerciasis (WHO 2015) because of serious adverse events associated with the administration of ivermectin to *L. loa* infected individuals or DEC to *O. volvulus* infected individuals.

A single dose of therapy should be administered yearly to all eligible people in endemic areas for a period of at least five years to reduce microfilarial density in infected people and the overall infection prevalence in the endemic area below a threshold at which transmission is unlikely to be maintained. In areas with low initial LF prevalence, such as some districts in Sierra Leone and Burkina Faso, MDA was stopped after 2-3 rounds per the RPRG recommendation according to the respective Ministry of health annual reports on NTD. Trained community members under supervision of health workers are preferred as distributers of the drugs to targeted endemic communities. The whole process requires a well organized and coordinated monitoring and evaluation at the local, regional and national levels (WHO 2015). MDA coverage should be ≥65% of the total population to be considered effective. It should be noted that reaching 65% effective coverage is not easy in urban cities (Mwakitalu et al. 2013; Simonsen and Mwakitalu 2013).

After three annual rounds of MDA, an optional mid-term assessment of the prevalence of microfilaraemia or CFA can be undertaken before the TAS eligibility assessment after the 5th MDA round. If the sentinel and spot-check sites are eligible for TAS, then a TAS can be undertaken followed by the 6th MDA round. That last round can be done even if the Evaluation Unit (EU) passes the TAS.

The TAS is a standardized method to assess the impact of MDA and provide national programme officials with technical information that can help to decide whether to stop MDA in an EU. The EU is the geographical area where TAS is implemented. It can be made of a combination of IUs, an entire IU or a part of an IU. The EU should not exceed two million inhabitants and should be made of contiguous geographical areas that share similar LF endemicity level and transmission dynamics (WHO 2015). After five to six years of annual MDA rounds, the LF elimination programmes' impact needs to be assessed to determine whether the programme has achieved its objective of reducing levels of microfilariae in endemic areas to a level where transmission is unlikely to occur even if the MDA are stopped. TAS are designed to help programme managers determine whether areas have reached this critical threshold of infection (Deming and Lee 2011). The evidence provided by the TAS should not divert the programme managers to thoughtfully consider the MDA stopping decision. The geographical area for the TAS is the evaluation unit (EU), which may comprise multiple IUs or part of an IU.

After the 5th MDA round, an assessment survey using blood films should be carried out at least six months after the MDA. The survey will target the inhabitants aged 5 years and above in sentinel and spot-check sites. A microfilaraemia rate <1% in all sites in order to continue to implement the TAS (WHO 2004)**.** Regardless of the results of this assessment, the sixth MDA round should be planned and carried out in the EU. In EUs with less than 1% microfilaraemia rate, programme managers should plan to conduct the TAS at least 6 months after the last MDA round (Gyapong et al. 2005).

The targeted population for the TAS is the 6-7 years children that should be free of infection if the MDA was successful in stopping LF transmission. Antigenemia using Immunochromatographic card tests or Filaria Test Strips is usually determined within all the surveyed individuals in *W. bancrofti* endemic area. The survey is implemented in each Evaluation Unit that underwent at least 5 MDA rounds with a coverage rate of at least 65%. To determine the TAS sample size and select both the required sampling strategy (school or community based) and the villages to be visited, the *Survey sample builder* tool (Horton et al. 2000) could help to automate the calculations. The survey is implemented at school-based or at and community-based according to the school enrollment rate that should be equal or higher than 75% for a school-based survey. In areas where *W. bancrofti* is endemic and *Anopheles* or *Culex* is the principal vector, the target threshold is <2% antigenaemia prevalence. Because *Aedes* species are known to be more efficient transmitters of the parasite, in Bancroftian areas where *Aedes* is the primary vector, the target threshold is <1% antigenaemia prevalence (WHO 2015).

1.2.4.3 Post-MDA surveillance

The post-MDA surveillance period starts after the EU has passed the TAS. The TAS is also the recommended survey to detect any resurgence of LF transmission after MDA stopping. For post-MDA surveillance, the TAS should be conducted three times every 2-3 years to ensure that transmission is not re-emerging. The TAS planning and reports should be shared with WHO and the Regional Programme Review Group (RPRG) for advice.

1.2.4.4 Verification of the disease elimination

The verification of LF elimination necessitates five main actions from the national programme managers, RPRG, WHO and the Strategic and Technical Advisory Group on Neglected Tropical Diseases (STAG-NTD):

(i) the compilation of all data related to LF before, during and after the national programme initiation from each IU;

(ii) the national dossier preparation by the national programme for LF elimination after data analyses;

(iii) the submission of the national dossier to the RPRG via WHO by the national programme;

(iv) the dossier review by the RPRG that will stipulate recommendations to the Monitoring and Evaluation Working Group (MEWG) of the STAG-NTD via WHO headquarters;

(v) the review of the RPRG recommendations by the MEWG that will make their recommendations to the STAG-NTD.

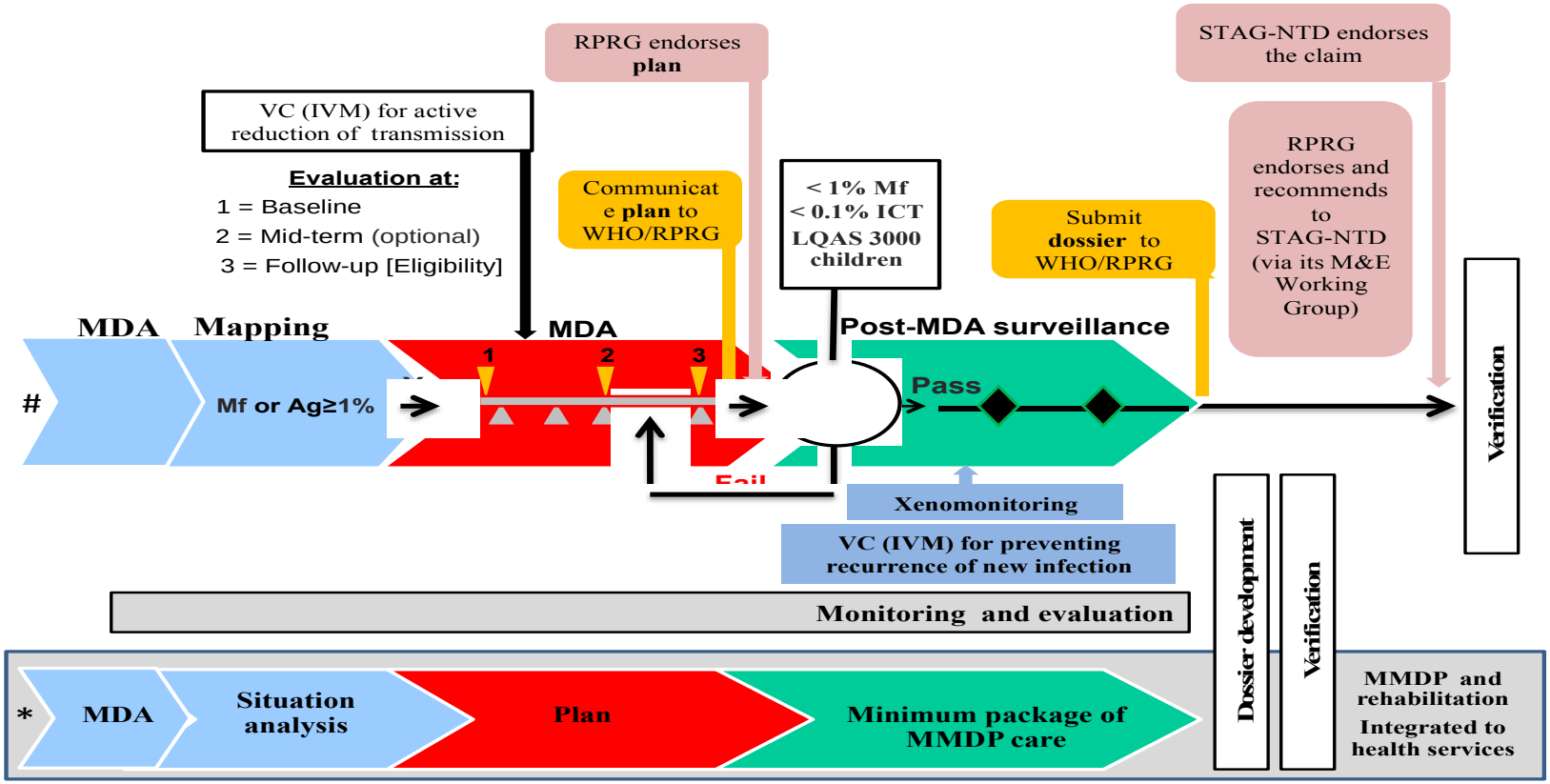


Figure 1.2 Overall framework and programme steps of the GPELF

1.2.4.5 Morbidity management and disability prevention

The GPELF's MMDP activities mainly focus on lymphedema and hydrocele. The other clinical manifestations of LF such as the adenolymphangitidis and the fever are supposed to be managed by standard practices and referral of individuals, because public-health approaches related to them have not yet been established (WHO 2011a). LF chronic clinical burden is estimated to be about 19.4 million cases and 16.7 million cases respectively for of hydrocele and lymphedema (Ramaiah and Ottesen 2014). Effective surgery is available that leads to significant improvement in the patients' economic situation and quality of life (WHO 2011a).

LF is an important contributor to the global disability burden with at least 2.8 million DALYs and additional mental disorders often experienced by affected peoples and their health workers (Ton et al. 2015; WHO 2015). Elimination of these disabilities will not only require implementation of MDA but also the care of afflicted individuals in all endemic communities.

Despite significant advances in terms of MDA implementation, little progress has been made regarding the care of people suffering from the clinical manifestations of LF. The lack of information with respect to morbidity control in endemic areas has contributed to the problem (WHO 2015). A minimal package of recommended assistances needs to be provided to people with LF:

(i) MDA or treatment to remove any remaining adult parasites and Mf;

(ii) Surgery for hydrocele (in *W. bancrofti* endemic areas);

(iii) Treatment for adenolymphangitis (ADL) when it occurs; management of lymphedema to avoid its worsening as well as the occurrence of ADL episodes (WHO 2016b).

Simple and self-administered hygiene and exercise applied to the affected parts are recommended for the basic management of lymphedema. The simplicity of the care should not impair its lifelong availability for patients in local primary health care facilities. Additional measures, such as properly caring the wound, elevating the affected limb and proper footwear, are important. These measures were found to be effective in decreasing the number of ADL episodes and providing patients with a better quality of life. Home-based care is encouraged (WHO 2016b).

The success of MMDP is currently assessed in terms of the geographical coverage of the basic management package for all known patients in endemic countries. As of 2014, only 24 endemic countries had sent MMDP service data to WHO, of which 10 seem to have organized surveillance of these services at the IU level (WHO 2015).

In order to obtain a certification of elimination, countries that show evidence of transmission interruption will also need to provide in their dossier the following MMDP details:

(i) the number of patients estimated by the IU;

(ii) the number of facilities designated to provide recommended care serving IUs with known cases;

(iii) an assessment of the readiness and quality of services provided in designated facilities.

Endemic countries are advised to start documenting the MMDP activities and follow the directions in documents available from WHO (WHO 2016b).

1.3 Transmission of LF

1.3.1 Life cycle of *W. bancrofti*

Arthropod vectors are needed for the maturation and the transmission of filarial worms from one vertebrate to another (Sasa et al. 1976; Schacher 1973). The female adult worms stay in the lymphatics and mature before producing live pre-larval forms called microfilariae.

The microfilariae produced by female adult worms are found in the host blood in the daytime (in large blood vessels) or at night (in peripheral blood vessels) depending on the characteristics of the filarial species. They are found in the blood or lymphatic vessels. When the microfilariae are circulating in the peripheral blood or moving in the cutaneous tissue, they get taken up by mosquito vectors during a blood meal. Once in the suitable mosquito vector, the microfilariae pass through the wall of the digestive tract into the mosquito haemocele. Later, the microfilariae pass through three maturation stages: the first larval stage (L1), the second larval stage (L2) and the infective third larval stage (L3) (Figure 1.3). Each stage in the vector has specific suitable locations, so the L1 and L2 are usually found in the thorax of the host vector. L3s are released into the hemolymph which is the circulatory system that baths allows the larvae to move freely inside the mosquito into head, thorax, abdomen, palps and even legs. The L3 in the head move into the

mosquito mouth before being deposited on the vertebrate host's skin where it penetrates actively the lymphatic vessels under the skin through the biting wound and goes to the deeper lymphatic vessels and lymph nodes (Beaver et al. 1984). In a suitable host, the infective larvae undergo a maturation process that takes nine to 14 days to reach the larval stage 4 (L4) that will become an adult worm after approximately 16 days.

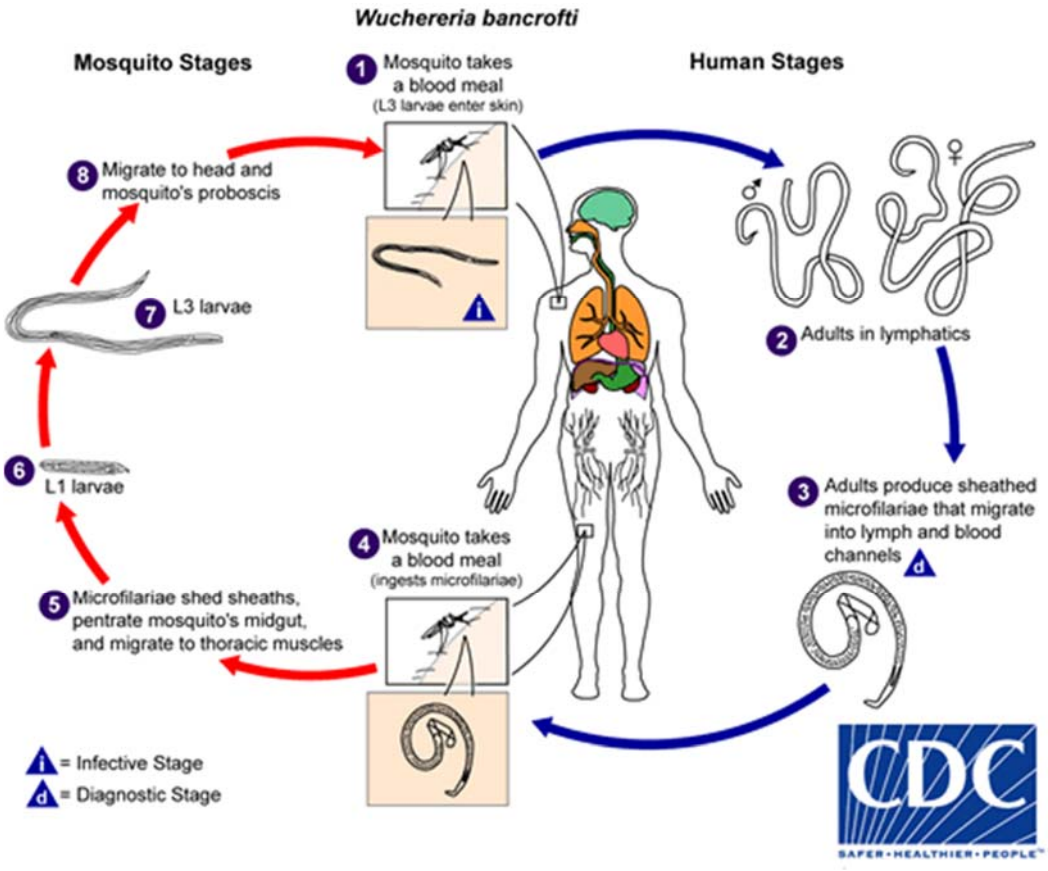


Figure 1.3 Life cycle of *W. bancrofti*
(Modified from Centers for Disease Control and Prevention (CDC 2016)

1. They become adults that commonly reside in the lymphatics
2. The adult worms (male and female) produce microfilariae that migrate into lymph and blood vessels moving actively through lymph and blood

❸ A mosquito ingests microfilariae when biting an infected human host

❹ After ingestion by the vector, the microfilariae undergoa series of changes asthey move through the mosquito's midgut and reach the thoracic muscles

❺ The microfilariae start a maturation process that leads to the larval stage 1 (L1)

❻ and later to the larval stages 2 (L2) and 3 (L3)

❼ The L3s go to the mosquito's proboscis

❽ and will be deposited on the skin of the host during a vector mosquito blood meal before migrating into the lymphatic vessels through the biting wound.

1.3.2 Life cycle of mosquitoes

The mosquito life cycle passes through four different stages, the egg, the larva, the pupa, and the adult (American Mosquito Control Association 2017).

Egg stage

This is the first stage of mosquito life cycle. Eggs can be laid one at a time (*Anopheles, Ochlerotatus* and *Aedes*) or attached together making what is called "rafts" and float on the surface of the water (*Culex* and *Culiseta* species). Several *Aedes* and *Ochlerotatus* lay eggs on humid topsoil that will be swamped by water. Eggs usually hatch into larvae within 48 hours; others might resist very cold winters before hatching. This habitat reqires water for the process to continue (American Mosquito Control Association 2017)..

Larva stage

The larva in the water needs to go to the surface for breathing. Larvae molt their skins four times and get larger after each molt. Most larvae have siphon tubes for breathing and hang upside down from the water surface. At this stage, some differences exist between species:

- Anopheles larvae do not have a siphon and lie parallel to the water surface to get a supply of oxygen through a breathing opening.

- *Coquillettidia* and *Mansonia* larvae attach to plants to obtain their air supply.

The larvae feed on microorganisms and organic matter in the water. During the fourth molt, the larva changes into a pupa (American Mosquito Control Association 2017).

Pupa stage

The pupal stage is a resting, non-feeding stage of development. Pupae are mobile, responding to light changes and moving (tumble) with a flip of their tails towards the bottom or protective areas. This is the time the mosquito changes into an adult. In *Culex* species, this takes about two days in the summer (South of the united States). At the end of the development, the pupal skin splits and the adult mosquito (imago) emerges (American Mosquito Control Association 2017)..

Adult stage

The newly emerged adult rests on the surface of the water for a short time to allow itself to dry and all its body parts to harden. The wings have to spread out and dry properly before it can fly. Blood feeding and mating will occur couple of days after the adults emerge.

The different stages of this cycle will usually last for a period that depends on both temperature and species characteristics. For exemple, *Culex tarsalis*, a common California (USA) mosquito, might go through its life cycle in 14 days or 10 days respectively at 70° F and 80° F. Additionally, some mosquitoes species adapted

their entire life cycle to be as short as four days or as long as one month (American Mosquito Control Association 2017).

1.3.3 Vectors of LF

LF is a mosquito-borne disease transmitted by more than 80 mosquito species (White 1975). The main mosquito vectors can be considered to be found in three different geographical areas: South Pacific islands and some limited areas of South East Asia (*Aedes*); West Africa, Papua New Guinea, Vanuatu and Solomon islands (*Anopheles*); China, South East Asia, Egypt, East Africa, the Caribbean and Latin America (*Culex*). The LF elimination strategy in any endemic area will depend upon the vector and their associated competence (White 1975).

Transmission intensity is dependent on ecological and socioeconomic characteristics of the area. In non-urban areas without pit latrines, anophelines are the main vectors of LF; whereas in urban areas, *Culex quinquefasciatus (Cx. quinquefasciatus)* is the principal vector (Hawking 1977; Mandsfied-aders 1927; Maxwell et al. 1999; White 1971). The permanence of these types of breeding sites creates a situation of continuous LF transmission over the year in urban areas, but in rural settings, LF transmission is intense during rainy season because of the non-permanent nature of the breeding sites (McMahon et al. 1981; Wijers and Kiilu 1977). Rapid expansion of urban areas without appropriate accompanying sanitation measures is the leading cause of the abundance of *Cx.quinquefasciatus*. These vectors are responsible for more or less biting nuisance depending on the number of breeding sites (wet pit latrines, cess pits and blocked open drains) (Curtis and Feachem 1981).

1.4 Measures of human infection and LF transmission

After the launch of the GPELF in 2000, an important improvement was observed in LF diagnosis that was previously limited to clinical examination, detection of antibodies against native-antigen preparations, observation of microfilariae in night blood samples using microscopic examination of giemsa stained thick smears or blood filtration membranes (Ramzy 2002; Rebollo and Bockarie 2014). The current human diagnostic tools aim at mapping *W. bancrofti* infection and assessing interventions' impact. They can be categorized into parasitological, immunological, medical imagery, and molecular biology methods.

1.4.1 Parasitological methods

Many epidemiological surveys and control programmes for filariasis determine the prevalence of microfilaraemia and assess the effect of interventions based on microfilariae (Mf) detection methods. *W. bancrofti* Mf has a nocturnal periodicity in many endemic areas (Sasa et al. 1976), requiring night time blood collection be conducted from around 10 pm to 2 am. This is not convenient for either the affected communities or the evaluation team. Examination of Giemsa-stained thick blood films is widely used for detection of microfilaraemia (Eberhard and Lammie 1991). Even though Mf can be lost during fixation and staining leading to erroneous results (Sabesan et al. 1991), the visualization, and counting of Mf in blood samples under a microscope remains the gold standard to diagnose active LF infection. However, microfilaraemia can be an insensitive method if microfilarial density is very low, especially after implementation of MDA as part of an elimination programme (Eberhard and Lammie 1991; Melrose et al. 2004). Nearly 50% of infected children are not detected using measurement of microfilaraemia alone. Moreover, a majority

of individuals with active LF infection have no detectable circulating Mf, but can perpetuate the transmission of infection (Lammie, Hightower, and Eberhard 1994; Rebollo and Bockarie 2014). Therefore, the prevalence of LF based solely on microfilaraemia underestimates the real burden of the disease (Turner et al. 1993). However, it is inexpensive and can be used in laboratories with limited facilities (Rebollo and Bockarie 2014). It is the method used for baseline LF endemicity level assessment as part of the elimination programme in endemic countries like Mali. The additional following three microfilarial concentration techniques are also used and may or may not increase the test sensitivity. They are used for research studies only.

1.4.1.1 ***The membrane filtration technique (Nucleopore®)*** (Chularerk and Desowitz 1970)

Usually, one mL of whole blood is haemolysed and filtered through a membrane with 5 µm pores that traps the Mf but allows smaller cells to pass through. The filter is then removed from the plastic holder, placed on a microscope slide and stained with Giemsa. Mf are counted under a microscope (Figure 1.4). This method allows quantification of Mf from a known amount of blood and morphologic identification of the microfilarial species, especially in areas where more than one filarial species are endemic. For this method, it is recommended to do two filtrations for each individual and use a mean to determine parasite load. This technique is not cheap and requires qualified techncians. Its use is limited in Mali to the clinical research activities for an accurate assessment of various interventions' impact.

1.4.1.2 ***The modified Knott's concentration method*** (Knott 1935)

One mL of blood is mixed with 9 mL of 2% formalin in a 15-mL tube and centrifuged to sediment the Mf. The supernatant is removed, a drop of 1% methylene blue is added to the sediment and the mix is transferred to a glass slide and visualized under a microscope for Mf. The precipitate can make Mf examination difficult and thus reduce its sensitivity.

1.4.1.3 The counting chamber technique (McMahon et al. 1979)

Widely used in East Africa, 100 µl of blood is mixed with 900 µl of 3% acetic acid and then transferred into a special chamber called "counting chamber" and Mf examination and counting is done under a microscope. This method does not distinguish between microfilarial species.

A: Filtration membrane box and the other filtration device components

B: The other filtration device components (membrane holder)

C: Blood sample filtration through the

D: recovery of the filtered blood into a

Figure 1.4 Nucleopore filtration technique set for blood filtration

1.4.2 Immunological Methods

1.4.2.1 CFA Detection

For Bancroftian filariasis, the development of a monoclonal antibody, Og4C3, for detecting CFA by ELISA (Enzyme-Linked Immuno-Sorbent Assay) in early 1990 (More and Copeman 1990) offered the convenience of daytime blood collection with a greater test sensitivity as compared to microfilaraemia (Simonsen and Dunyo 1999; Turner et al. 1993). The CFA assay is aimed at showing the presence of the circulating filarial antigen in the tested blood sample. It has the advantage of detecting infection in children (or adults) with LF who were tested negative for microfilaraemia (Steel et al. 2001). The Og4C3 assay approaches 100% sensitivity for patent infection as assessed by Giemsa-stained thick blood films, which makes it suitable for field studies (Lammie et al. 1994). It can also be used for quantitative assessment of LF infection. In its current format, this ELISA, in addition to its operating cost, requires a well-trained technician and specialized equipment such as an ELISA reader, making it not very user friendly for fieldwork.

The ICT, based on the monoclonal antibody AD12.1, was developed later in the 90's (Weil et al. 1997). It has comparable sensitivity and specificity to the Og4C3 assay, but revolutionized the diagnosis of Bancroftian filariasis due to its rapid test format, readily applicable in field studies (Chandrasena et al. 2002; Njenga and Wamae 2001; Pani et al. 2000; Simonsen and Dunyo 1999). The ICT test is currently the preferred method for LF mapping (Gyapong et al. 2002; Onapa et al. 2005). This test is qualitative and semi-quantitative (Chesnais et al. 2013) and provides with the result in 10 minutes. However, it has some backlogs such as the need of a cold chain (2-8 degrees Celsius), the necessity to read the card at exactly

10 minutes after blood application, a short shelf life (no more than nine months in suitable storage conditions) and the fact that one will remain positive several months after worms and Mf dying (Bockarie et al. 2002; Reimer et al. 2013).

Recently, a new CFA test, the Alere Filariasis Test Strip (FTS) was produced. It uses similar reagents of those of the ICT, but on a different platform. The FTS is cheaper than the ICT and can be stored for a longer period of time. The two tests are comparable in terms of ease of use in the field, but laboratory studies demonstrated that the FTS could detect lower concentrations of CFA than ICT. In a subsequent study, the FTS detected CFA in 27% more samples than ICT in a highly LF endemic area in Liberia (Weil et al. 2013).

Although the available tests for CFA can detect infection as early as the first month of patency (Weil et al. 1996), their utility in the early detection of recrudescence of transmission is compromised by the fact that microfilaraemia and antigenaemia develop months or years after exposure (Lammie et al. 1998; More and Copeman 1990; Weil et al. 1987, 1997; Witt and Ottesen 2001).

1.4.2.2 Filarial Antibody Detection

Methods for filarial antibody detection targeting crude filarial antigens, have been available and used for epidemiological and diagnostic purposes since the1960s (Harnett, Bradley, and Garate 1998; Melrose et al. 2004). Antibody assays provide a cumulative measure of exposure to filarial infection and may circumvent some limitations of methods based on direct detection of the parasite or its antigens (Lammie et al. 2004). In filarial endemic areas, isotype-specific antifilarial

antibody responses against parasite antigens across populations characteristically correlate with LF infection status (Ottesen 1992), and that fact has driven the development of antibody assays. For example, filarial-specific IgG4 levels correlate well with antigenaemia (Addiss et al. 1995), making its measurement a potential useful strategy for assessing the impact of mass chemotherapy on filarial infection. Antifilarial IgG4 antibody assays reduce cross-reactions with antibodies against non-filarial helminths and most nematodes except *Strongyloides* (Melrose et al. 2004; Muck, Pires, and Lammie 2003). Many recombinant filarial antigens have been developed and used in filarial antibody assays with a presumably greater specificity for either the diagnostic or exposure assays (Lammie et al. 2004; Wang et al. 1999). Bm14, WbSXP and BmR1 are three recombinant antigens commercially available that demonstrated a >90% sensitivity in field studies without any cross-reactivity due to non-filarial helminth infections. Unlike the two other assays, Bm14 ELISA assay demonstrated antibody reactivity with sera from patients with *W. bancrofti*, *B. malayi*, *L. loa* and *O. volvulus.* The Filariasis Cellabs ELISA kit (CELISA) is based on Bm14. In Samoan villages, CELISA was found to be a potential diagnostic tool that could be used for LF surveillance in the South Pacific (Joseph et al. 2011). BmR1 assays are relatively insensitive for *W. bancrofti* infection, but sensitive for *B. malayi* infection, making the assay suitable and useful in areas with mostly or only Brugian filariasis (Lammie et al. 2004).

More recently, Wb123 IgG4, an immunoassay based on detection of antibody to a *W. bancrofti* L3-specific antigen, was found very specific and sensitive (>90%) in detecting *W. bancrofti* infection. It exists in a laboratory based ELISA format and in a field-friendly strips format with comparable performance (Kubofcik, Fink, and

Nutman 2012; Steel et al. 2012, 2013). A biplex format of the strips exists for LF (Wb123) and onchocerciasis (Ov16) simultaneous diagnosis on the same test (Steel et al. 2013).

Urinary antibody tests also exist for LF endemicity determination and assessment of children exposure to *W. bancrofti* infection. These tests exist only on laboratory-based format and the antibodies are not available for sale (Samad et al. 2013; Yahathugoda et al. 2014).

In sum, antifilarial antibody detection provides a good assessment tool of LF transmission after MDA cessation (Lammie et al. 2004). This assertion is sustained by the fact that antibodies are detectable in infected humans before CFA or any other manifestation or sign associated with the infection. Antibody responses and their detection should be more sensitive than microfilaraemia or CFA detection (Lammie et al. 2004) . Additionally, children born after transmission is stopped should not be positive for antibody, providing the optimal population for testing during the post MDA surveillance (Gao, Cao, and Chen 1994; Rodríguez-Pérez et al. 1999; Weil et al. 2000). A limitation of this test is the fact that antibody remains detectable after treatment (Steel et al. 2013).

1.4.3 **Medical imagery method**

Ultrasound has been successfully used to visualize adult *W. bancrofti* worms in the scrotal lymphatics of living patients (Amaral et al. 1994). Adult worm characteristic movements within the lymphatics, otherwise known as “the filarial-dance sign” allow its detection. This method has a sensitivity of about 80% (Norões

et al. 1996). It was found suitable for assessing the effects of antifilarial drugs on adult worms especially in men (Dreyer et al. 1998; Hussein et al. 2004), but is not practical for large epidemiologic screening and requires considerable expertise.

1.5 Measures of entomoloigical infection and tranmission

Dissection is performed on freshly collected mosquitoes or on fixed and stained mosquitoes if dissection is not possible at the collection time.

1.5.1 Measures of entomological infection

1.5.1.1 Dissection

Freshly collected mosquitoes

Dead mosquitoes can be dissected within six hours following the collection or the next day if they are kept at four degrees celcius (usually from pyrethrum spray catch or light trap). If the collected mosquitoes are alive (usually from the human landing catch (HLC) or any other method that allows live mosquito collection), they can be kept for a longer time before dissection if they are in a humid environment and allowed to feed on a sugar solution on cotton wool. Prior to dissection, mosquitoes are sorted and containers (collection tubes or cups) labelled by species, collection date, collection site, collection point and collection time. A technician can process about 50–100 specimens daily. The tube or cup containing the mosquitoes can be shaken gently or placed in a refrigerator for few minutes to kill mosquitoes. The killed mosquitoes are then placed in a petri dish on a wet wipe before individual dissection.

The mosquito is deposited on a clean microscope slide before being separated into three parts (the head, the thorax and the abdomen segments) that should be placed individually in each of the three different drops of saline (0.9%NaCl) on the same slide. The wings and legs are removed beforehand and placed in a tube if further molecular processing is needed, especially for *An. gambiae sensu lato (s.l)* to determine the specific species. These steps are done using a dissecting needle under a dissecting microscope with mirror or lateral light.

For the head, the mouth part should be opened cautiously to allow any L3 to actively move into the saline water. After the head, the two other parts should be lacerated with caution not to damage any larval stage (L1 or L2 usually) that does not move. Larvae can be seen at this stage of the process, but to avoid missing any larvae, the three drops are covered with a cover slip before an observation under a compound microscope at x40 (WHO 2013a).

For recently blood-fed mosquitoes, the mid-gut is removed and placed in distilled water to lyse the red blood cells. The solution is then covered with a cover slip and observed under a compound microscope at x10 firstly followed by x40 (WHO 2013a). Quality control should be done by reading 10% of the slides randomly selected by a senior experienced technician.

Fixed and stained mosquitoes

When part or all the collected mosquitoes cannot be processed soon after the collection, the specimens are sorted according to the information sought by the investigators, fixed and stained for later dissection as described by Nelson (1958) (Nelson 1958) and modified by Chambers et al (2009) (Chambers et al. 2009).

Specimens are fixed individually or in pools of interest (on a 80% or more ethanol solution or a 70% ethanol solution with 5% glycerol) in decreasing ethanol dilutions (70%, 55% and 25%) for 30 min in each dilution before a final 30 minutes wash in distilled water. The fixed specimens can be stored for a long period before staining. The mosquitoes are then stained in Mayer's haemalum stain for a week at room temperature followed by washing the specimens in distilled water for three days and storage in pure glycerol. Dissection can occur at any time on fixed specimens.

Items needed: Ethanol (ethyl alcohol) ≥ 80%; Distilled water; Haemalum (Mayer's) stain (VWR, West Chester, Pennsylvania, USA); Glycerol; Glass screw-top vials; measuring cylinder to make up alcohol dilutions of 70%, 55% and 25%; Glass slides and cover slips; Dissecting microscope with mirror or light source and compound microscope.

Method: After collection and identification, place mosquitoes in 80–98% ethanol in tightly sealed vials. Alternatively, 70% ethanol with 5% glycerol (v/v) can be used. They can be placed in individual vials or grouped by species and collection site or time. Up to 200 mosquitoes can be placed together in one 2.5 x 7.6 cm tube. They can be transported and stored indefinitely in alcohol until staining. The dissection is done under a stereoscopic zoom dissecting microscope (at x8 to x35) by separating each mosquito in three parts (head, thorax and abdomen) and carefully opening each part on a microscope slide. Larval stage can be seen at this step but the positive or doubtful slides need further observation under a microscope

at 20x–40x magnification or even at x100 as well as a help from an expert if doubts persist. The number of the different larval stages recovered can then be recorded.

For verification or training purposes, positive and negative slides can be permanently mounted in glycerol if desired.

1.5.1.2 Molecular Biology Methods

The Polymerase chain reaction (PCR) is a molecular biology technique that can detect the parasite DNA or RNA (reverse transcriptase PCR or RT-PCR) in ground mosquitoes and amplify them. It requires well-trained technicians and specialized equipment and laboratories. The PCR method can detect the DNA without distinction between the different larval stages (Fischer et al. 2007; Rao et al. 2006) while the RT-PCR can detect the infective larvae RNA as well as the other larval stages ones (Laney et al. 2010). Given the operating cost and the vectors' low infection rates, these PCR are done on pools of mosquitoes (5-50 usually) gathered according to the parameters of interest and grounded mechanically in a tube. For DNA extraction, samples can be sun dried, put on silica gel or on an alcohol solution (100%) while for the RNA extraction, they should be kept on an RNAlater^{R} solution (Laney et al. 2010). A DNA or RNA extraction kit is used for the release in the tube and the reaction takes place in a thermal cycler machine that assures the DNA or RNA fragment replication to allow its detection.

Filaria genomic DNA can be detected after amplification using the loop-mediated isothermal amplification (LAMP). This sensitive and highly specific method allows DNA detection within 30 minutes and can be used on field as a point of care

(POC) tool. The targeted product amplification causes a turbidity of the solution if the tested human blood or mosquito sample contains *W. bancrofti* DNA. This method is cheaper (no need of thermocycler) and less sensitive to inhibitors in the samples as the conventional PCR (Rebollo and Bockarie 2014; Takagi et al. 2011).

The tested mosquitoes infection or infectivity rates are estimated in term of maximum infection likelihood using statistical software PoolScreen 2.0 (Katholi and Barker 2010). The software uses the number of positive and negative pools as well as the number of mosquitoes in each pool. The pool screening methods are used to reduce the number of tests to perform to estimate the infection prevalence within a large population. The pool sizes are chosen to minimize the number of tests required (Charles R. Katholi et al. 1995). When processing pools of mosquitoes, it is not possible to know how many mosquitoes were positive once a pool becomes positive. Thus, it is not possible to directly calculate the prevalence of infected flies from the proportion of positive pool samples. However, an algorithm developed by Katholi et al in 1995 enables an estimate of the prevalence of infection from the available data. The pool screen software was made based on that algorithm.

1.5.2 Measures of LF transmission intensity and elimination endpoints

LF transmission intensity and its elimination endpoints are measured using indices related to the vector abundance and bioecology and to the transmission.

1.5.2.1 Related to the vector abundance and bioecology

• Vector biting density: number of female mosquitoes collected when attempting to feed on human for a specified period of time (usually hour or day).

• Vector resting density: number of female mosquitoes resting in a room or house and collected per a specified period of time.

• Parity rate: percentage of mosquitoes that laid eggs at least once (parous), calculated by dividing the number of parous mosquitoes of the species of interest by the total number of dissected females of the same species (parous and nulliparous) and multiplying the obtained number by 100.

• Monthly vector biting rate (MBR): number of female mosquitoes collected when attempting to feed on human per month. If collections were not done every night of the month, the daily vector biting density can be multiplied by the mean number of days in a month (30.5 days) (WHO 2013a).

• Annual vector biting rate (ABR): estimated number of female mosquitoes collected when attempting to feed on human per year. If collections were not done every night of the year, the vector biting density per person per day can be multiplied by 365, the number of days in a year. It can also be determined by multiplying the MBR by 12, the number of months in a year. Seasonal variations should be taken in account.

• Human blood index: percentage of female mosquitoes that really fed on human. It is determined by dividing the number of female mosquitoes whose blood meal was identified as human blood by the total number of female mosquitoes whose blood meal was tested for blood origin (from human or animal). An ELISA usually determines the blood origin.

1.5.2.2 Related to LF transmission

• Vector infection rate: percentage of female mosquitoes found infected with any filarial larval stage. It is determined by multiplying the number of female mosquitoes found harbouring a L1, L2 or L3 by 100 and dividing the obtained number by the total number of dissected females of the same species.

• Vector infective rate: percentage of female mosquitoes found infected with a L3 larval stage. It is determined by multiplying the number of female mosquitoes found harbouring a L3 of the parasite by 100 and dividing the obtained number by the total number of dissected females of the same species.

• Monthly infective biting rate (MIBR): estimated number of mosquitoes harbouring at least one L3 biting a human per month. It is determined by multiplying the vector infective rate by the monthly vector-biting rate.

• Annual infective biting rate (AIBR): estimated number of mosquitoes harbouring at least one L3 biting a human per year. It is determined by multiplying the vector infective rate by the annual vector-biting rate.

• Monthly Transmission Potential (MTP): indicator of risk for infection per month. It is determined by dividing the total number of L3 recovered by the number of mosquitoes dissected and multiplying the obtained number by the MBR.

• Annual transmission potential (ATP): indicator of risk for infection per year. It is determined by dividing the total number of L3 recovered by the number of mosquitoes dissected and multiplying the obtained number by the yearly vector-biting rate. It is also the sum of the 12 MTP calculated for the 12 months of the year (WHO 2013a).

The cut-off values of 0.25%, 0.5% and 1% were suggested for *Culex* transmission areas (Farid et al. 2007; Michael et al. 2006; Rao et al. 2014) while 0.65% was

suggested for *Anopheles* transmission areas (Pedersen et al. 2009). These cut-offs are only suggestive.

1.5.2.3 Specific to Aedes mosquitoes

The standard methods for assessing the effect of Vector Control (VC) on *Aedes* mosquitoes is based on collection of larval stages (immature specimens, including pupae) rather than collection of eggs or adults. The usual sampling unit is a house where a careful search, count and characteristic recording of all potentially water-containing containers is implemented. The containers' main characteristics of interest are the presence or absence of water and the presence or absence of *Aedes* larvae, pupae and larval and pupal skins.

• House (premises) index: proportion of houses infested with *Aedes* larvae or pupae. It is determined by multiplying the number of infested houses by 100 before diving the obtained number by the total number of houses inspected.

• Container index: percentage of containers infested with *Aedes* larvae or pupae. It is determined by multiplying the number of positive containers by 100 and dividing the obtained number by the total number of containers inspected.

• Breteau index: number of containers infested with *Aedes* larvae or pupae per 100 houses inspected. It is calculated by multiplying the number of positive containers by 100 and dividing the obtained number by the total number of houses inspected (WHO 2013a).

LF transmission intensity and the impact of elimination interventions (mass drug administration, vector control) in an area can be quantified by examining blood samples to determine the prevalence of microfilarial or filarial antigen carriage

(WHO 2011b). The microfilarial prevalence and the antigen prevalence are determined by dividing the number of people found positive for Mf or for antigen (using ICT, FTS or Og4C3 antigen tests) by the total number of people tested for each method. To have the prevalence in per cent, the obtained number is multiplied by 100.

1.6 Potential vector control

A total of four categories of methods are used for lymphatic filariasis vector control: chemical control and impregnated materials; environmental management; biological control; and integrated control.

1.6.1 Chemical control and impregnated materials

Several categories of chemical products are being used for LF vector control, namely oils, chlorinated hydrocarbons, organophosphates, carbamates, natural pyrethrins (pyrethrum extract) and synthetic pyrethroids, insect growth regulators, and systemic insecticides. In urban settings, mosquito larval stages in breeding sites can be targeted using a thin film of petroleum oils at the surface of the water body. Larvae are deprived of air and die because of that or by poisoning when the oils penetrate into their respiratory orifice.

Dichloro-diphenyl-trichloroethane (DDT) was the most important and commonly used organochlorine. Its use in houses against *Anopheles* mosquitosas part of malaria control programmes allowed drastic reduction of LF transmission levels and even achieved elimination in some areas like Solomon Islands (Webber 1977,

1979). DDT could not be recommended in most of West and Central African countries because of high resistance of the main LF vectors.

The use of insecticide treated nets to prevent mosquito bites is becoming a more common means of vector control because of its efficiency in preventing malaria and ease of use. The nets are currently well known in tropical areas even if their actual usage rate is not ideal (Atieli et al. 2011). For insecticide treated nets to get a significant impact the coverage should be at least 60%, which is not the case in most of LF endemic countries in Africa. Togo and Malawi are the exceptions. Coverage of long lasting insecticidal nets (LLIN) distributed by the national malaria control programme was >75%, contributuing significantly to the fact that these two countries got Mf rate below 1% after five consecutive MDA rounds.

For controlling *Cx. quinquefasciatus,* only organophosphate molecules were used for a long period in some areas like Dar es Salaam and Rangoon. *Cx. quinquefasciatus* is difficult to control because of the variety of breeding sites and the absence of an effective strategy to stop its breeding in a sustainable manner. Using the most effective control measure available would require retreatment of all the numerous potential breeding sites at least four times per year (Hougard et al. 1993), a substantial expenditure in term of money and employees. Polystyrene bead treatment of non-open water bodies can be effective in stopping *Culex* breeding for a long period (Maxwell et al. 1990; Reiter 1978) if the water does not overflow.

Ivermectin, the mainstay of MDA due to its activity against Mf in humans, has also been demonstrated to be toxic for several arthropod species, especially mosquitoes

(Wilson et al. 1993). Mosquitoes that bite people treated with this drug exhibited a shorter survival (Bockarie et al. 1999; Cartel et al. 1990; Foley, Bryan, and Lawrence 2000). This effect was also seen in *Ae. polynesiensis* in French Polynesia up to a four month period after the drug absorption (Cartel et al. 1991). Mortality rates of up to 41% and 82%, respectively, were observed in *Ae. vexans* and *Psorophora confinnis* after a blood meal on animals treated with ivermectin (Loftin et al. 1996).

1.6.2 Environmental management

Environmental management is the sustained modification or change one can do to the environment to circumvent the multiplication of mosquito vectors of pathogens in the concerned area. These modifications are oriented towards the clearance of water bodies or vegetation that are suitable to the reproduction of local vector species.

In places where mosquitoes that transmit LF (eg. *Anopheles* and *Mansonia* mosquitoes) lay eggs; proper management of used water to avoid marshes will prevent multiplication of these vectors. If the principal mosquito species transmitting LF in an area are *Mansonia* species, discarding plants like *Pista* (used by the mosquito eggs and larval stages to develop) will decrease the number of bites that a person receives daily. These plants can be removed manually or by depriving them of water, dramatically reducing the *Mansonia* mosquito population.

As in the case of *Mansonia*, other mosquito vectors may need special control methods targeting specific developmental stages. For example, *Ae. scutellaris*

group mosquitoes lay eggs in small receptacles that contain rain water. So, removal of empty bottles, coconut shells, and used tyres around habitation help reduce *Aedes* densities.

1.6.3 Biological control

The biological control of LF vectors consists of the use of pathogens and predators of mosquito vectors, such as viruses, bacteria, protozoa, fungi, nematodes and fishes that feed on mosquito larval stages. The implementation of biological control can have more impact if done at the same time as environmental handling.

The two important bacteria currently in large use are *Bacillus thuringiensis israelensis* (*Bti*) and *Bacillus sphaericus*. The first uses toxin to kill various mosquito species larvae, including *Anopheles* (Majori, Sabatinelli, and Coluzzi 1987), *Culex* (Kar et al. 1997) and *Mansonia* (Chang, Ho, and Chan 1990), while the second is very good in killing *Culex* genera from polluted and non-polluted water bodies by inducing an osmotic shock caused by the interaction between the toxin and intestinal proteins of the insect (Pardo-López, Soberón, and Bravo 2013). *Gambusia affinis* is a fish that feeds on mosquito larvae pullulating rice cultivation areas or wells and helps considerably in reducing the number of mosquito produced by these water bodies.

Romanomermis culicivorax (a mermithid nematode pathogen for adult mosquito) and *Lagenidium giganteum* (a fungus) were successfully used in rice fields to control mosquitoes (Lacey and Lacey 1990).

Introduction of sterile male *Culex* mosquitoes allowed the elimination of this species in an island of Florida (Patterson et al. 1970). Genetic control of mosquitoes was first used successfully (Grover et al. 1976; Jayasekera et al. 1980; Yasuno et al. 1978) for *Cx. quinquefasciatus*. The use of a chemosterilisant (Sharma et al. 1973), creation of an intrinsic "cytoplasmic incompatibility" (Curtis and Adak 1974; Laven 1967) and the elimination of female *Culex* based on the larval stage pupae size has also been used successfully (Sharma et al. 1973) with this species. Unfortunately, females from other areas (≥3km from the intervention area) were found to migrate to the intervention area.

Although there has recently been considerable interest inthe development of transgenic mosquitoes unable to transmit *Plasmodium,* this approach has not yet been explored for *W. bancrofti*.

These methods and technologies can be used to control LF transmission. Some of these technologies such as the bi-directional "cytoplasmic incompatibility" (gene driving system) exist only for *Culex* (not for *Anopheles*).

1.6.4 Integrated vector management

Integrated vector management involves the combination of two or more of the methods mentioned above so that they act in a complementary way to achieve a high degree of control. Control of mosquitoes using chemical pesticides has generated several problems including insecticide resistance, safety risks for humans and domestic animals, and other environmental concerns. These problems and the high cost and sustainability issues of programmes based predominantly on

conventional insecticides have stimulated increased interest in integrated vector control.

1.6.5 The role of vector control against LF transmission

Besides the common mosquito control measures evocated prior to this section, many other recent measures exist and can be used as part of an integrated vector management (IVM) that can foster the LF elimination process.

Because of the presence of more than one LF vector species (each of which may have different behaviour) in several endemic areas, the use of several IVM measures will likely be needed for sustained elimination in most cases. Nevertheless, the use of a single method for LF vector control was successful in eliminating LF in some *Anopheles*-transmitted countries. Examples include the Solomon Islands using indoor spraying (residual) of DDT (Webber 1977, 1979) and The Gambia using ITN (Rebollo, Sambou, et al. 2015). In PNG, use of LLIN was successful to drastically reduce LF transmission (Reimer et al. 2013).

Spraying DDT in houses is the most commonly used vector control measure for malaria control. It has also been used successfully to reduce *W. bancrofti* transmission by *An. punctulatus* group in several countries, including the Solomon Islands (Webber 1979), PNG (Bockarie 1994) and Indonesia (Iyengar, De Rook, and Van Dijk 1959). It has also been found to decrease LF transmission by *An. gambiae s.l* and *An. funestus* in Togo (Brengues, Subra, and Bouchite 1969; Scheiber et al. 1976).

An. mosquitoes are characterized by a facilitation transmission pattern (Southgate and Bryan 1992; Webber 1991) and were found to be very sensitive to vector control. Only in *Anopheles* and *Mansonia* LF transmission areas had transmission interruption achieved using vector control alone. This phenomenon was observed in some areas of Central America where *An. darlingi* is the vector, and in some countries of South-East Asia where *B. malayi* was transmitted by *An. sinensis*, *An. barbirostris* and other mosquitoes that bite indoors (WHO 1984). Thus, reducing the vector density, the parasite load or both, can interrupt *Anopheles* transmission.

In Sri Lanka, where Brugian filariasis was transmitted by *Mansonia* species, the countrywide use of DDT spraying (from 1959 to 1965) in houses to fight malaria induced the complete disappearance of microfilaraemia (Abdulcader and Sasa 1966) despite a prevalence of 6.8% in 1939 before any intervention (Sasa 1976).

1.7 The type of vector control in endemic countries

In sub-Saharan Africa, elimination of LF and malaria will require a combination of chemotherapy and decreased human-vector contact through vector control with LLIN and indoor residual spraying (IRS) (Onyango et al. 2013). LLINs are currently provided at no cost for endemic communities, with a focus on pregnant mothers and children < 5 years, as part of the malaria elimination actions in many countries (Rebollo, Mohammed, et al. 2015). Several authors have pointed out the important reduction of microfilaraemia prevalence due to vector control alone and discussed how this can be improved by adding MDA (Bockarie et al. 2002; Reimer et al. 2013; Sunish et al. 2007). In areas where bed net usage rates are high and vector populations are still susceptible, bed net use may eliminate LF if the

reservoir of microfilaraemia has first been reduced by MDA or if the baseline endemicity level was low, as in the Solomon Islands (Reimer et al. 2013; Webber 1979). Recently, national governments and their funding partners achieved better coverage of LLIN in many malaria and LF endemic areas (Rebollo, Sambou, et al. 2015), creating a real opportunity for universal coverage (one net for every two people) and fostering of the elimination of these diseases (Rebollo, Sambou, et al. 2015). Nevertheless, in many hyper endemic areas, the addition of MDA to vector control measures is needed, because a small increase in mosquito vector biting rate with no change in the human microfilaraemia rate can induce re-emergence of transmission, especially when *Anopheles* mosquitoes are not the main transmitting vectors and the MDA coverage is low (WHO 2013a).

1.8 The insecticide resistance issue

About 70 million LLIN were distributed at no cost in 88 countries worldwide, including 39 countries in Africa (WHO 2013a). These free distributions targeted malaria although they also impacted other mosquito borne diseases, such as LF (Blackburn et al. 2006). Unfortunately, this useful control measure is threatened by an increasing frequency of resistance of mosquito vectors to the insecticides recommended by the WHO for LLIN and IRS (Hemingway, Field, and Vontas 2002; WHO global malaria program 2012). These resistance issues are due to a combination of factors, including chemical vector control pressure (LLIN; IRS) (Mathias et al. 2011), the use of insecticides in agriculture (Baleta 2009; Lines 1988) and environmental pollution (Xenobiotic pollution) that has been reported to accelerate the selection for insecticide resistance in malaria vectors (Djouaka et al. 2008; Nkya et al. 2013).

Studies are underway to determine how to best overcome the resistance issue. Currently, Chlorfenapyr is available for vector control in the form of ITN or residual spraying in areas where mosquitoes are showing pyrethroid resistance (N'Guessan et al. 2007).

Due to the paucity of entomological studies targeting LF, the impact of insecticide resistance on the efficacy of ITN and IRS has not been established in the context of LF elimination. Since there has been geographical expansion of resistance of mosquitoes to multiple classes of insecticide across Africa (Ranson et al. 2016), the impact of this on LF control requires assessment.

1.9 Vector competence and transmission

The competence of a mosquito vector in transmitting LF is its ability to pick up microfilariae and allow its maturation up to the third stage larvae (L3) before transmitting this L3 to a human (Boakye et al. 2004). Vector-parasite relationship in terms of *W. bancrofti* transmission is characterized by two main schemes, the facilitation and the limitation.

The facilitation that is observed with *An. gambiae* is associated with an increase in the vector's capacity to uptake and bring microfilariae to the L3 stage when the microfilarial load is high. On a biological point of view, the well developed teeth-like cibarial armatures damage some of the ingested microfilariae providing the remaining ones with more chance to get to a mature L3 stage. The limitation is the pattern that allows more efficient development to the L3 stage when the blood

microfilarial load is low. This characterize vectors species with relatively less developed cibarial armatures for which too many microfilariae will impede the capacity of the vector to bring many of them to the mature L3 stage. Limitation is observed within the *Culicine* species. A third relationship, deemed less epidemiologically important, is the proportionality that is characterized by a constant parasite yield ratio that does not change as microfilarial intake increases (Bockarie, Pedersen, et al. 2009; WHO 2013a).

LF due to *W. bancrofti* is transmitted by several mosquito species exhibiting different level of competence in transmitting. The vector competence becomes particularly important currently with the MDA impact on infection and parasite load. The main described LF transmission patterns are all linked with the blood parasite load and the vector species (Erickson et al. 2013; Southgate and Bryan 1992). The elimination thresholds in the context of the LF TAS are currently driven by the local vectors' competence (Erickson et al. 2013; WHO 2013a). They are different for the vectors showing limitation (*Culex* and *Aedes* genera) and those showing facilitation (*Anopheles* genus) (Erickson et al. 2013; WHO 2013a).

Several studies showed the vector-parasite relationship in anophelines, as the facilitation but in other areas, *Anopheles* species might be characterized by the limitation as the type of relationship (Amuzu, Wilson, and Boakye 2010; Boakye et al. 2004). In Papua New Guinea at low parasite density exposures, *An. farauti s.s* was carrying five times more parasites than African *Anopheles* species (Erickson et al. 2013).

Differences in vector competence mainly reside in the structures of the cibarial armatures that are more developed within those showing limitation (Bryan and Southgate 1988; Erickson et al. 2013), the host immune responses as a reaction to an external element by inducing a melanization, the Mf uptake impact on mosquito survival that becomes more important when the ingested number is high (Erickson et al. 2013) and the vectors' resistance to insecticides on vector side (Hemingway and Ranson 2000). On the human blood side, vector competence may be impacted by the presence or absence of coinfection with the malaria parasite, *Plasmodium falciparum* (*P. falciparum*) (M T Aliota et al. 2011; Muturi et al. 2006) and previous treatment with ivermectin (Foley et al. 2000).

Some of the vector related factors are listed below:

1.9.1 Structure of cibarial armature

In many LF vectors, the digestive tract contains teeth-like structures (armatures) protruding from the gut wall into the lumen. At which extent these armatures are developed may determine the variation in vectorial efficiency between and within mosquito species (Amuzu et al. 2010; Bryan and Southgate 1988). The more developed the refractory apparatus, like the cibarial armature in *Anopheles* species, the higher the threshold density of Mf sufficient to interrupt transmission of LF (Bryan and Southgate 1988). McGreevy et al (1982) demonstrated that the African vector *An. gambiae* with a well-developed cibarial armature kills about 50% of ingested *W. bancrofti* microfilaraemia while *Cx. quinquefasciatus* and *Ae. aegypti*, with less prominent ones, kill only about 6% (McGreevy et al. 1982). The complexity of the structure of the cibarial armature also varies among anopheline

vectors (McGreevy et al. 1978) but it is not known to what extent it affects vector competence within the same genera.

1.9.2 Melanization

In partially susceptible and in refractory mosquito species, filarial larvae encounter host immune responses in the form of melanization at different stages during their development. Extracellular and intracellular melanization of *Brugia* larvae have been reported in different species of mosquitoes. However, this phenomenon has not previously been observed in wild caught anopheline mosquitoes infected with *W. bancrofti* in Africa. Melanized microfilariae have been observed in wild caught *An. punctulatus* in Papua New Guinea (*M. Bockarie personnal communication, December 2009*). In the same country, Erickson et al (2013) did not observe this phenomenon and found very few *An. farauti* harbouring melanized *W. bancrofti* (Erickson et al. 2013).

1.9.3 Infection impact on mosquito survival

As reported by Michael, Snow and Bockarie (2009) from a meta-analysis study that took in account the available data on mosquito infection and infectivity mostly in controlled conditions, no apparent relationship was found between the mortality of the three main genera of mosquito involved in LF transmission (*Culex, Aedes and Anopheles*) and the Mf uptake (Michael, Snow, and Bockarie 2009). Some of the available individual studies observed an impact of the infection intensity on mosquito survival (Erickson et al. 2013; Jordan and Goatly 1962) while others did not (Michael et al. 2009). In Papua New Guinea, an increased mortality rate was observed for *An. farauti s.s* that fed on medium to high mf load blood (Erickson et

al. 2013) but this phenomenon was not observed for *An. punctulatus* (Michael et al. 2009).

The rarity of data regarding *Anopheles* genus mortality allows saying very little about the role played by filarial infection (Michael et al. 2009). These vectors' characteristics appear to be specific not only for area but also for species in the same area.

1.9.4 Insecticide resistance impact on LF transmission

The extremely high levels of esterases associated with insecticide can have an impact on the Mf maturation within the mosquito vector as observed in *Culex* in the form of Mf development inhibition by very elevated esterases levels (McCarroll et al. 2000). Additionally, Hemingway and Ranson reported that insecticide resistant *Anopheles* may exhibit the same phenomenon (Hemingway and Ranson 2000).

1.10 The *Loa loa* co endemicity with LF

In WHO African Region countries like Cameroon are faced with life-threatening adverse reactions experienced by certain persons with high levels of *Loa loa* Mf in the blood receiving ivermectin. A new strategy has been introduced in *L. loa* and LF co-endemic areas where elimination program is needed (WHO 2015). Given the importance of the bed net especially in areas where loasis is coendemic with LF, the hard to reach remote areas deserve alternative ITN that are part of the main component of WHO strategy in these areas (Cano et al. 2014).

Research is ongoing to find new strategies to detect these individuals with very high microfilarial loads in the suspected areas to exclude them from ivermectin treatment or to provide them with alternative drug regimen like albendazole alone given twice a year as a MDA plus the IVC (WHO 2016a). The WHO has proposed the last two points, but only one of the nine countries facing this issue had implemented this strategy by the end of 2015 except few research studies on these strategies (WHO 2016a).

1.11 LF transmission in non-rural settings in Africa

Many urban areas in West Africa were undergoing annual MDA or started in 2015 (WHO 2016a). The mosquito species composition in these settings is dominated by *Culex spp* that may not be vectors or have not been proven to transmit LF despite the fact that they have been experimentally infected with *W. bancrofti* up to the L3 stage in Liberia (Gelfand 1955). In West Africa, the main vectors of LF are members of the *An. gambiae* complex that are relatively uncommon in urban areas because of the nature of available breeding sites (de Souza et al. 2014). Recently, the same authors reported a lack of evidence for LF transmission in big cities of Sierra Leone and Liberia where rural-urban migration occurred because of the political conflict. The reallocation of the resources used to implement MDA in such settings is an important issue (de Souza et al. 2014) and assessing urban settings based on parasitological assessment and xenomonitoring before a final decision is taken regarding MDA is crucial.

Chapter 2

Rationale and objectives

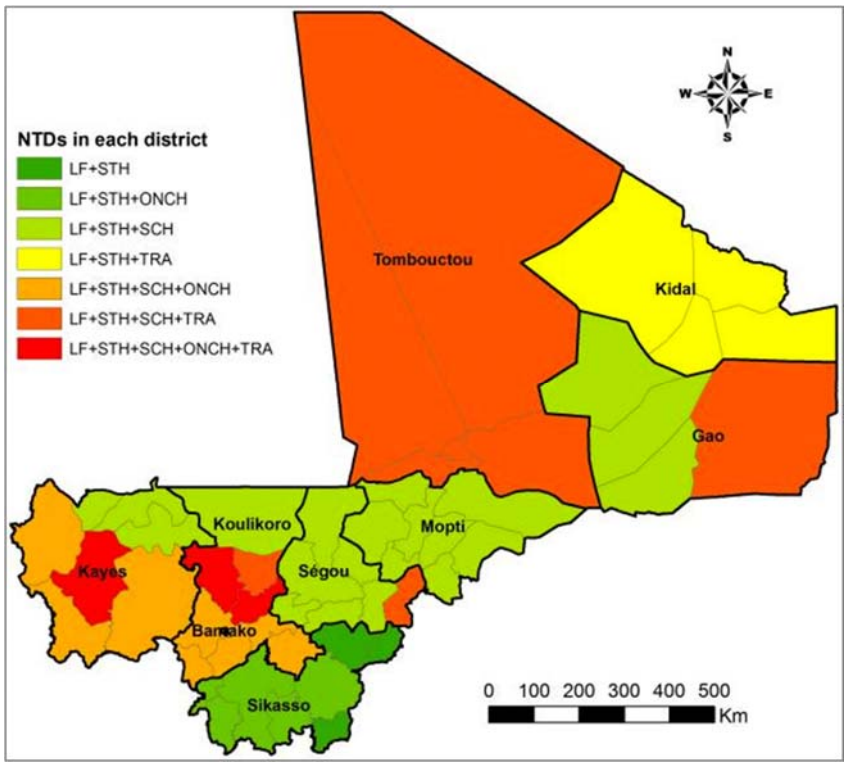
2.1 Mali situation

2.1.1 LF elimination progress in the country

In Mali, although the public health importance of LF was noted as early as the 1970′s (Touré 1979), the prevalence and distribution were not studied again until 2002. This was due to the lack of orginzation of the health system and the priotization given to diseases that are associated to higher mortality rates such as malaria. Based on Point of care diagnosis using the Immunochromatographic card test for LF antigen detection, all eight administrative regions of Mali were shown to be endemic for lymphatic filariasis with an overall prevalence of 7.07%, ranging from 1% in the north to 18.6% in the south in 2004 with approximately 10 million people at risk of the disease (Dembélé, Bamani, Dembélé, M. O. N. O. Traoré, et al. 2012). More than one thousand cases of chronic LF clinical manifestations were reported by the census survey undertaken by the NPELF (Dembele M, personal communication, June 2014). The MDA was initiated in 2005 with few districts and reached the countrywide coverage in 2009. As of 2016, 49 of the 65 endemic health districts have passed the TAS1, stopped MDA and entered the program surveillance phase. In 2012, the first two health districts that initiated the pilot MDA implementation met the criteria and stopped the MDA.

Except the three administrative regions of the Northern part of the country where safety issue are oingoing since 2012, the EU of the other regions passed the TAS as of 2016. As part of the post-MDA surveillance, in 2015, the first two districts that stopped the MDA passed their TAS2 using ICT within the 6-7 years old children three years after their last MDA. In Mali, the NPELF and its technical arm that is the Filariasis Research Unit (FRU) of the Faculty of Medicine of Bamako

are implementing the LF elimination activities. The FRU plans and implements the national programme's activities impact assessment as well as clinical research activities. As part of the reasearch activities, the FRU conducted clinical trials on the doxycycline impact on *Mansonella perstans*, the impact of the higher dose and higher frequency on *Wuchereria bancrofti* and the triple co administration of albendazole, ivermectin and azithromycin for the MDA targeting LF and trachoma. Additional studies on entomological and immunological aspects of LF are being conducted by the FRU.



LF: lymphatic filariasis; ONCH: onchocerciasis; SCH: schistosomiasis; STH: soil-transmitted helminthiasis; TRA: trachoma. In Kidal region, the endemicity level of schistosomiasis in each district is not yet clear and further mapping is planned.

Figure 2.1 Map of Mali showing the endemicity areas for lymphatic Filariasis (Dembélé, Bamani, Dembélé, M. O. Traoré, et al. 2012)

2.1.2 Vectors of LF in Mali

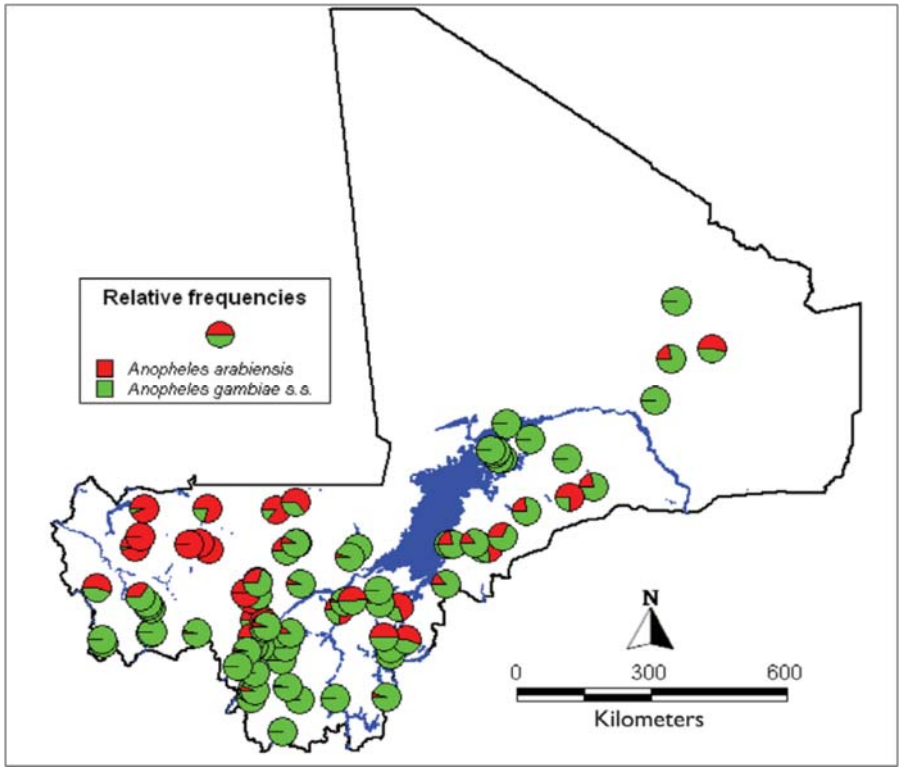
The main vectors of LF in Mali belong to the *An. gambiae* group *(An. gambiae s.l* also called *An. gambiae* complex), followed by *An. funestus* complex (Coulibaly et al. 2006; Toure et al. 1996). In Mali, the *An. gambiae* complex of mosquitoes is composed of *An. arabiensis* Patton and three chromosomal forms of *An. gambiae Giles sensu stricto* (*s.s*) named Bamako, Savanna and Mopti (Coluzzi, Petrarca, and Di Deco 1985; Ranque et al. 1984). Based on molecular and bionomical evidence, the *An. gambiae* molecular "M form" (chromosomal form Mopti) is named *Anopheles coluzzii Coetzee* & *Wilkerson sp. n*., while the "S form" (chromosomal forms Bamako and Savanna) retains the nominotypical name *Anopheles gambiae Giles* (Coetzee et al. 2013).

The members of *An. gambiae s.l* exhibit noticeable differences in spatial and seasonal distribution (Coluzzi et al. 1985; Sogoba et al. 2008a). They are all susceptible vectors for malaria and LF (Toure et al. 1996). The eco-climatic characteristics drive the density and the specific species of *Anophes* mosquito frequency distribution in Mali.

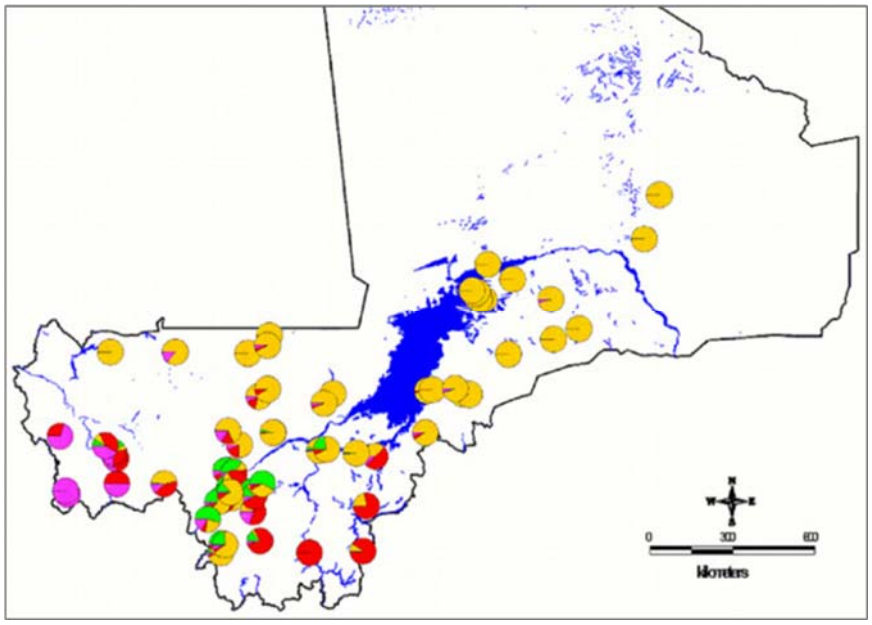
Besides this complex, the second important vector of LF in Mali is the *An. funestus* complex. Its members are not as well-known as those of *An.gambiae s.l* because of the difficulty with rearing *An. funestus* in the insectary (Koekemoer et al. 2014). Of note, *An. funestus* was shown to be the main vector of LF in certain regions of Mali (Coulibaly et al. 2006; Toure 1979) and played a more prominent role than *An. gambiae s.l* during the dry cold season (Coulibaly et al. 2013; Toure 1979).

Although other *Anopheles* species, such as *An. nili, An. rufipes and An. pharaoensis*, exist in West African countries, they have never or rarely been found infected with LF due to their relatively low numbers (Hamon et al. 1968).

An. gambiae and *An. funestus* are both highly anthropophilic, improving their capacity as vectors (Toure 1979). They have different types of breeding sites with small temporary rain-dependent pools and puddles for *An. gambiae* and large non-temporary water bodies with plants for *An. funestus*. The two vectors also have different high-density periods ensuring a long period of transmission (Coulibaly et al. 2006; Toure 1979). *An. gambiae s.s* and *An. arabiensis* are more frequent from the beginning of the rainy season to the end of the rainy season (May-June to September-October) while *An. funestus* start to be more common at the end of the rainy season (October to December) (Coulibaly et al. 2006; Toure 1979). To be successful, vector control strategies targeting malaria and LF in the country, should take into account the biology and behaviour of the common species as well as the relatively less common vector species.



A: Source: (Sogoba et al. 2007)



B: Source: (Sogoba et al. 2008b)

Figure 2.2 Map of Mali showing the distribution of different *Anopheles* mosquitoes species relative frequencies

A: Observed relative frequencies of An. arabiensis and An. gambiae s.s. in 94 sampling locations in Mali, West Africa

The green color represents the relative frequencies of *An. gambiae s.s.* and the red the relative frequencies of *An. arabiensis.*

B: Observed relative frequencies of the chromosomal forms in 71 locations in Mali, West Africa

The orange represents Mopti, the red Savanna, the green Bamako and the purple the hybrids/recombinants relative frequencies. (Source Spatial distribution of the chromosomal forms of anopheles gambiae in Mali

2.2 Rationale

2.2.1 MDA with albendazole/ivermectin combination impact on *Anopheles* transmitted LF

One of the key questions that has emerged as the GPELF has grown globally since 2000 (WHO 2015) is the impact of annual MDA using albendazole plus ivermectin (ALB/IVER) on the transmission of the *W. bancrofti* infection in different epidemiological settings in Africa.

Whereas the long-term impact of combination albendazole/diethylcarbamazine (ALB/DEC) on transmission of LF has been demonstrated in multiple epidemiologic settings (Ramzy et al. 2006; Supali et al. 2013), few studies have examined the long term impact of repeated annual administration of ALB/IVER on LF transmission by *An. gambiae* complex members in West Africa (CDC 2011; Richards et al. 2011). This regimen is the one recommended in West Africa because of the overlapping geographic distributions of LF and onchocerciasis.

An infection rate of <2% in 6-7 year old children is believed to be the level that is unable to sustain transmission (WHO 2011b) in an *Anopheles* transmission area.

Although not part of most of the post-MDA TAS, entomological assessment of LF transmission can be of great importance, because it directly measures the transmission potential. For this thesis, the ICT was used, as it is the gold standard CFA prevalence assessment tool recommended for TAS. Given some of the features of this test, additional data on LF prevalence were obtained using a variety of other techniques to ascertain whether or not transmission has been interrupted in this previously highly LF-endemic region (Sikasso) of Mali. Our methodology was based on an integrated approach to surveillance, using a newly available alternative diagnostic method (Wb123) that detects antibody against *W. bancrofti* (Steel et al. 2012) as well as xenomonitoring to allow clearer identification of any potential resurgence of transmission.

Xenomonitoring is used to measure level of Mf reservoir in the community during and after MDA through the determination of infection rates in mosquitos. Although the use of xenomonitoring as a monitoring tool holds promise as an important component of post-MDA surveillance in the LF elimination process, it requires a safe and effective way of collecting mosquitoes at the community level that is representative of the vector fauna.

2.2.2 *Anopheles* mosquito collection methods

Several LF endemic countries have stopped or are about to stop MDA in many endemic districts. Given the increasing role of entomology in determining where MDA alone is not as effective as expected in eliminating LF, it is important to study local vector behaviours and adapt the tools used for vector collection to meet local and current needs, including ethical concerns. Ideally, examination of vector

abundance, distribution, species composition and infectivity should be assessed prior to initiation and at the end of MDA. To date, the HLC has been the most frequently used method for *Anopheles* collections in many endemic areas of West Africa. This is due in large part to the fact that it mimics the natural situation of mosquitoes trying to bite humans. However, HLC raises ethical concerns including the possibility that infected mosquitoes can bite the collectors (Govella, Moore, and Killeen 2010; Sikaala et al. 2013). Additionally, HLC is labour intensive and the mosquito yield may depend on the collector's attractiveness to mosquitoes, ability and experience (Govella et al. 2010; Sikaala et al. 2013). Thus, despite the fact that most of the existing mosquito data were generated using this method, its use is controversial and many ethics committees are reluctant to continue to approve its use for sampling mosquitoes.

To overcome these issues, alternative trapping methods have been explored with regard to ease of use, operator independence, cost of implementation and safety to the operator. The Biogents sentinel trap (BGST) (Maciel-de-Freitas, Eiras, and Lourenço-de-Oliveira 2006), and a human-baited tent trap, the Ifakara tent trap type C (ITTC) (Govella et al. 2011, 2010) represent alternative collection methods.

2.2.3 Spatial distribution of LF endemicity foci

Kouassi et al (2015) in Guinea (Kouassi et al. 2015) and De Souza et al, 2014 in Sierra Leone and Liberia (de Souza et al. 2014) reported a lack of evidence for LF transmission in urban big cities. In pre-MDA era studies, dispersal of endemicity foci within endemic areas was reported as focally distributed in areas where *Anopheles* species act as vectors of *W. bancrofti* (Graves et al. 2013; Kazura and Bockarie

2003). However, the impact of *An. gambiae* complex density on LF endemicity at a fine scale in endemic areas under MDA is unknown, especially after more than five annual MDA rounds. Such knowledge coupled with distribution maps may help increase our understanding of the natural history of LF endemicity and provide with information to help decision makers allocate scarce resources.

2.3 Aims and specific objectives

The main aims of the research were to i) investigate LF vector population and associated transmission patterns before, during and after the initiation of MDA ii) assess efficacy of new entomological trapping tools for LF post-MDA xeno-monitoring and iii) determine transmission potential in an urban environment in Mali.

To achieve this goal, six specific objectives have been defined:

1. To assess LF transmission pattern in a pilot area in southern Mali prior to and following the institution of MDA;
2. To determine the impact of six annual rounds of MDA on vector fauna competence to transmit LF in the pilot area of Sikasso district;
3. To assess the dynamics of antigenemia and transmission intensity of *W. bancrofti* following cessation of mass drug administration in a formerly highly endemic region of Mali
4. To compare the efficacy of human landing catch method for sampling mosquitoes against a human baited tent trap and the Biogents sentinel trap in two villages with different vector densities in the the Sudan savannah district of Kolondieba, Mali;

5. To compare LF transmission intensity after six MDA rounds in two neighbouring villages of Kolondieba district with different *Anopheles* densities;
6. To assess LF transmission in urban settings in Mali.

The pilot site was selected three years prior to the launch of the MDA by the National Programme to provide the decision makers locally (Ministry of Health) and globally (GPELF) with information and advice on the outcome of their intervention. In anticipation of the launch of NPELF activities in the country, a pilot study of the impact of MDA with ALB/IVER on *W. bancrofti* transmission was initiated in collaboration with the WHO in Mali, Ghana (West Africa) and Kenya (East Africa) in 2001.

2.4 General methods

2.4.1 Study overall design

These studies were predominantly descriptive and included cross sectional entomological surveys along with longitudinal human surveys. Additionally, the impact of an intervention (the MDA) on both human and vector infection rates were explored. Standard infection status assessment methods were used as well as a recently developed method, antibody testing for Wb123, which detects infection earlier than the previous tests because it targets antibody responses instead of CFA (Kubofcik et al. 2012; Steel et al. 2013).

2.4.2 Study sites

This study was conducted in rural and non-rural settings. The rural endemic areas were in Sikasso and Kolondieba districts, in the administrative region of Sikasso, the most highly endemic area in Mali before any LF oriented intervention (Dembélé, Bamani, Dembélé, M. O. N. O. Traoré, et al. 2012). Bamako, the capital city, was used for the urban LF transmission assessment.

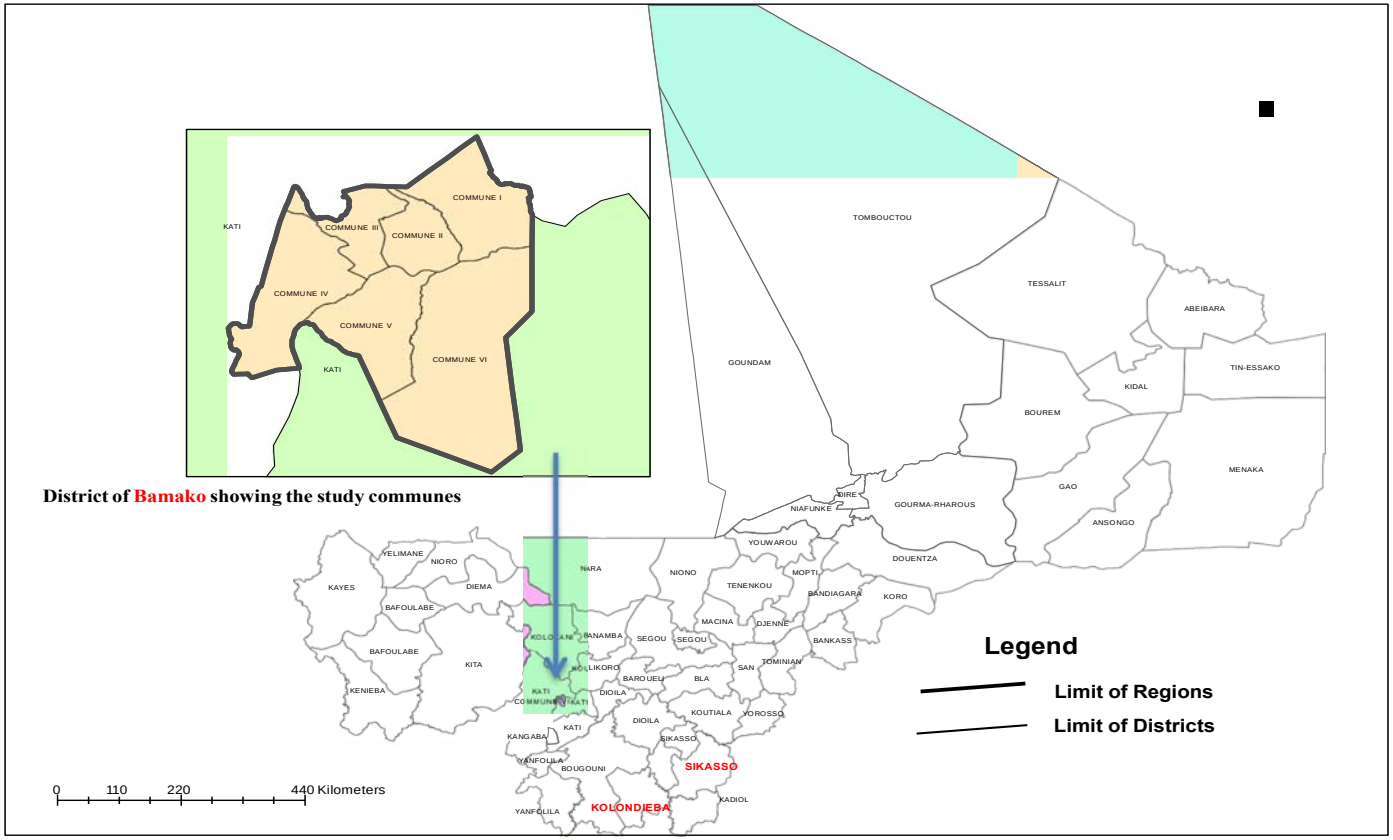


Figure 2.3 Map of Mali showing the three study districts (Sikasso, Kolondieba and Bamako)
The 3 study sites names are in Red and the Bamako area was enlarged to show the communes that were visited during the study

2.4.3 Collection methods

The sample collection was done based on a quantitative data collection scheme using entomological tools, such as the HLC, the BGST and ITTC. The collected mosquitoes were either individually dissected or processed using qPCR or Reverse Transcriptase PCR. For the parasitological component, finger prick blood samples were collected on filter paper for subsequent testing, on a glass slide for the night blood thick smear and/or directly using a pipette for the ICT. The dried blood spot on the filter paper was used in the laboratory for the Og4C3 ELISA (Das et al. 2006) and Wb123 ELISA (Steel et al. 2012) and PCR (Williams et al. 1996).

Methodologies and guidelines from the WHO and GPELF's documents were extensively used, especially the "Monitoring and epidemiological assessment of mass drug administration in the global programme to eliminate lymphatic filariasis: a manual for national elimination programmes" (WHO 2011b), the manuals for National Elimination Programmes on "Transmission assessment surveys training" (WHO 2013b) and on "Practical entomology" (WHO 2013a).

The geographical information system (GIS) was developed to determine the coordinates of the houses and main breeding sites in the study villages through a Global Positioning System or GPS (GPS, Garmin 12 (Garmin, Olathe, KS, USA)). The maps were produced using ArcGIS Software.

The MDA impact assessment of this thesis was a 7-year long study that initially assessed the capacity of *Anopheles* to transmit *W. bancrofti* after five to six annual

MDA rounds. After transmission interruption was demonstrated, the Task Force for Global Health (TFGH) based in Atlanta, US, supported the surveillance component for three years followed by an additional four years of support from the Division of Intramural Research of the National Institutes of Health (NIH), Bethesda, MD. The second year of WHO funding was obtained from the Filarial Programmes Support Unit (FPSU) located at the Liverpool School of Tropical Medicine (LSTM), UK. FPSU, formerly known as the Centre for Neglected Tropical Diseases (CNTD) and the NIH supported the comparison surveys of *Anopheles* collection methods in Kolondieba district.

Data management and statistical analyses were performed using the following software: Poolscreen version 2.0 (C R Katholi et al. 1995) for mosquito pools' infection probability estimation, GraphPad Prism software version 5 (GraphPad Software, La Jolla, CA), Microsoft Excel® version 2010, SPSS version 14 (Statistical Package for Social Sciences) (SPSS Inc., Chicago, IL) for data entry and statistical analyses (comparison of rates, proportions and continuous numbers such as the number of mosquito collected) and ArcGIS 10.1 (ESRI, Redland, CA) for mapping.

A collective village-wide oral consent was obtained from the villages' elders. Signed individual written consent was also obtained from all study participants and/or guardians. Protocol and consent forms were approved by the Institutional Review Boards of the WHO/Tropical Diseases Research and LSTM and the Ethics

committees of the Faculty of Medicine of Bamako and National Institute for Research in Public Health, in Mali.

Some data were collected before the PhD starting (Chapters 3 and 4) but all data sets were reviewed, cleaned and final analyses conducted after the thesis initiation under the supervision of the thesis supervisors. More detailed specific descriptions of the materials and methods are embedded within individual chapters.

Chapter 3

Objective 1. Wuchereria bancrofti transmission pattern in southern Mali prior to and following the institution of MDA

3.1 Introduction

The LF vectors in Mali, as in other areas of sub-Saharan Africa, are members of the *An. gambiae* and *An. funestus* complexes (Coulibaly et al. 2006; Touré 1979). Whereas *An. gambiae* is the predominant species described in most studies, Touré (1979) noted a high prevalence of *An. funestus* especially at the end of the rainy season (from June-July to December) in a transmission study in a North Sudan savannah area. Though other *Anopheles* species are more common in rural areas, they are not present in high enough densities to be vectors of public health importance (Coulibaly et al. 2006; Touré 1979). Since *An.gambiae* complex is the main vector in Mali, its characteristics and behaviour regarding LF transmission need to be elucidated in order to better plan adapted elimination measures (Bockarie, Pedersen, et al. 2009; WHO 2013a).

This chapter aimed to assess the vector fauna abundance and the main species involved in LF transmission in the sentinel area composed of six neighbouring hyper endemic villages in the district of Sikasso before and after the first round of MDA.

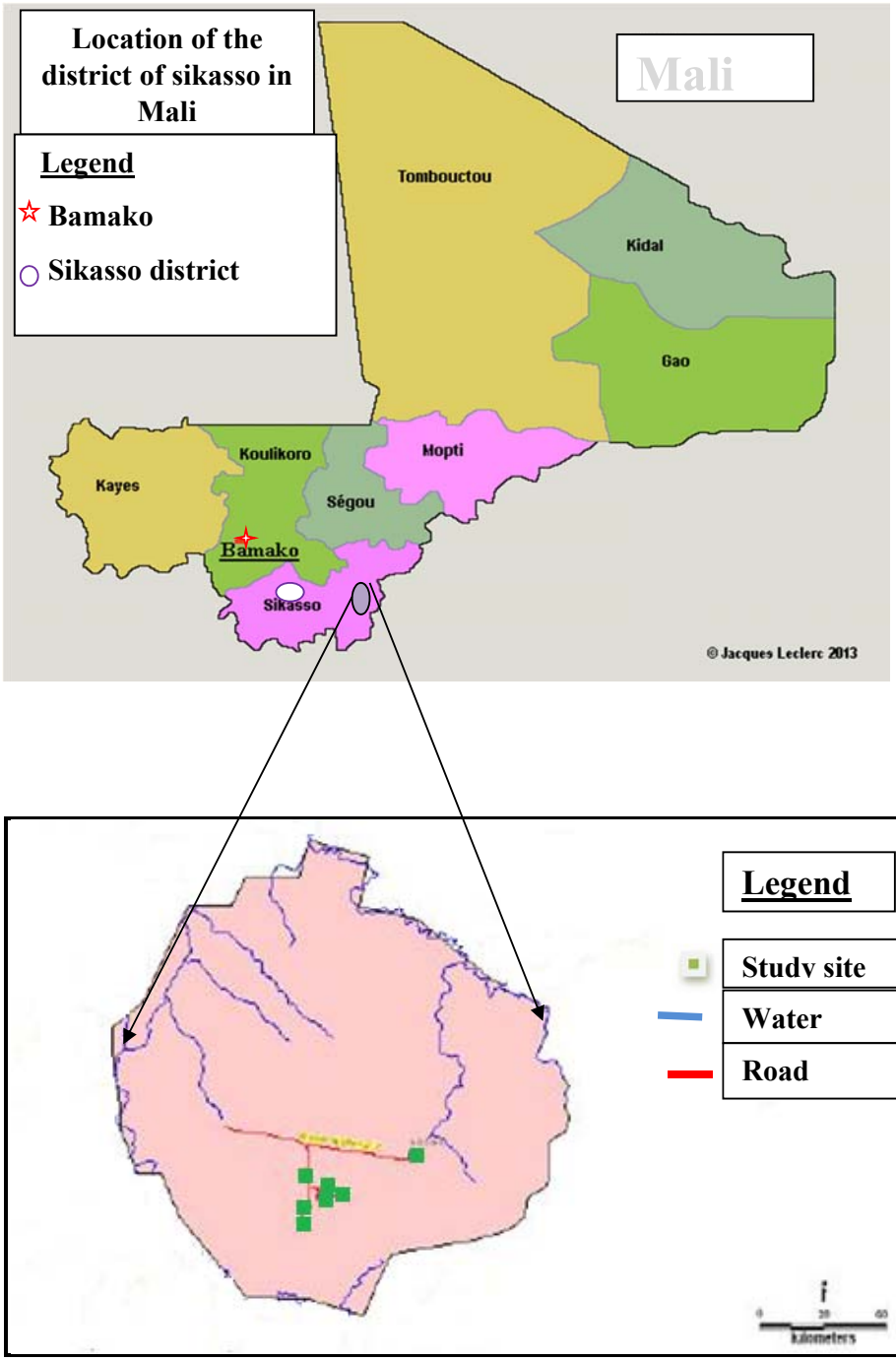
3.2 Methods

3.2.1 Study site identification and characterization

The study was initiated in the Sikasso district in southern Mali prior to the introduction of MDA for the elimination of LF. This area was historically known to be endemic for *W. bancrofti* (Bogoba 1981), and mapping surveys performed by the NPELF confirmed a high prevalence of CFA positivity (as assessed by ICT on 50-100 individuals/village) in the village of Dozanso and a neighbouring village in 2001 (unpublished data). Additional ICT based surveys were subsequently carried out in the

larger villages surrounding Dozanso in 2001 and led to the selection of six high prevalence villages (Dozanso, Gondaga Missasso, N'Torla, Niantansso and Zanadougou) for baseline entomological studies (Figure 3.1).

The study villages are comparable with regard to socio-cultural indicators, health care seeking behaviour and disease perception. The distance between the villages and the community health care centre of Kolokoba ranges from six to 15 km (mean 9.5 km), occupied by cotton fields, backwaters, and trees typical of the dense Sudan Savannah vegetation. The administrative region of Sikasso covers 76,480 km^2. Rainfall in this region ranges between 1200 and 1500 mm per year, with a rainy season from July to December. Due to the high levels of transmission observed during the first year of the study, yearly MDA was instituted in the six study villages in June 2002, one month prior to the second entomological survey. The region has a total population of 2.45 million, the highest population density in the country with 32 inhabitants per km^2 in 2008. Prior to this study, there had been no MDA implemented in this area.



Location of the study sites within the district of Sikasso

Figure 3.1 Spatial repartition of the study villages

3.2.2 Study population

A complete census, including the name, age, sex and profession of each inhabitant, was performed in all six villages. All dwellings were recorded and assigned an identification number. A GPS device was used to develop basic maps showing locations of the six villages within the Central District of Sikasso. The total population of the six study villages was 3,681 in 2001, consisting primarily of farmers, whose main occupations are agriculture (cotton, maize, millet and peanut) and domestic animal breeding. The ICT surveys carried out in 2001 revealed CFA prevalence rates varying from 81.8% in Niantanso (165/202) to 24.6% in Zanadougou (50/202) (Table 3.1). The prevalence of microfilaraemia was assessed in 2002 (prior to the initiation of MDA) by examination of three slides of 20 µl of night blood/subject and ranged from 40% in Dozanso (48/120) to 13.8% in N'Torla (27/196).

Table 3.1 Characteristics of the study population prior to the MDA

Villages	Total tested	Male	Female	Mf positive	CFA positive
		%	%	%	%
Dozanso	120	54.2	45.8	40	61.7
Missasso	207	35.3	64.7	20.3	36.9
Gondaga	212	45.8	54.2	15.1	43.4
Niantanso	202	42.1	57.9	29.7	81.8
N'Torla	196	50.5	49.5	13.8	40.3
Zanadougou	202	30.7	69.3	17.3	24.6

Mf =Microfilaraemia, CFA =Circulating filarial antigen.

A collective village-wide oral consent was obtained from the villages' elders, and all study participants signed individual written informed consent. The study protocol and consent forms were approved by both the Institutional Review Board (IRB) of the WHO/TDR and the ethics committee of the Faculty of Medicine, University of Bamako, Mali.

3.2.3 Study design

This was a longitudinal study during which monthly entomological surveys were performed in six study villages from July to December in 2001 (prior to initiation of MDA with ALB/IVER) and during the same period in 2002 (one month after the first MDA conducted in May 2002).

3.2.4 Laboratory analyses

Entomological surveys were performed for 12 days during each calendar month (2 days/village/month) by the same team. Mosquitoes were collected by two trained field personnel in one room in each of four different houses in each village using the HLC method. One collection team worked from 6:00 pm to midnight and the second from midnight to 6:00 am. Mosquitoes were captured using a Colluzi and Petrarca type mouth aspirator connected to a paper cup as the storage container (Coluzzi and Petrarca 1973). A supervisor retrieved the containers at two-hour intervals. The captured mosquitoes were kept overnight at ambient temperature in a paper cup under a damp cloth and dissected the following morning.

Mosquitoes were sorted morphologically for species identification (*An. gambiae* and *An. funestus* complexes). Some *An. gambiae* complex specimens were processed by PCR method to distinguish between the two members of the complex (*An. arabiensis* and *An. gambiae s.s*). The *An. gambiae s.s* were further processed by PCR to identify the molecular forms, M and S, as described by Favia et al (Favia et al. 1997; Favia and Louis 1999).

The selected specimen for the species identification using PCR were stored into a Carnoy's solution (3 parts pure ethanol:1 part glacial acetic acid) on field before a storage at -20 °C in the laboratory. They were later processed individually by PCR using the following steps:

- The mosquito is placed in a 1.5ml Eppendorf tube containing 25µl of "fly grinding buffer": (the stock of 500 ml of this product contains: NaCl: 2.92 g, Sucrose: 34.25g, Tris: 6.05 g, adjusted to pH 9.1 with HCl, EDTA: 50ml of 0.5M, 25 ml 10% SDS, Deionized water);

- Using a sterilized pestle, the mosquito is crushed and the pestle rinsed with another 25 microliters of "Fly Grinding Buffer";

- Place the tube in a water bath at 65 ºC for 30 minutes to inhibit DNase activity;

- Add 7µl of potassium acetate and incubate on ice or at 4ºC in refrigerator for 30 minutes. At this stage, the proteins are precipitated;

- Centrifuge at 14000 rpm for 15 minutes;

- Collect the supernatant in a new Eppendorf tube and pour over 100 µl of 100% ethanol for 5mn. Adding alcohol precipitates the DNA to the bottom of the tube;

- Centrifuge at 14,000 rpm for 15 min;

- Pour the 100% ethanol and add ethanol (but fresh 70% this time);

- Centrifuge at 14000 rpm for 5 min;

- Pour the ethanol, and let the DNA pellet dry at room temperature;

- Suspend the DNA in 100 µl of a solution of TE (Tris-EDTA) or deionized and sterile water. The DNA thus extracted can be directly amplified or kept for several months in the refrigerator;

- Amplification of DNA: An amplification reaction comprises a total volume of 25 µl of reagents such as Water, specific primers and DNTPs.

- Amplification parameters: denaturation 94°C for 30 seconds, pairing 50 ° C for 30s and extension 72 ° C for 30s. This cycle is repeated 30 times.

- specimens will then be identified according to the migration levels of the bands on an agorose gel: *An. gambiae s.s.* has 390 bp and *An. Arabiensis* 315 bp. The *An. gambiae* complex members are characterized by these specific DNA bands which make it possible to identify them. For the molecular forms of *An. gambiae s.s*, the 390 bp band is cleaved to give two bands, the molecular form (S) with 110 bp and the molecular form (M) with 280 bp. The gel is read on an UV light source and the number of bp is estimated according to a marker that size is known on the first and last well of each of the 2 lines of each gel.

For the remaining mosquitoes, the head, thorax and abdomen were dissected separately for each mosquito and recovered parasite larvae were categorized into L1, L2 or L3 stages. A fine needle was used for that dissection as previously described (Coulibaly et al., 2006; Toure, 1979).

Entomologic parameters assessed included infection rate, infectivity rate, Monthly biting rate, Monthly transmission potential and the Annual transmission potential that were calculated as previously described (Rwegoshora et al. 2005; Walsh et al. 1978; WHO 2013a):

- Infection rate: proportion of mosquitoes found infected after dissection with any *W. bancrofti* larval stage (L1–L3).

- Infectivity rate: proportion of mosquitoes found infected with one or more infective larvae (L3).

- MBR (Monthly biting rate): number of mosquitoes caught during the HLC x 30/ (total number of collectors used per collection x number of collections in the month).

- MTP (Monthly transmission potential): MBR x (number of L3 recovered divided by the number of mosquito dissected). It provides for a given vector species with an estimate of the number of infective bites for *W. bancrofti* a human would receive per month.

- The ATP (Annual transmission potential) is the annual total of the individual MTP.

For this study, the 6-month transmission period data are used as the annual data since from January to May, LF vectors densities are too low to detect any transmission (Coulibaly et al. 2006; Lehmann et al. 2010).

3.2.5 Data management and analysis

Data were analysed using SPSS version 14 (SPSS Inc., Chicago, IL) and GraphPad Prism software version 5 (GraphPad Software, La Jolla, CA). The Chi^2 test or the Fisher's exact test was used as appropriate to compare proportions as well as the 95% confidence intervals.

3.3 Results

3.3.1 Monthly variations in vector densities

The total number of mosquitoes collected in the six villages of Sikasso District, from July to December in 2001 and 2002, were 23,265 and 12,986, respectively. *Anopheles*

gambiae complex members were the most commonly collected mosquitoes each collection year. Overall, *An. gambiae s.l* (20,957 in 2001 and 11,190 in 2002) was more common than *An. funestus* complex (2,308 in 2001 and 1,796 in 2002) among the known vectors in the study area (Tables 3.2 and 3.3). At the beginning of the transmission season, *An. gambiae s.l* was more abundant than *An. funestus* complex (158 fold more in July 2001 and 138 fold more in August 2002). However, by the end of the transmission season in December 2001, the two species were equally abundant (Table 3.2) and only a two-fold increase in collection of *An. gambiae s.l* was observed in November and December 2002 (Table 3.3).

Table 3.2: Monthly variation of the entomological parameters for the transmission of lymphatic filariasis in six villages of the District of Sikasso in 2001

***Anopheles funestus* complex**								
Month	**Collected**	**Dissected**	**Infected**		**Infective**		**MBR**	**MTP**
			N (%)	**95% CI**	**N (%)**	**95% CI**		
July	25	25	0 (0)	[0-11.29]	0 (0)	[0-11.29]	4	0
Aug	33	33	0 (0)	[0-8.68]	0 (0)	[0-8.68]	5	0
Sep	278	148	4 (2.7)	[0.86-6.39]	2 (1.4)	[0.23-4.40]	43	0.6
Oct	1,402	789	51 (6.5)	[4.90-8.35]	15 (1.9)	[1.11-3.05]	219	4.2
Nov	514	432	17 (3.9)	[2.39-6.10]	13 (3)	[1.68-4.96]	80	2.4
Dec	56	44	0 (0)	[0-6.58]	0 (0)	[0-6.58]	9	0
Total	**2,308**	**1,471**	**72 (4.9)**	[3.88-6.09]	**30 (2)**	[1.41-2.86]	**60**	**1.2**
Anopheles gambiae s.l								
Month	**Collected**	**Dissected**	**Infected**		**Infective**		**MBR**	**MTP**
			N (%)	**95% CI**	**N (%)**	**95% CI**		
July	3960	3959	123 (3.1)	[2.60-3.70]	88 (2.2)	[1.80-2.72]	618.75	13.75
Aug	4971	4948	137 (2.8)	[2.34-3.25]	91 (1.8)	[1.49-2.24]	776.72	14.28
Sep	9096	4708	211 (4.5)	[3.92-5.10]	120 (2.5)	[2.13-3.03]	1421.25	35.53
Oct	2,320	2005	137 (6.8)	[5.79-8.00]	61 (3)	[2.36-3.86]	362.5	10.9
Nov	544	544	36 (6.6)	[4.75-8.95]	12 (2.2)	[1.20-3.72]	85	1.88
Dec	66	66	2 (3)	[0.51-9.65]	0 (0)	[0.00-4.44]	10.31	0
Total	**20,957**	**16,230**	**646 (4)**	[3.69-4.29]	**372 (2.3)**	[2.07-2.53]	**545.76**	**12.55**

N=Number, % =Percent, MBR =Monthly vector biting rate, MTP =Monthly transmission potential.

Table 3.3: Monthly variation in the entomological parameters related to the transmission of lymphatic filariasis in six villages of the District of Sikasso in 2002

			***Anopheles funestus* complex**					
Months	**Collected**	**Dissected**	**Infected**		**Infective**		**MBR**	**MTP**
			N (%)	**95% CI**	**N (%)**	**95% CI**		
July	14	14	2 (14.3)	[2.47-39.74]	0 (0)	[0-19.26]	2.2	0
Aug	18	18	2 (11.1)	[1.91-32.11]	0 (0)	[0-15.33]	2.8	0
Sep	342	342	22 (6.4)	[4.18-9.42]	4 (1.2)	[0.37-2.80]	53.4	0.64
Oct	786	786	38 (4.8)	[3.49-6.51]	16 (2)	[1.21-3.22]	122.8	2.46
Nov	600	600	26 (4.3)	[2.91-6.20]	20 (3.3)	[2.11-5.01]	93.8	3.1
Dec	36	36	2 (5.6)	[0.94-17.16]	0 (0)	[0-7.98]	5.6	0
Total	**1,796**	**1,796**	**92 (5.1)**	[4.17-6.22]	**40 (2.2)**	[1.62-2.99]	**46.8**	**1.03**
			Anopheles gambiae s.l					
Months	**Collected**	**Dissected**	**Infected**		**Infective**		**MBR**	**MTP**
			N (%)	**95% CI**	**N (%)**	**95% CI**		
July	1,646	1,646	18 (1.1)	[0.67-1.69]	2 (0.1)	[0.02-0.40]	257.2	0.26
Aug	2,488	2,488	37 (1.5)	[1.06-2.02]	5 (0.2)	[0.07-0.44]	388.8	0.78
Sep	2,846	2,846	244 (8.6)	[7.60-9.64]	40 (1.4)	[1.02-1.89]	444.7	6.23
Oct	3,214	3,214	160 (5)	[4.27-5.77]	70 (2.2)	[1.71-2.73]	502.2	11.05
Nov	924	924	34 (3.7)	[2.60-5.05]	22 (2.4)	[1.54-3.52]	144.4	3.46
Dec	72	72	12 (16.7)	[9.40-26.61]	2 (2.8)	[0.47-8.87]	11.3	0.31
Total	**11,190**	**11,190**	**505 (4.5)**	[4.14-4.91]	**141 (1.3)**	[1.07-1.48]	**291.4**	**3.79**

N =Number, % =Percent, MBR =Monthly vector biting rate, MTP =Monthly transmission potential.

Relative frequencies of *An. gambiae s.l* members and *An. gambiae s.s* molecular forms

Among the 15,869 *An. gambiae* complex members examined by PCR for specific species identification, 99.02% [98.85-99.16] (15,713/15,869) were *An. gambiae s.s* and 0.98% [0.84-1.15] (156/15,869) were *An. arabiensis*. The relative frequency of *An. gambiae s.s* varied from 95.5% in December to 99.7% in July without statistically significant monthly variation (Table 3.4). The same scenario was observed for the relative frequency of *An. arabiensis* (Table 3.4). The overwhelming majority (95.09% [94.75-95.42]; 14,942/15,713) of the *An. gambiae s.s* collected in 2001 was the S molecular form (Table 3.5). This molecular form showed comparable relative frequency between collection months excepted in December when significant lower relative frequency was observed 66.7% [54.38-77.45] (Table 3.5).This high relative frequency of the S molecular form was observed in all of the study villages (data not shown).

Table 3.4: Monthly variation in the relative frequencies of *An. gambiae s.l* members in 2001

Months	Total tested	*An. gambiae s.s*			*An. arabiensis*		
		N	(%)	95% CI	N	(%)	95% CI
July	3,907	3,895	99.7	[99.48-99.83]	12	0.3	[0.17-0.52]
August	4,883	4,859	99.5	[99.28-99.68]	24	0.5	[0.32-0.72]
September	4,543	4,480	98.6	[98.24-98.92]	63	1.4	[1.08-1.76]
October	1,926	1,895	98.4	[97.75-98.88]	31	1.6	[1.12-2.25]
November	544	521	95.8	[93.82-97.24]	23	4.2	[2.76-6.18]
December	66	63	95.5	[88.13-98.83]	3	4.5	[1.17-11.87]
Total	15,869	**15,713**	**99.02**	**[98.85-99.16]**	**156**	**0.98**	**[0.84-1.15]**

N =Number; *%* =Percent

Table 3.5: Monthly variation in the relative frequencies of the molecular forms of *An. gambiae s.s* in 2001

Months	Total tested	M Form			S Form		
		N	(%)	95% CI	N	(%)	95% CI
July	3,895	195	5	[4.35-5.73]	3,700	95	[94.27-95.65]
August	4,859	233	4.8	[4.22-5.42]	4,626	95.2	[94.58-95.78]
September	4,480	211	4.7	[4.12-5.36]	4,269	95.3	[94.64-95.88]
October	1,895	80	4.2	[3.38-5.2]	1,815	95.8	[94.8-96.62]
November	521	31	6	[4.15-8.24]	490	94.05	[91.76-95.85]
December	63	21	33.3	[22.55-45.62]	42	66.7	[54.38-77.45]
Total	15,713	**771**	**4.91**	**[4.58-5.25]**	**14,942**	**95.09**	[94.75-95.42]

N =Number; *%* =Percent; S Form = *An. gambiae* Form Bamako or Savannah; M Form = *An. gambiae* Form Mopti.

3.3.2 Vector infection rates and transmission intensity

Both *An. gambiae* and *An. funestus* complexes were found to be harbouring infective larvae during the two years of study (2001 and 2002). In July and August, infective *W. bancrofti* larvae were recovered only from *An. gambiae s.l* (Figure 3.2). *An. funestus* complex harboured infective larvae from September to November. No infective mosquito was recovered in December 2001 (Table 3.2). In both 2001 and 2002, the *An. funestus s.l* monthly infectivity rate remained comparable from September to November (Table 3.2 and 3.3).

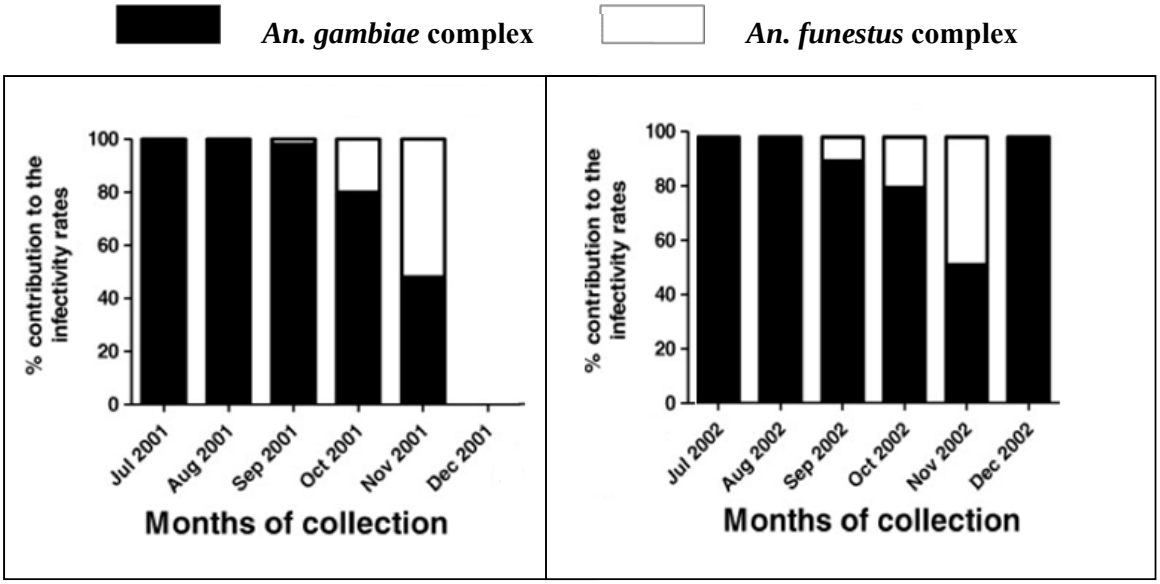


Figure 3.2 Species contribution to the overall monthly infectivity rates in 2001 and 2002.

The black represents the contribution of *An. gambiae s.l* and the white the contribution of *An. funestus* complex.

Overall, infection rates were comparable between *An. gambiae* and *An. funestus* complexes with 4% [3.69-4.29] versus 4.9% [3.88-6.09] in 2001 (Table 3.2) and 4.5% [4.14-4.91] versus 5.1% [4.17-6.22] in 2002 (Table 3.3). In 2001, LF infected *An. funestus* complex species were observed from September to November with monthly infection rates ranging from 2.7% [0.86-6.39] to 6.5% [4.9-8.35] while infected *An. gambiae* complex was observed during each month from July to December 2001 with rates ranging from 2.8% [2.34-3.25] to 6.8% [5.79-8] (Table 3.2). In 2002, both *An. funestus* and *An. gambiae* mosquitoes were found infected during July to December with monthly infection rates ranging from 4.3% [2.91-6.20] to 14.3% [2.47-39.74] and 1.1% [0.67-1.67] to 16.7% [9.4-26.61], respectively (Table 3.3). The infectivity rate for *An. gambiae s.l* significantly decreased from 2.2% [1.62-

2.99] in 2001 to (1.3% [1.07-1.48] in 2002 following MDA, but only a non statistically significant increase in infection rate was observed from 2001 (4.0% [3.69-4.29]) to (4.5% [4.14-4.91]) in 2002. For *An. funestus* complex, infection (4.9% [3.88-6.09] versus 5.1% [4.17-6.22]) and infectivity (2% [1.41-2.86] versus 2.2% [1.62-2.99]) rates did not significant change from 2001 to 2002 (Tables 3.2 and 3.3). In 2001, the overall infctivity rates of the two species complexes were comparable but in 2002, after the first MDA round, *An. funestus* complex was significantly higher than the one of *An. gambiae* complex.

In 2001, the overall MTP was more than 10 fold greater for *An. gambiae* complex than *An. funestus* complex. The *An. gambiae* complex was responsible for 0 to 35.53 infective bites per human per month. The *An. funestus* complex was responsible of 0 to 4.2 infective bites per human per month. In November 2001, the *An. funestus* complex was responsible for 2.4 infective bites while *An. gambiae* complex was responsible for 1.9. No infective bite was recorded in December 2001 (Table 3.2). In 2002, there was a dramatic decrease in the ATP for *An. gambiae* complex as compared to 2001 (from 12.55 to 3.79 infective bites per person during the transmission season). The same scenario was observed for *An. funestus* complex with the ATP in 2001 (84 infective bites/person) (Table 3.2) and the one recorded in 2002 (28.1 infective bites/person) (Table 3.3).

3.4 Discussion

The baseline entomological data collected in this longitudinal study confirmed exposure to infective mosquitoes in the six study villages in Sikasso District prior to

the initiation of MDA. As it had been reported previously in Mali, *An. gambiae* and *An. funestus* complexes are the predominant vectors of LF (Coulibaly et al. 2006; Toure 1979). In keeping with the high prevalence of human infection in these villages, the recorded vector densities were higher, especially for *An. gambiae* complex, than those reported in Banambani (Sudan savannah area) in Mali, where *W. bancrofti* is endemic but the prevalence of infection is lower (Coulibaly et al. 2006). Other *Anopheles* species (*An. pharaoensis*, *An. nili*, *An. rufipes*) were collected but not systematically processed during this study because of their very low relative densities, precluding no epidemiologically significant role in the transmission of LF, and the fact that they have not been recognized as vectors of *W. bancrofti* in Mali and other neighbouring West African countries (Coulibaly et al. 2006; Lenhart et al. 2007).

Among the *An. gambiae* complex members examined by PCR for specific species identification, the S form of *An. gambiae s.s* was predominant. A predominance of the S form of *An. gambiae s.s* among vectors of LF has also been observed in Ghana (Amuzu et al. 2010). Although the PCR identification of *An. gambiae* complex species and *An. gambiae s.s* molecular forms was not performed on all the collected mosquitoes for logistical reasons, at least 76% of the mosquitoes collected each month were dissected to ensure that the samples tested were temporally and geographically representative. Mosquitoes were sent to our laboratory in Bamako for PCR analyses without identity numbers that could link them to the dissection results precluding the determination of infection rates for the different molecular forms. This was mainly due to the fact that molecular forms identification using the carcasses was not planned at the beginning of this multi-country study.

Overall, the highest monthly vector densities for *An. gambiae s.l* were found in July and August (at least 99% of the vectors collected in the month), while those of *An. funestus* complex were observed in November and December (at least 33% of the vectors collected in the month). Similar variations in the densities of the two vectors were reported in Banambani that is located in a different eco-climatic area in Mali (Coulibaly et al. 2006) and are related to differences in environmental conditions during the transmission season and the breeding preferences of each species (*An. funestus* complex prefers shadowed and vegetated breeding sites while *An. gambiae* complex prefers sunny breeding sites with limited vegetation) (Coulibaly et al. 2006; Toure 1979). The frequencies of *An. gambiae s.s* and *An. arabiensis*, two members of *An. gambiae s.l*, also showed differing patterns during the transmission season.

Due to the relatively low infectivity rates and high number of mosquitoes, processing pools of *Anopheles* vectors for *W. bancrofti* infection is the most efficient strategy as compared to individual mosquito processing for following vector transmission rates during MDA (Chanteau et al. 1994; Kuhlow and Zielke 1978). L3 specific RT-PCR allows infective pools to be distinguished from infected pools and provides a more accurate determination of the transmission potential for *W. bancrofti* (Laney et al. 2010; Lenhart et al. 2007). In the present study, the infection and infectivity profiles of the two morphologically distinct *Anopheles* species complexes (*An. gambiae* and *An. funestus* complexes) showed some variability over time, suggesting that the two species complexes should be processed for PCR in separate pools if

detailed information regarding their relative contributions to monthly transmission is desired. Nonetheless, in the setting of post MDA assessment, where human-vector contact is the main factor of interest, *An. gambiae* and *An. funestus* complexes can be processed in the same pool (Laney et al. 2010; Lenhart et al. 2007).

In 2002 (post MDA), the number of mosquitoes caught was approximately half that in 2001 (before MDA). This effect was most dramatic for *An. gambiae s.l* where the number captured decreased by almost 50%. Potential reasons for this decrease in mosquito numbers include changes in climate, increased awareness of the study area population with respect to the role of mosquitoes in disease transmission (resulting in less breeding sites and increased use of insecticide treated nets), and the effect of ivermectin on mosquito survivorship. Although decreases in mosquito numbers following the initiation of MDA (Simonsen et al. 2010) and an effect of ivermectin on mosquito survivorship (Kobylinski et al. 2010; Sylla et al. 2010) have both been described, these factors were not directly addressed in this thesis.

Whereas the decreased vector numbers in 2002 (post-MDA) clearly contributed to the overall decrease in ATP observed for the *An. gambiae* complex, infectivity also declined significantly in 2002, suggesting that multiple factors may have played a role in the observed decrease in transmission including the decrease of the Mf prevalence and loads consecutive to the MDA (Simonsen et al. 2010). The fact that a similar decrease in ATP was not seen for *An. funestus* complex may have been due to the low overall numbers of *An. funestus* complex mosquitoes captured, although a higher degree of facilitation by *An. funestus* complex as compared to *An. gambiae* complex

cannot be excluded. Unfortunately, the study was not designed to address this issue, and published data comparing facilitation between the two species are limited (Southgate and Bryan 1992; De Souza et al. 2012).

Despite the fact that the overall mosquito infection rates were relatively stable during the six months of collection in each of the two transmission seasons, the ATP for *W. bancrofti* varied considerably over the course of the seasons as a result of the large differences in vector densities and MBR (Chanteau et al. 1994). This has important implications for the timing of MDA for LF in this region, since drug administration conducted at the beginning of the rainy season would be predicted to be most effective in decreasing transmission due to maximal reduction in Mf prevalence and loads at the precise time that vector density and biting rates are beginning to rise.

Ethical approval for this study was obtained from WHO and University of Bamako. At the time that the study was performed, HLC was considered an ethically acceptable method of mosquito collection. The collectors in this study were adult village residents normally exposed to mosquito bites. The collectors were not given antimalarial prophylaxis, but were provided access to a health practitioner (nurse) during the study in the event of malaria infection as recommended for adult subjects living in malaria endemic area. Since the goal of HLC is to collect the mosquito before it bites, the risk of infective bite is actually quite low. Although HLC is still used in some settings, research is actively ongoing in our Center and others to find a comparable method that does not involve human (Govella et al. 2011; Okumu et al. 2010).

3.5 Conclusion

In conclusion, the entomological data confirmed the district of Sikasso as an area of high *W. bancrofti* transmission. This led to the selection of this area as one site in a multi-country study on the effects of MDA on LF transmission by anopheline vectors and as the first region in Mali for implementation of MDA with ALB/IVER to eliminate transmission of LF. Comparison of the vector infectivity rate prior to and immediately following the first round of MDA showed a significant overall decrease after institution of MDA. Importantly, the dramatic variability in MTP over the transmission season suggests that the efficacy of MDA can be maximized by delivering drug at the beginning of the rainy season (just prior to the peak of transmission).

Chapter 4

Objective 2. The Impact of six annual rounds of MDA on *W. bancrofti* infections in humans and in mosquitoes in Mali

4.1 Introduction

The mass drug administration (MDA) is the main pillar of the Global Programme to Eliminate Lymphatic Filariasis (GPELF). In addition to MDA, LF transmission is affected by mosquito competence, which is related to the vector-parasite relationship. This vector parasite relationship is characterized by two epidemiologically important patterns: facilitation (anophelines) and limitation (culicines) (Bockarie, Pedersen, et al. 2009; WHO 2013a). Thus, the important decrease in the peripheral blood microfilarial load after MDA implementation is associated with an improved capacity of *Culex* vectors to continue LF transmission; while theoretically, *An. gambiae s.l* fail to do so after a certain number of MDA rounds. Taking this into account, the threshold for LF transmission in terms of vector density and required microfilarial load are different for the two groups of vectors.

The concept of limitation and facilitation arose from experimental studies (Bain 1971; Bain and Brengues 1972; Bain and Philippon 1970) and was later confirmed by empirical observations in some *Anopheles* transmission areas (Bockarie, Pedersen, et al. 2009; WHO 2013a). Mathematical analyses (Mougey and Bain 1976; Pichon 1974; Pichon et al. 1980; Pichon, Perrault, and Laigret 1974) subsequently showed that the limitation system has only one positive stable equilibrium. There will be a threshold, for interruption of transmission, which depends on vector density, but there is no threshold that depends on parasite density (Dye 1992) because a very low parasite load rather increases vector ability to transmit the disease if the limitation is the transmission system.

Within the *An. gambiae s.l*, there are data on the overall impact of the ALB/IVER regimen over a long period (Kyelem et al. 2003, 2005; Ramaiah and Ottesen 2014; Simonsen et al. 2010) but no close yearly data on the impact on both parasitological and entomological indicators over a long period of time. The objective of this chapter is to determine the vector fauna competence to transmit LF after one or more annual rounds of MDA.

4.2 Methods

4.2.1 Study sites

The study was undertaken in six neighbouring villages of the district of Sikasso previously described in the chapter 3 of the thesis.

4.2.2 Study Design

To assess *W. bancrofti* infection and transmission dynamics and the impact of six consecutive annual MDA rounds on the vectors' competence in these six villages of Sikasso district, a monthly cross sectional entomological survey was undertaken from July to December each year, as well as a parasitological assessment in July each year just prior to the MDA and the entomological survey, from 2002 to 2008 before this PhD. All six villages received MDA for six years. During the seventh year (2008), ALB/IVER was not distributed in two of the villages that did not have any evidence of ongoing transmission to provide preliminary data in anticipation of stopping MDA in the remaining villages the following year.

4.2.3 Study population

A complete census, including the name, age, sex and profession of every inhabitant, was performed in the study villages every year before the parasitological assessment. All subjects ≥ 2 years of age who presented for evaluation were included in the study.

4.2.4 MDA

As part of the NPELF activities, ALB/IVER was administered to all eligible subjects (not pregnant or breastfeeding within a week of delivery, taller than 90 cm and aged five years and above) in collaboration with the district and community health care staff using the health workers as drug distributors. MDA coverage rates were calculated based on the number of eligible subjects.

4.2.5 Entomological studies

Villagers were trained to collect mosquitoes from 6:00 pm to 6:00 am using the HLC method. A 12-day monthly entomological survey was carried out concomitantly by different teams in each of the six villages to determine village-wide *W. bancrofti* transmission potential. The parameters assessed included the MBR, ABR, infection rate, infectivity rate and the ATP during the study period. From July to December each year, mosquitoes were collected by two collectors per room in four different rooms in each village at night. The first collection team worked from 6:00 pm to midnight and the second from midnight to 6:00 am in each room. The collector caught the mosquitoes as they tried to land using a mouth aspirator connected to a paper cup as the storage container, as developed by Coluzzi and Petrarca (Coluzzi and Petrarca 1973). The mosquitoes that were collected during the night were kept in ideal

conditions (temperature, relative humidity using wet wipes) and dissected early the following morning.

4.2.6 Laboratory analysis

Mosquito samples were sorted by species (*An. gambiae s.l* and *An. funestus* complex) on the basis of morphology (Diagne et al. 1994). In 2002, a subset of the dissected *An. gambiae s.l* was tested using PCR techniques (Favia and Louis 1999) to determine the sibling species proportions.

During the dissection, the head and thorax were dissected separately for each mosquito, and recovered parasite larval stages were categorized into L1, L2 and L3. Female mosquito's dissection and observation of the ovaries tracheal coils (Beklemishev, Detinova, and Polovodova 1959) were done for parity status determination.

4.2.7 Parasitological and clinical assessment

Although the main focus of this study was on entomological surveys, parasitological and clinical data were also collected each year to complete the LF endemicity level assessment. Sixty microliters of night blood were obtained by finger prick from adult volunteers (15 years and above) for preparation of three thick smears. The slides were stained with Giemsa for identification and quantification of *W. bancrofti* microfilariae. The adult volunteers, as well as children ≤ 5 years old, were tested for *W. bancrofti* CFA using ICT during the first year (chapter 3) and after the sixth MDA. The clinical assessment consisted of a brief interview and physical examination focusing on

characteristic manifestations of LF, namely lymphedema, elephantiasis and hydrocele. Any clinical stage of lymphedema (from reversible pitting oedema to elephantiasis) or hydrocele (small, big, unilateral or bilateral) was considered as a case and recorded without additional information.

4.2.8 Data management and analysis

All data were recorded on standard data sheets and entered into the computer using SPSS version 14 (SPSS Inc., Chicago, IL) and GraphPad Prism software version 5 (GraphPad Software, La Jolla, CA). The Chi^2 test or Fisher exact test was used as appropriate for the comparison of proportions. The confidence level was set at 95% for all statistical tests.

A collective village-wide oral consent was obtained from the villages' elders as well as a signed individual written consent form from all study participants and/or guardians. Both the IRB of the WHO/TDR and the Ethics committee of the Faculty of Medicine of Bamako in Mali approved the protocol and the consent forms.

4.3 Results

4.3.1 Clinical, parasitological, and MDA

A total of 1,333 subjects from six villages aged two years and above have been included in the analysis. Females represented 57.8% of this population. Lymphedema and hydrocele had prevalences of 0.3 and 2.8 %, respectively (Table 4.1). Cross-sectional assessment of the human microfilaraemia prevalence rate showed a dramatic decrease in the prevalence of *W. bancrofti* microfilaraemia over the course of the study ($p< 10^{-4}$) from 21.4% (244/1139) in 2002 before the first MDA to 0.2% (2/856) in 2007 and 0.0% (0/760) in 2008 after the 6^{th} MDA round (Table 4.2). The geometric

mean microfilarial densities in microfilaria-positive individuals also decreased from 103 Mf/ml in 2002 (Figure 4.1) to 23 Mf/ml in 2006 and 0 Mf /ml in 2007 and 2008.

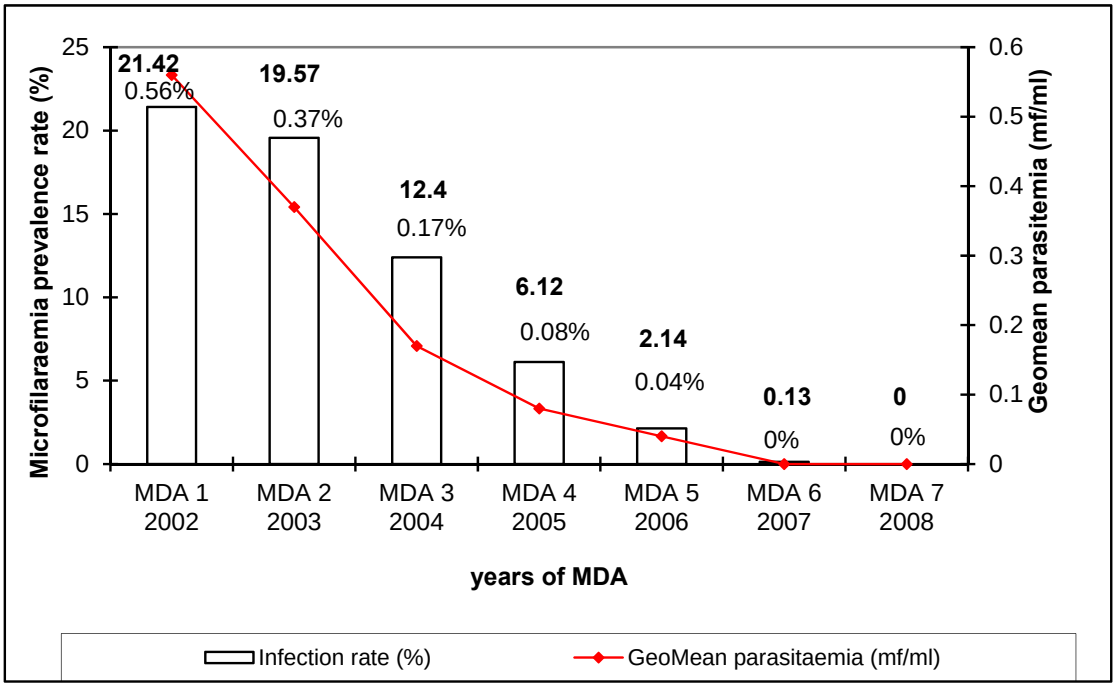


Figure 4.1 *Wuchereria bancrofti* microfilaraemia prevalence and geometric mean changes throughout the six consecutive MDA rounds in the study area]
W. bancrofti microfilaraemia prevalence (white bar and bolded numbers) and geometric mean curve changes throughout the six consecutive MDA rounds in the study area.

The coverage rate for the eligible population varied from 67% to 78% during the first five MDA. In the four villages treated in 2008, coverage remained high at 89.6% (3,201/3,574). Mild adverse events were reported by 0.6% (13/2,135) of the subjects in 2002 and the frequency decreased over time with only a few cases of mild headache reported in 2008 (data not shown). No severe adverse events were recorded during the study.

Table 4.1: Baseline characteristics of the study population

		Male	Female	Elephantiasis	Hydrocele	Mf +	CFA +
	Total	**%**	**%**	**%**	**%**	**%**	**%**
Age group							
<5 years	194	47.9	52.1	0	0	ND	53.1
≥5 years	1139	42.2	57.8	0.3	6.7	21.4	47.1
Villages							
Dozanso	120	54.2	45.8	1.7	7.7	40	61.7
Missasso	207	35.3	64.7	0	11	20.3	36.9
Gondaga	212	45.8	54.2	0	6.2	15.1	43.4
Niantanso	202	42.1	57.9	0.5	7.1	29.7	81.8
N'Torla	196	50.5	49.5	0	3	13.8	40.3
Zanadougou	202	30.7	69.3	0	6.5	17.3	24.6

Mf= microfilaraemia, CFA= circulating filarial antigen, ND=Not Done

Table 4.2: Variation in the *W. bancrofti* microfilaraemia carriage rate within the five years and above in 2002 and 2008

	Baseline (2002)				Post 6 MDA (2008)			
Localities	**N tested**	**Pos**	**%**	**95% CI**	**N tested**	**Pos**	**%**	**95% CI**
Dozanso	120	48	40	[31.52-48.96]	86	0	0	[0-5.33]
Missasso	207	42	20.2	[15.23-26.18]	138	0	0	[0-2.15]
Gondaga	212	32	15.1	[10.74-20.40]	167	0	0	[0-1.78]
Niantanso	202	60	24.8	[23.71-36.28]	129	0	0	[0-2.30]
N'Torla	196	27	13.7	[9.47-19.15]	141	0	0	[0-2.10]
Zanadougou	202	35	17.3	[12.57-23.01]	99	0	0	[0-2.98]
Total	**1,139**	**244**	**21.4**	**[19.11-23.88]**	**760**	**0**	**0**	**[0-0.39]**

Pos= positive, N= number

4.3.2 Entomological indices

An. gambiae s.l was the most common vector in the study area (90.1%). At baseline, 2.3% of *A. gambiae* complex vectors were infective. *An. funestus* complex had an infectivity rate of 2%. The ATP was 8 and 76 infective bites per human per year, respectively, for *An. funestus* and *An. gambiae s.l* (Table 4.3). Among the 15,869 *An. gambiae s.l* examined by PCR for specific species identification, 99.02% (15,713/15,869) were *An. gambiae s.s* and 0.98% (156/15,869) were *An. arabiensis*. The most common sibling species of *An. gambiae s.s* in all localities were the Bamako/Savannah molecular form (S form) also called *An. coluzzii*, which comprised 95.09% (14,942/15,713) of the mosquitoes examined, followed by the Mopti molecular form (M form), which accounted for 3.8 % (data not shown).

Table 4.3: Baseline vector characteristics in the study area prior to MDA

Species	No. collected	No. Dissected	No. Infected (%)	95% CI	No. Infective (%)	95% CI	ABR	ATP
An. gambiae s.l	**20,957**	**16,230**	646 (4.0)	[3.69-4.28]	372 (2.3)	[2.07-2.53]	545.8	76
An. funestus	**2,308**	**1,471**	72 (4.9)	[3.88-6.09]	30 (2)	[1.41-2.86]	60.1	8
Overall	**23,265**	**17,701**	**718 (4.1)**	**[3.77-4.35]**	**402 (2.3)**	**[2.06-2.50]**	**605.9**	**84**

No. = number; ABR= annual biting rate; ATP= annual transmission potential

The ABR decreased over time from 605.9 bites per human per year in 2001 (Table 4.3) to 203.96 bites per human per year in 2007 (Table 4.4). The vector infection rate (*An. gambiae s.l* and *An. funestus* complexes) also decreased dramatically (by more than 98.11%) from 4.1% [3.77-4.35] (718/17,701) in 2001 (Table 4.3) to 0.04% [0-0.14] (2/4,680) in 2007, twelve months after the 6th MDA (Table 4.4). Of the two infected *An. gambiae s.l* mosquitoes, one harboured a single infective L3 larva and the second one, a single non-infective L2 larva. Thus, *An. gambiae* complex members infectivity rate in 2007 was 0.02% [0-0.11] (1/4,624) (Table 4.5). The ATP for *An. funestus* was equal to 0 in 2006 and 2007 (Table 4.6). Due to the combination of a decrease in mosquito biting rates and lower numbers of infective mosquitoes, the ATP (number of infective bites per human per year) decreased by 99.6% from 84 in 2001 to 0.3 in 2006 and 2007, twleve months after the 6th MDA (Table 4.4).

Table 4.4: *An. gambiae* and *An. funestus* annual variation of the LF transmission level over the six MDA rounds

Years	Number collected	Number dissected	ABR	Infection rate (L1/L2 pos)		Infectivity rate (L3 pos)		ATP
				% (positive/N)	95% CI	% (positive/N)	95% CI	
Before	23,265	17,701	605.9	4.1 (718/17,701)	[3.77-4.35]	2.3 (402/17,701)	[2.06-2.50]	84
MDA 1	12,986	12,986	338.2	4.6 (597/12,986)	[4.25-4.97]	1.4 (181/12,986)	[1.20-1.61]	28.1
MDA 2	18,394	18,394	479	1.2 (222/18,394)	[1.06-1.37]	0.2 (44/18,394)	[0.18-0.32]	6.9
MDA 3	13,021	13,021	339	1.1 (143/13,021)	[0.93-1.29]	0.1 (16/13,021)	[0.07-0.20]	2.5
MDA 4	10,622	9,578	276.61	0.17 (16/9,578)	[0.10-0.27]	0.05 (5/9,578)	[0.02-0.12]	0.9
MDA 5	10,604	10,604	276.1	0.06 (6/10,604)	[0.02-0.12]	0.02 (2/10,604)	[0-0.06]	0.3
MDA 6	7,832	4,680	203.96	0.04 (2/4,680)	[0-0.14]	0.02 (1/4,680)	[0-0.11]	0.3

N= total number; MDA= mass drug administration; ABR=annual biting rate; ATP= annual transmission potential;

pos= positive

Table 4.5: *Anopheles gambiae s.l* annual variation of the LF transmission level over the six MDA rounds

Years	Number of mosquito collected	Number of mosquito dissected	ABR	Infection rate		Infectivity rate (L3 pos)		ATP
				% (positive/N)	95% CI	% (positive/N)	95% CI	
Before	20,957	16,230	545.8	4 (646/16,230)	[3.69-4.29]	2.3 (372/16,230)	[2.07-2.53]	75
MDA 1	11,190	11,190	291.4	4.5 (505/11,190)	[4.14-4.91]	1.3 (141/11,190)	[1.07-1.48]	22
MDA 2	17,825	17,825	464.2	1.2 (213/17,825)	[1.04-1.36]	0.2 (42/17,825)	[0.17-0.32]	6.63
MDA 3	11,818	11,818	307.8	1.1 (128/11,818)	[0.91-1.28]	0.1 (15/11,818)	[0.07-0.20]	2.32
MDA 4	10,072	9,080	262.29	0.15 (14/9,080)	[0.09-0.25]	0.04 (4/9,080)	[0.01-0.11]	0.72
MDA 5	10,514	10,514	273.8	0.06 (6/10,514)	[0.02-0.12]	0.02 (2/10,514)	[0-0.06]	0.31
MDA 6	7,755	4,624	201.95	0.04 (2/4,624)	[0-0.14]	0.02 (1/4,624)	[0-0.11]	0.28

N= total number; MDA= mass drug administration; ABR=annual biting rate; ATP= annual transmission potential;

pos= positive

Table 4.6: *Anopheles funestus* complex annual variation of the LF transmission level over the six MDA rounds

Years	Number collected	Number dissected	ABR	Infection rate		Infectivity rate (L3 pos)		ATP
				% (positive/N)	95% CI	% (positive/N)	95% CI	
Before	2,308	1,471	60.1	4.9 (72/1,471)	[3.88-6.09]	2 (30/1,471)	[1.41-2.86]	7
MDA 1	1,796	1,796	46.8	5.1 (92/1,796)	[4.17-6.22]	2.2 (40/1,796)	[1.62-2.99]	6.2
MDA 2	569	569	14	1.6 (9/569)	[0.77-2.88]	0.4 (2/569)	[0.06-1.16]	0.3
MDA 3	1,203	1,203	31.3	1.2 (15/1,203)	[0.73-2.0]	0.1 (1/1,203)	[0.004-0.41]	0.18
MDA 4	550	498	14.32	0.4 (2/498)	[0.07-1.32]	0.2 (1/498)	[0.01-0.99]	0.16
MDA 5	90	90	2.3	0 (0/90)	[0-3.27]	0 (0/90)	[0-3.27]	0
MDA 6	77	56	2.01	0 (0/56)	[0-5.21]	0 (0/56)	[0-5.21]	0

N= total number; MDA= mass drug administration; ABR=annual biting rate; ATP= annual transmission potential;

pos= positive

4.4 Discussion

Consistent with the data from other studies (CDC 2011; Richards et al. 2011), six rounds of MDA with ALB/IVER were extremely effective in reducing the prevalence of *W. bancrofti* microfilaraemia in residents of a highly endemic area of Mali. Although testing for microfilaraemia was limited to 53.02% (604 /1139) of the total population eligible for MDA in the six villages, it is unlikely that the infection rate in the remaining population was substantially higher than that in the tested subjects. Thus, the observed impact of MDA on *W. bancrofti* microfilaraemia in the present study is compatible with the long-term objective of the GPELF to interrupt transmission using MDA alone.

As previously reported in the baseline study in this area (chapter 3), the dominant vector, *An. gambiae s.l,* continued to account for more than 90% of the mosquito vectors collected in this area over the seven years of the study, followed by *An.funestus* (data not shown). The overall trend in any given year was characterized by a high frequency of *An. gambiae s.l* early in the rainy season followed by a gradual decrease in *An. gambiae s.l* and a gradual increase in the abundance of *An. funestus* towards the end of the rainy season (Coulibaly et al. 2013). These changes are related to the climatic conditions over the year and not a result of the current interventions such as the MDA (Toure et al. 1996).

In addition to seasonal variation, the vector density showed significant yearly variation from 2001 to 2008. The dramatic decrease in ABR following the first MDA in the study area has previously been reported and was most likely due to increased

awareness of the study area population with respect to the role of mosquitoes in disease transmission (resulting in less breeding sites and increased use of insecticide treated nets). LLIN were provided for free only to mothers just after delivery at the community health centre from 2002 to 2004. Beginning in 2005, LLIN availability in the six villages increased because of the free distribution campaigns for vector control related to malaria prevention. Although yearly variations in ABR are not unusual in Mali and have been observed in the neighbouring sites of Pimperena (unpublished data), this would not be expected to have a significant effect on the ATP without a concomitant change in the vector infectivity rate.

Despite high levels of transmission prior to the institution of MDA, the vector infection and infectivity rates decreased to a very low, but detectable, level in 2007. Only two captured mosquitoes were infected with *W. bancrofti*, of which only one was infective, representing a more than 99% reduction in the infectivity rate. No differences were apparent between the two villages that continued to have infected mosquitoes and the four other villages with respect to overall compliance with the program or distance to non-MDA villages.

The mean ATP was also reduced by more than 99% after six MDA rounds. Although persistence of transmission despite low levels of microfilaraemia in the human population has been reported with *Culex* species that exhibit limitation (decreasing yield of infective larvae per Mf as the number of ingested Mf increases), *An. gambiae* and *An. funestus* complexes demonstrate facilitation (increasing yield of infective larvae per Mf as the number of ingested Mf increases) (Pichon 2002; Southgate and

Bryan 1992). Consequently, the dramatic reduction in transmission intensity (only one infective larvae recovered and an infectivity rate of the *Anopheles* vectors of 0.02% (the cut-off of 0.03% has been proposed) is likely sufficient to interrupt transmission in this rural area of Sikasso district (De Souza et al. 2012) as sexual reproduction is required in the human host to produce microfilariae.

Nevertheless, caution should be exercised in stopping MDA, as there might be variation in the efficiency of the different sibling species within the *An. gambiae* group of mosquitoes; thus continued close surveillance for resurgence of transmission will be essential. In this regard, a staggered approach to stopping, as undertaken in this study, may be most prudent.

4.5 Conclusion

In summary, the data to date suggest that six rounds of MDA with ALB/IVER may be sufficient to interrupt transmission in a highly endemic region of Mali where *Anopheles* is the main vector. Annual evaluation of the human and vector populations for evidence of *W. bancrofti* infection continues in the study villages following cessation of MDA and will be essential to validate this conclusion.

Chapter 5

Objective 3. Dynamics of antigenemia and transmission intensity of *Wuchereria bancrofti* following cessation of MDA in a formerly highly endemic region of Mali

5.1 Introduction

MDA is aimed at interrupting LF transmission through clearing of peripheral blood microfilariae that prevent human-to-human vector-borne transmission (WHO 2015). As Bancroftian filariasis was found to be endemic in all eight administrative districts of Mali, ranging from 1% in Timbuktu (northern part of Mali) to >18% in Sikasso (southern part of the country) (Dembélé, Bamani, Dembélé, M. O. N. O. Traoré, et al. 2012), annual MDA using ivermectin and albendazole was initiated sequentially starting from the most highly endemic district in the country (Dembélé, Bamani, Dembélé, M. O. N. O. Traoré, et al. 2012). These sentinel area data would be used to guide the NPELF for the potential outcomes of the post-MDA surveillance in previously hyper endemic areas.

The current chapter reports data collected to assess transmission after MDA was stopped in 2007 (after seven rounds of MDA). Although this study was initiated prior to the formal WHO recommendations for TAS, which require demonstration of an infection rate of <1% in >400 children aged 6-7 years using the ICT to document interruption of transmission (WHO 2011b), a similar approach was taken using ICT testing of children aged 6-7 years. ICT testing of a cohort of children ≥8 years old and adults and entomological assessment of LF transmission were performed. Finally, the use of several additional methods (Og4C3 ELISA, PCR targeting *W. bancrofti* DNA and *W. bancrofti* infective larval stage specific antigen Wb123-based IgG4 immunoassays) to assess transmission interruption in this previously highly LF-endemic region (Sikasso) of Mali was explored. Our data support an integrated approach to surveillance.

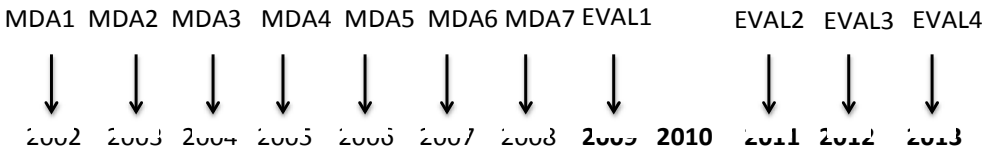
5.2 Methods

5.2.1 Study sites

The study was conducted in the same area as the chapters 3, 4 and 5 of the thesis (Coulibaly et al. 2013;Coulibaly et al. 2015; Coulibaly et al. 2016).

5.2.2 Study design

As post-MDA surveillance, a yearly cross-sectional parasitological assessment of all children 6-7 years of age and all eligible older volunteers aged eight years and above was performed in July from 2009 to 2012. In addition, a monthly entomological assessment of LF transmission (from July to December) was conducted in the six study villages in 2009, 2011 and 2013. In 2013, only children aged 6-7 years were tested with ICT, along with a thick smear from night blood. Infective stage *W. bancrofti* larvae (L3) were assessed in mosquitoes using an L3-specific RT-PCR technique as previously described (Laney et al. 2010). The study design is illustrated in Figure 5.1.



ICT	+	+	+	+
CFA ELISA	+		+	
MF	+	+	+	+
Wb blood PCR	+			
WB123 Antibody			+	
HLC Vector Collection/Dissection	+	+	+	+
PSC/RT-PCR			+	

Figure 5.1 Timeline of treatment, EVAL, and monitoring
The years in which MDA and EVAL surveys were performed are shown by the arrows and time at which monitoring tools in support of EVAL are shown by the + sign

5.2.3 Parasitological and serological assessments

Infection status was assessed using the ICT for the detection of circulating *W. bancrofti* antigen (Allere, Portland, ME). Dried blood spots were collected for additional laboratory analyses. Microfilaraemia was assessed by finger prick at night (between 10 p.m. and 2 a.m.) among ICT positive volunteers using a calibrated thick smear. Yearly parasitological studies were conducted in July, at the beginning of the transmission period, except in 2009 when, for logistical reasons, this assessment was performed in October. Because of the concern of potential transmission, other diagnostic tests were performed among the 6-7 years in 2012, namely the Og4C3 (TropbioTownsville, Australia) and ELISA testing for antibodies to Wb123 on eluted blood spots as previously described (Steel et al. 2013).

5.2.4 Entomological assessment

Each month a 12-day entomological survey was conducted in the six villages to assess the village wide *W. bancrofti* transmission pattern during the LF transmission period in Mali from July to December (Coulibaly et al. 2013). Two HLC sessions were organized per month and per village. Two collectors worked inside each of four collection rooms per session. Because *An. gambiae* is endophilic, collections were performed indoors to maximize yield. A total of 72 collection rounds were undertaken with the HLC. The collection was done from 6 pm to 6 am, and, for ethical reasons, the collectors were replaced at midnight at each collection site. All *An. gambiae* and *An. funestus* complexes collected were freshly dissected for parity status based on techniques previously described (Detinova and Gillies 1964) and for infection (any larval stage) and infectivity (L3 stage) status by individual mosquito dissection as previously described (Goodman et al. 2003).

In 2012, Pyrethrum Spray Catches (PSC) were carried out to collect mosquito vectors in addition to the HLC using Premium®, a pyrethroid based insecticide, in 30 randomly selected rooms per village in each of the six collection months. The random selection consisted to select randomly 30 numbers from the list of numbers corresponding to the total number of rooms in each study village using Microsoft Excel. During each of the 36 PSC collection rounds, the number of persons sleeping in each visited room was recorded on the mosquito collection sheet. The collected mosquitoes during the PSC were pooled (1 to 20 mosquitoes) in the field and stored in tubes containing RNAlater® and sent to Smith College, Northampton, Massachusetts, USA) for *W. bancrofti* RNA detection by RT-PCR as previously described (Laney et al. 2010).

For PSC, the MBR was determined by dividing the number of fed and half-gravid female *Anopheles* collected in a room by the number of sleepers in the room the night before the collection multiplied by 30 (WHO 2013a). The ABR was the sum of all the MBR calculated over the year (WHO 2013a). From HLC collected *Anopheles*, the parameters were determined as previously reported (Walsh et al. 1978; WHO 2013a).

5.2.5 Sampling

The present study predated the official WHO guidelines for TAS (WHO 2013b). Because the evaluation unit was small (< 300 children aged 6-7 years), all of the available eligible children were screened.

5.2.6 Data analysis

The collected data were entered using Microsoft Access 2007 and analysed using GraphPad Prism software version 5 (GraphPad Software, La Jolla, CA) and SPSS version 20 (SPSS Inc., Chicago, IL). To compare the infection prevalences between villages or mosquito species, we used the Pearson Chi^2 or the Fisher exact test if necessary. The trend Chi^2 was used to test the statistical significance of any frequency or proportion's trend over time.

5.3 Results

5.3.1 Study demographics

A total of 289 children aged 6-7 years were assessed in 2009, 301 in 2011, 285 in 2012 and 309 in 2013. Concomitantly, available older children and adults were assessed in 2009 (n=800), 2011 (n=795), and 2012 (n=1,812) (Tables 5.1 and 5.2). In 2013, testing of older children and adults was restricted to those who were positive by ICT in 2012

(n=50). Although the sizes of the six study villages differed, the study populations within the villages were quite well balanced in terms of gender within both the 6-7 year old children and the ≥8 year old throughout the study period (Table 5.2).

Table 5.1: Sampling activities by year

	Study human sample			Mosquito collection		
Year	**Total population**	**6–7 years old**	**≥ 8 years old**	**Number collected**	**Technique used**	**Number collection rounds**
2009	4,431	289	800	4,448	HLC	72
2011[a]	4,761	301	795	2,962	HLC	72
2012	5,044	285	1,812	7,168/1,907	HLC/PSC	72/36[b]
2013[c]	5,225	309	50	nd	nd	nd

[a]In 2011, a random sample of 92 subjects from the 6 villages was tested with Wb123 ELISA

[b]In 2012, the 6 villages were visited once a month from July to December (collection in 30 rooms per visit per village)

[c]In 2013 the 50 subjects ≥ 8 years tested were the ones found positive using ICT in 2012

nd= not done; HLC=Human landing catch; PSC= pyrethrum spray catch

Table 5.2: Characteristics of the study population per village throughout the surveillance period in the six study villages of the Sikasso district

Village	6–7 years		8 years and above			Overall
	M/F	Total	M/F	Median age (Range)	Total	
Survey 1 in 2009						
Dozanso	20/29	49	60/73	34 (12–79)	133	182
Missasso	26/20	46	64/94	40 (15–76)	158	204
Gondaga	22/21	43	55/64	33 (12–75)	119	162
Niatanso	30/24	54	91/106	31 (12–69)	197	251
N'Torla	23/16	39	50/49	37 (12–72)	99	138
Zanadougou	28/30	58	31/63	37.5 (13–77)	94	152
Total	149/140	289	351/449	35 (12–79)	800	**1,089**
Survey 2 in 2011						
Dozanso	21/17	38	42/71	32 (15–82)	113	151
Missasso	22/31	53	51/99	35 (15–86)	150	203
Gondaga	21/17	38	58/73	29 (15–84)	131	169
Niatanso	25/29	54	73/60	31 (15–82)	133	187
N'Torla	35/26	61	53/80	31 (15–88)	133	194
Zanadougou	26/31	57	49/86	31 (15–89)	135	192
Total	150/151	301	326/469	38 (15–89)	795	**1,096**
Survey 3 in 2012						
Dozanso	20/16	36	95/137	32 (15–82)	232	268
Missasso	21/27	48	101/171	33 (15–79)	272	320
Gondaga	27/21	48	100/177	28 (15–85)	277	325
Niatanso	26/25	51	134/182	28 (15–83)	316	367
N'Torla	22/15	37	127/208	30 (15–89)	335	372
Zanadougou	34/31	65	137/243	30 (15–80)	380	445
Total	150/135	285	694/1,118	30 (15–89)	1,812	2,097
Survey 4 in 2013						
Dozanso	24/24	48	8/15	41 (8–75)	23	73
Missasso	26/21	47	1/4	38 (31–68)	5	52
Gondaga	30/25	55	0/5	28 (8–58)	5	60
Niatanso	32/23	55	1/3	25.5 (8–63)	4	60
N'Torla	18/24	42	1/6	46 (24–66)	7	49
Zanadougou	31/31	62	4/2	29 (8–58)	6	68
Total	161/148	309	15/35	38 (8–75)	50	359

M/F= male/female

5.3.2 CFA and Wb123 antibody prevalence assessment over the surveillance period

The CFA prevalence in 6-7 year old children did not increase significantly over the surveillance period. It went from 0% [0- 1.64] (0/289) in 2009 to 2.7 % [1.24-5.37] (8/301) in 2011 and 4.5% [2.6-7.66] (14/309) in 2013 (Table 5.3). There was also a non statistically significant decrease in CFA positivity over the study period in the ≥8 year olds, from 4.9% [3.53-6.67] (39/800) in 2009 to 3.5% [2.4-5.12] (28/795) in 2011, and 2.8% [2.08-3.65] (50/1,812) in 2012. Whereas none of the ICT-positive 6-7 year olds had detectable microfilaraemia, one of 39 (2.6%) [0.06-13.48] individuals in the older group was microfilaraemic in 2009, and 3/28 (10.7%) [2.81-29.37] were microfilaraemic in 2011. In 2012, none of the 50 ICT-positive older subjects was microfilaraemic 0% [0-8.89] (0/50) (Table 5.3). Forty-four of the previously ICT-positive older subjects, as well as six of the 6-7 year olds who were ICT-positive and eight years old at the time of the 2013 survey, were reassessed in 2013. None of the 28 subjects who remained ICT-positive in 2013 had detectable microfilaraemia (data not shown). In 2012, within the 6-7 year olds, positivity rates for both the Og4C3 ELISA for CFA (1.8% [0.65-4.27] (5/285)) and testing for antibodies to the *W. bancrofti*-specific antigen, Wb 123 (1.8% [0.65-4.27] (5/285)) were similar and comparable to the results obtained using the ICT test (3.9% [2.04-7] (11/285) even though the later showed a higher number of positive.

5.3.3 Entomological assessment

The number of mosquitoes collected using the HLC over the study period is detailed in Table 5.4. The highest ABR using the HLC was 374 bites per person in 2012 and the lowest was in 2011 with 155 bites per person. The parity rates for *Anopheles*

gambiae complex were comparable between the first two yearly entomological surveys with 83.9% [82.78-84.98] (3,675/4,380) in 2009, 84.34% [82.92- 85.65] (2,406/2,853) in 2011 but, in 2012, the observed parity rate 88.9% [88.05-89.70] (5,032/5,718) was significantly higher than those of the two previous years. In 2009, two (0.05%) filaria-infected *Anopheles* females were observed without any infective larval stage recovered. In 2011 and 2012, no *W. bancrofti* larvae were found in the dissected mosquitoes (Table 5.4).

Table 5.3: CFA and microfilaraemia prevalence rates in six to seven year old children and those eight years and above from 2009 to 2013

		Survey 1 (2009)	Survey 2 (2011)	Survey 3 (2012)[a]	Survey 4 (2013)
Sample size and target	Targeted sample size	1,107	1,107	2,530	372
	Total population	4,431	4,761	5,044	5,225
	Number tested (*n*)	1,089	1,096	2,097	359
ICT	≥ 8 years % Positive (*n*/*N*)	4.9% (39/800)	3.5% (28/795)	2.8% (50/1,812)	
	[95% CI]	[3.53–6.67]	[2.40–5.12]	[2.08–3.65]	–
	6–7 years % Positive (*n*/*N*)	0% (0/289)	2.7% (8/301)	3.9% (11/285)	4.5% (14/309)
	[95% CI]	[0–1.64]	[1.24–5.37]	[2.04–7.00]	[2.60–7.66]
Mf	≥ 8 years % Positive (*n*/*N*)[b]	2.6% (1/39)	10.7% (3/28)	0% (0/50)	
	[95% CI]	[0.06–13.48]	[2.81–29.37]	[0–8.89]	–
	6–7 years % Positive (*n*/*N*)[b]	0	0% (0/8)	0% (0/11)	0% (0/14)
	[95% CI]		[0–40.23]	[0–32.15]	[0–26.76]
PCR	≥ 8 years % Positive (*n*/*N*)	5.13% (2/39)	np	np	np
	[95% CI]	[0.89–18.63]			
	6–7 years % Positive (*n*/*N*)	0	np	np	np
	[95% CI]				
Wb123	≥ 8 years %Positive (*n*/*N*)	np	np	4.7% (2/43)	nd
	[95% CI]			[0.81–17.06]	
	6–7 years % Positive (*n*/*N*)	np	np	1.8% (5/285)	nd
	[95% CI]			[0.65–4.27]	
Og4C3	≥ 8 years ICT % Positive (*n*/*N*)	np	np	4% (2/50)	np
	[95% CI]			[0.70–14.86]	
	6–7 years % Positive (*n*/*N*)	np	np	1.8% (5/285)	np
	[95 % CI]			[0.65–4.27]	

[a]In 2012, the ELISA test was done on all the children and the 50 ICT positive adults

[b]Only the ICT positive subjects were tested for Mf

ELISA= Enzyme-Linked Immuno-Sorbent Assay; ICT= Immunochromatographic Card Test; ICT+= ICT positive; Mf= microfilaraemia; n= number positive; N= number examined; nd= not done; np= not planned; PCR= polymerase chain reaction; Wb123= filarial antibody test

Table 5.4 Annual variation of mosquito densities and biting rates over the surveillance period from 2009 to 2012

Collection method	Year	Species	No. of collected	No. and proportion of dissected [95% CI]	ABR	Parity Frequency [95% CI]	No. infected Infection % [95% CI]
HLC	**2009**	**GA**	4,443	4,375	232	3,671	2
				98.47 [98.05–98.8]		83.9 [82.78–84.98]	0.05 [0.01–0.18]
		FU	5	5	0	4	0
				100 [46.29–100]		80 [29.88–98.94]	
		PH	0	0	0	0	0
		RU	0	0	0	0	0
	2011	**GA**	2,911	2,803	152	2,364	0
				96.29 [95.52–96.93]		84.34 [82.92–85.65]	
		FU	3	3	0	3	0
				100 [31.00–100]		100 [31–100]	
		PH	39	38	2	30	0
				97.44 [84.92–99.87]		78.95 [62.22–89.86]	
		RU	9	9	1	9	0
				100 [62.88–100]		100 [62.88–100]	
	2012	**GA**	7,138	5,691	368	5,006	0
				79.82 [78.86–80.74]		88.9 [88.05–89.70]	
		FU	3	3	0	3	0
				100 [31–100]		100 [31–100]	
		PH	23	23	1	22	0
				100 [77.08–100]		94.1 [69.23–99.69]	
		RU	1	1	0	1	0
				100 [5.46–100]		100 [5.46–100]	
PSC	**2009**		nd	nd	nd	nd	nd
	2011		nd	nd	nd	nd	nd
	2012	***An.* spp.**	1,907	115[a]	12[b]	nd	0

[a]Number of pools of 20 mosquitoes tested with the RT-PCR; **No.= number**

[b]The number of half gravid and blood fed mosquitoes divided by the number of sleepers in the rooms visited the night before the collection
An. spp.= *Anopheles* species; HBR= human biting rate; HLC= Human landing catch; FU= *Anopheles funestus*; GA= *Anopheles gambiae*; PH= *Anopheles pharaoensis*; PSC= Pyrethrum spray catch; RU= *Anopheles rufipes*; nd= not done

With the PSC method during the six months of collection in 2012, 1,907 mosquitoes were collected and the ABR was 100 bites per person per year. The number of mosquitoes collected with the PSC technique was 3.75 times less than that collected with the HLC in 2012. Moreover, both the infection and infectivity of the PSC-collected mosquitoes were 0 (Table 5.4). Of note, *An. gambiae s.l* was the most frequent vector comprising more than 99% of the active vector fauna each year as compared to *An. funestus* complex (data not shown). This is due to the decline of *An. funestus* population over time related at least partially to the environmental changes in the study area.

We observed the highest vector density (12 mosquitoes per collector per night) in 2012 with 7,165 mosquitoes collected by 576 collectors over the study period. This density was 2.4 times higher than that in 2011 (2,962 mosquitoes) and 1.6 times more than that in 2009 (4,448 mosquitoes). Of the 2,962 and 7,165 mosquitoes collected respectively in 2011 and 2012, the frequencies of *An. pharaoensis* varied from 1.31% in 2011 to 0.32% in 2012 while the frequencies of *An. rufipes* varied from 0.30% in 2011 to 0.01% in 2012. These species were very rare during the previous collection years in this area and were never found to be infected with *W. bancrofti* (data not shown).

5.4 Discussion

The current study investigated the LF transmission patterns following cessation of MDA during the surveillance period from 2009 to 2013 in six neighbouring previously highly LF endemic villages in the Sikasso region in Mali. In 2008, after seven rounds of MDA, the *W. bancrofti* microfilaraemia and ICT positivity in children (6 – 7 years)

was reduced to 0%. By 2011 and 2012, the prevalence of ICT-positivity in 6-7 year old children showed an increase that was not statistically significant, although microfilaraemia was not detected. Despite this increase in CFA prevalence in 6-7 year old children, there was a statistically significant decrease in CFA prevalence rates over the same 5-year period among those ≥8 years of age. This decrease is consistent with attrition over time of established worms. These data are most consistent with interruption of LF transmission based on the absence of detectable microfilaraemia, the lack of infective *Anopheles*, and the decreased CFA prevalence in the older age group. Nonetheless, close monitoring in areas of previously high transmission is necessary to detect early resurgence of transmission and to generate data that may guide and improve the LF elimination process.

When prevalence was estimated using different tools (Og4C3 ELISA and Wb123 immunoassays) at a single time point (2012), ICT consistently gave a higher prevalence rate compared to the two other tests, although the differences in prevalence were not statistically significant. Higher prevalences using ICT compared to Og4C3 ELISA were also observed in Togo during a school-based TAS conducted three years after stopping MDA (Dorkenoo et al. 2015), although the reasons for this are unclear. *L. loa* microfilaraemia has been shown to be associated with ICT-positivity at both the community and individual levels (Bakajika et al. 2014; Wanji et al. 2015); however, the same studies showed no association between ICT-positivity and the prevalence of *M. perstans*, the only other filarial parasite endemic in the study area (Keiser et al. 2003).

Re-emergence of infection after just a few years of surveillance has been reported in Nigeria in some but not in all districts (Richards et al. 2011). In India after 10 years following MDA implementation, new infection among children was also reported (Ramaiah and Vanamail 2013). Using 6-7 year old children as the sentinel population makes sense in the Malian context because this group remains in the villages, whereas many adults travel from place to place because of seasonal migration for agriculture and may acquire infection in areas that have not yet started MDA (Kia et al. 2014).

The approach to post-MDA surveillance is still being perfected. Antibody testing (e.g. Wb123) has been proposed as a potential better tool than antigen testing for the early identification of on-going transmission, as antibody positivity typically occurs months prior to positivity in adult antigen-based circulating antigen testing (Harnett et al. 1998; Kubofcik et al. 2012; Weil and Ramzy 2007). As there was good concordance between Wb123 prevalence and that of the CFA testing in the children (Table 5.3) and with both tests now being POC (Golden et al. 2013; Steel et al. 2013) , it is possible that the Wb123 rapid diagnostic test may be considered as a major surveillance tool in the near future.

Although screening of vector populations for the presence of infective larvae has been one of the two pillars of assessing transmission interruption in onchocerciasis (Lamberton et al. 2014; Lovato et al. 2014), its widespread use in LF has not taken hold to date. However, using both standard (dissection) and molecular techniques on both HLC and PSC collected mosquitoes (n=9,072) only a few positives were found (and only just after the cessation of MDA). This is probably due to the drastic reduction

of microfilaraemia prevalence after the seven consecutive MDA treatments and to the relatively low number of mosquitoes collected and the low sensitivity of the dissection (Laney et al. 2010). Since RT-PCR, a more sensitive method to detect infective stage L3 larvae in the vector, is available (Laney et al. 2010), screening of larger numbers of mosquitoes and pool screen-based molecular techniques will need to be assessed. The entomological data in the study area are in a very good concordance with the final conclusion from the parasitological data that are currently the WHO recommended transmission assessment criteria (mainly based on 6-7 year old children infection status).

The observation that *An. Pharaoensis* and *An. Rufipes* were more frequently biting humans and their identification as secondary vectors of *W. bancrofti* in West Africa (Brengues et al. 1974), raises the possibility that transmission can be sustained by a number of vectors other than the most prevalent (*An. Gambiae* complex). The rain pattern in 2012 (number of precipitations and average rainfall) likely played a role in the increased vector density, as well as in the increase in *An. pharaoensis* and *An. rufipes* frequencies (Ngom et al. 2014; Talla et al. 2014). However, what is needed is an adequately designed prospective study of *W. bancrofti* transmission dynamics and vector control in this region of Mali. In addition, HLC was much more effective at collecting *Anopheles* than PSC; because of potential ethical issues related to HLC (Govella et al. 2010), better collection methods are needed.

With very low human infection and vector infectivity rates, there is no evidence that *W. bancrofti* transmission has re-emerged in the study villages in the present study (WHO 2011b, 2013a). Nevertheless, new entomological studies are needed to

understand transmission dynamics in the context of post MDA surveillance. Mosquito vectors transmit *W. bancrofti* in two primary patterns, limitation and facilitation. Limitation is typically exhibited by *Culex* species and allows more efficient L3 development when microfilaraemia loads are low. Conversely, facilitation (usually exhibited by *Anopheles* species) leads to decreased numbers of developing L3 when microfilaraemia loads are low. Because limitation of *An. gambiae sensu stricto* has been observed in Ghana (Amuzu et al. 2010), it should also be assessed in other geographic locations (e.g. Mali) given the possibility of adaptation or specific mutation that can modify mosquito's transmission pattern (Southgate and Bryan 1992). From our previous studies, in the same area, WHO criteria were met but the mosquitoes were still infective (infectivity rate of 0.02 %) when the MDA was stopped (Coulibaly et al. 2015). Taking into account the entomological data and determining a threshold could be beneficial to be able to safely stop MDA in highly LF endemic areas.

5.5 Conclusion

Despite a dramatic and stable decrease in the prevalence of infection in the older age groups and in mosquitoes five years following the cessation of MDA in six villages previously highly endemic for LF, a non statistically significant increase in the prevalence of LF antigenaemia occurred among 6-7 year old children. The observed non significant prevalence increase within this group was in concordance with the entomological data that showed an absence of LF transmission and with two other LF testing methods. The strength and weakness of tests used in post-MDA assessments should be known and taken in account before a final testing strategy is selected. By applying multiple different tests to the assessment of transmission interruption

following cessation of MDA in LF-endemic areas, an integrated assessment strategy can be suggested that combines serologic and vector-based techniques.

Chapter 6

Objective 4. Comparison of the human landing catch method for sampling mosquitoes against a human baited tent trap and Biogents sentinel trap in a Sudan savannah area of Mali

6.1 Introduction

The human-biting rate (ma) is obtained by multiplying the female mosquito density per person (m) times the number of blood meals a single mosquito take on humans (a). The human-biting rate, also expressed as HBR, is directly and accurately provided by human bait collections as the number of bites per human per period of time (night, month or year). When using non-human-bait mosquito collection methods, the parameters (a) and (m) have to be calculated independently. The parameter (a) depends on host preference, host availability and oviposition cycle duration. Usually, it is calculated by dividing the human blood index by the duration of the gonotrophic cycle in days. Blood-meal analyses are not needed when human-baited collection methods are used to estimate HBR. Human bait collections typically yield higher HBR than pyrethrum spray catch; although it remains unclear which method yields the best estimate of mosquito-human contact.

In terms of vector abundance and mosquito-human contact estimation, caution is needed due to the inherent biases that need to be taken in account. For example, children do not receive as many mosquito bites as the adult baits used for the collection. This can lead to an overestimation of the overall human-biting rate (Bryan and Smalley 1978; Carnevale and Molinier 1978; Port et al. 1980). Additionally, not taking in account the time people usually spend outdoors before going to bed can lead to bias in the estimation of the indoor human-biting rate. Even if no definitive relationship has been established between the number of sleepers and the room's attractiveness to vectors, unfed mosquitoes are more attracted to rooms with more sleepers (Haddow, Gibbins, and Gibbins 1942).

Transmission potential can be determined by multiplying the number of infective larvae (L3) recovered by the average human biting rate of the same vector for a specified period of time and dividing this by the number of dissected mosquitoes (Rwegoshora et al. 2005). This method combines important vector and parasite characteristics in a unique measure of the likelihood of monthly or yearly LF transmission. A small decrease in the biting rate can translate into a huge decrease in the transmission potential because the transmission potential is determined by multiplying the mosquito biting rate by the infectivity rate. At equal infectivity rate, lower biting rate can then considerably decrease the transmission potential. Local environmental conditions also affect transmission: rainfall, temperature, humidity and soil type can all affect the production of breeding sites and the survival of adult mosquitoes.

Thus, the scenario is complex given the high number of genera and species involved in LF transmission, each vector–parasites combination having different characteristics sometimes in the same ecological area. In West Africa, for example, it has been observed that *An.gambiae s.l* transmits LF up to a certain period from which *An.funestus* take over. When the density of the first begins to decrease, that of the second starts to increase. This maintains a certain level of transmission throughout the transmission season (Coulibaly et al. 2013; Toure 1979).

GPELF interventions are expected to lead to a decrease to less than 1% of the human microfilariae carriage rate or <2% for *W. bancrofti* antigen prevalence (within the population > five years old) at the follow-up survey that takes place about six months after the fifth MDA round. If this condition is satisfied, a final MDA can be done

followed by the TAS that will check the infection rate in children between 6-7 years old or those from the first and second grade if the primary school crude enrolment rate is higher or equal to 75% (WHO 2013b) .

One of the main challenges for GPELF has been the monitoring of transmission intensity during and after MDA. Since stopping transmission relies primarily on MDA and vector control in areas where MDA alone failed or is taking too long to achieve the elimination (WHO 2013b), vector control and the use of xenomonitoring as a monitoring tool are an important component of post MDA surveillance in the LF elimination process. Thus, a safe and effective way of collecting mosquitoes at the community level that is representative of the vector fauna is required (Pedersen et al. 2009).

Studies comparing human-baited tent traps to HLC found good correlation between the yields of these two collection methods when studying malaria vectors (Govella et al. 2009, 2011). LF is unique because it is transmitted by four genera of mosquitoes including *Anopheles, Culex, Aedes and Mansonia*. *Anopheles* mosquitoes are the principal vectors in rural areas in Africa, but *Culex* species play an important role in LF transmission in urban communities in East Africa. *Mansonia* has also been incriminated as a vector of LF in Ghana (Ughasi et al. 2012). Despite this, the value of the different traps in sampling the vectors of LF has not been studied previously. The BGST have been included in this study because of their efficiency in sampling *culicidae*.

The BGST is essentially a collapsible, white fabric container with an opening that is partially covered by white gauze. With a diameter of 36 cm and a height of 40 cm, the trap has a fan that sucks air into the trap through a black catch pipe. The airflow draws approaching mosquitoes into a catch bag (Figure 6.1).

To date, HLC is the most frequently used method for *Anopheles* collections in many endemic areas of West Africa, due in large part to the fact that it mimics the natural situation of mosquitoes trying to bite humans. HLC, however, raises ethical concerns including the possibility that infected mosquitoes can bite the collectors (Govella et al. 2010; Service 2009; Sikaala et al. 2013). Additionally, HLC is labour intensive and the mosquito yield is dependent on the collector's attractiveness to mosquitoes, ability and experience (Sikaala et al. 2014). Thus, despite the fact that most of the existing mosquito data were generated using this method, its use is controversial and many ethics committees are reluctant to approve its use for sampling mosquitoes (Figure 6.2).

To overcome these issues, alternative trapping methods have been explored with regard to ease of use, operator independence, cost of implementation and safety for the operator. Human-baited tent traps, like ITTC, represent alternative collection methods that, like HLC, allow fresh specimen collection for live dissections and adequate storage for PCR or RT-PCR processing. ITTC has been reported to have yields more similar to those of the HLC as compared to several other methods (Govella et al. 2011, 2010; Krajacich et al. 2015) (Figure 6.3).

Ideally, examination of vector abundance, distribution, species composition and infection rate should be assessed prior to initiation and at the end of MDA. Several LF endemic countries have stopped or are about to stop MDA in many implementation units (districts). Given the increasing evidence for the importance of the association between vector competence and outcome of interventions against LF, effective vector sampling is becoming increasingly important (Gambhir et al. 2010). Most of the data on ITTC are from Tanzania (Chaki et al. 2012; Govella et al. 2011, 2010; Krajacich et al. 2015), Zambia (Sikaala et al. 2013) and Kenya (Mukabana et al. 2012) that are populated by mosquito species other than members of the *An. gambiae* complex, which is the predominant one in many West African countries. The BGST, a CO2 or human odor baited trap, has been shown as a quite good alternative sampling tool for *An. darlingi* and *Culex* in Suriname (Maciel-de-Freitas et al. 2006) but turned out to be weak in collecting *An. aquasalis* in the same country (Schmaedick et al. 2008). So, it may be good at collecting *An. gambiae* and *An. funestus* complexes, the main vector of LF in Mali.

None of the prior studies compared the collected *Anopheles* infection rate for *W. bancrofti* and *P. falciparum.* To confirm the good reported correlation between ITTC yields and that of HLC in West African settings, this chapter assesses alternative LF vectors collection methods such as the BGST and a human baited tent trap, ITTC, and how they compare to the HLC in two villages in Mali that have different mosquito densities in the context of MDA impact on LF endemicity and transmission levels after the fifth MDA round.

A

A: Mosquito sucking fan of a Biogents sentinel trap (BioGents, Regensburg, Germany, http://www.biogents.com/) with BG Lure (BioGents, GmbH, Regensburg, Germany, http://www.biogents.com/)

B

B: Mosquito collection bag in a Biogents sentinel trap

C

C: An operating Biogents sentinel trap

Figure 6.1 Biogents sentinel trap

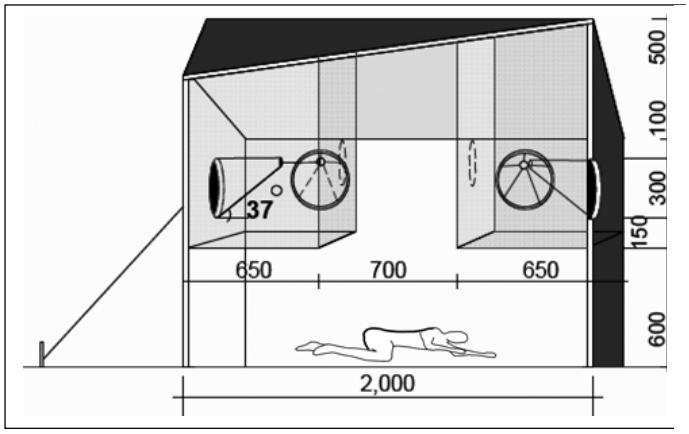
A: A colluzi and Petrarca type mouth aspirator for HLC with its different parts including in the middle a cardboard paper soup cup whose basis is replaced by a net in order not to block the air flow produced by the collector sucking.

B: A mouth aspirator ready to operate

C: A collector acting as bait to collect mosquito trying to bit his legs using a mouth aspirator and a lighting lamp

Figure 6.2 Mouth aspirator for human landing catch

A



A: Picture from Govella et al, 2010 showing the Ifakara tent trap type C design with dimensions in millimeters. Mosquitoes get into the trap through 6 funnel shaped entrances (2 on each side of the rectangle length and one on each width of the rectangle). Each entrance is maintained by a wire bar that has soft caps outside of the plastic rings, forming an inner small aperture of the funnel end (Govella et al. 2010).

B

B: The different parts of a tent trap type C

C

C: An operating tent trap type C with protected human bait

Figure 6.3 Ifakara tent trap type C

6.2 Methods

6.2.1 Study site identification and characteristics

Kolondieba district has an estimated population of 216,260 inhabitants distributed over 205 villages. The study was conducted in the villages of Bougoula (1,906 inhabitants) (longitude 11.045155758 and latitude -6.982963281) and Boundioba (3,201 inhabitants) (longitude 11.04218429 and latitude -6.984337661) that are located ~ 15 km apart, 276 km at the south of Bamako in the district of Kolondieba, region of Sikasso. This area has the highest annual rainfall in the country, ranging from 1,200 to 1,500 mm, with a rainy season that extends from July to December. Subsistence agriculture is the main occupation followed by panning for gold and wood harvesting from the forests. The district had already received five consecutive annual MDA rounds with ~80% annual epidemiological coverage rate when the present study was initiated in 2011. The endemicity levels of the study villages before MDA were unknown, although the sentinel site representing both villages, another neighbouring village, was highly endemic before the initiation of MDA with a *W. bancrofti* anigen prevalence rate of 60% in 2000) (Dembele M, personal communication, June 2014). The two study villages share several important characteristics (climate, vegetation and housing style, ethnic group composition, and socio-cultural and health care seeking behaviours), despite the existence of a permanent backwater in Boundioba (but not Bougoula) that is an important potential larval habitat for mosquitoes.

6.2.2 Study Design

A longitudinal study with monthly mosquito collections was conducted from July to December in 2011 and 2012 in the two study villages to compare the ITTC and BGST

to the commonly used HLC in this previously LF endemic area. Mosquito collections were conducted three times a month in each study village in 2011 and six times a month in 2012 (in order to increase the number of collected mosquitoes). This frequency was driven by logistical constrains such as the number of ITTC available and the long delay to have them delivered in Mali from Tanzania.

6.2.3 Vector collection methods

Local teams were trained to set up the traps and collect the mosquitoes. The three collection tools were:

1) The all-night HLC method - Mosquitoes attempting to feed were captured by adults seated on benches, with their feet and legs bared to the knee, using mechanical mouth aspirators and aided by light from torches. One collector operated indoors and the other outdoors at each collection point. These two collectors operated from 6 p.m to midnight before being replaced by two others who operated from midnight to 6 a.m

2) The newly developed BGST- A simple suction trap constructed to use upward-directed air currents as well as visual cues to attract mosquitoes. The trap was used with a dispenser system (BG-Lure) that releases artificial human skin odours and needs no CO_2 (Krockel et al. 2006).

3) The ITTC - It does not require electricity or moving parts and has been found to be able to collect well correlated numbers of *Anopheles* with the HLC yields in rural and urban settings in Tanzania (Govella et al. 2011, 2010). An attractant, a villager, slept under each ITTC and was responsible for collecting the trapped mosquitoes using a mechanical mouth aspirator every two hours.

6.2.4 **Logistics**

Vectors were collected during the last two weeks of each collection month (from July to December). To control for random effects, the three trapping methods were implemented simultaneously at each of the three collection Zones, first in one village for three consecutive days and then in the other village for another three days in 2011 and every other day in each village in 2012. All of the collections occurred between 6:00 pm and 6:00 am at each of the three collection sites in the two villages selected according to the village environmental characteristics and separated from each other by ≥200 m. Overall, the three areas we named 'Zones' were at the Northern side (Zone A), at the middle (Zone B) and at the Southern side of the village (Zone C). One of them (Zone A) was close to the main breeding site in the village, the second (Zone B) was close to the original settlement area corresponding to the middle of the village, and the third (Zone C) was located close to the recently occupied area of the village. In each Zone, the locations for the three sampling methods were separated by ~ 100 m due to the relatively small size of the villages inhabited areas. Collectors worked in two shifts (from 18:00 to 24:00 and from 24:00 to 6:00) for the HLC and ITTC. Only *An. gambiae s.l* members were further processed because the other species are of little epidemiological importance (do not transmit disease or were present in very low numbers). Collected mosquitoes were stored in labelled screw top tubes containing a solution of 70% ethanol in 2011 or RNALater® solution in 2012 after sorting according to morphologically identified species, collection site and method. Whereas the specimens from 2012 were freshly stored and frozen the next day, those from 2011 were freshly dissected for parity rate and *W. bancrofti* infection status in the field before preservation of the carcass in alcohol and storage at room temperature thereafter.

6.2.5 Processing of specimens

Infection status and species identity were determined for the 2011 specimens stored in alcohol using the hemalum staining technique. In 2012, the mosquitoes were sorted and directly stored in RNALater® solution for subsequent screening using PCR in the laboratory (Laney et al. 2010). Given the fact that no infective mosquito was recovered in 2011, the 2012 collected mosquitoes tested using PCR provided with an opportunity to test the same mosquitoes pools for the co endemic malaria parasite plasmodium faliciparum. This allowed to not only ascertain good DNA extraction but also allowed to compare the tree collection methods yields as related to the infection rate for one or both co endemic parasites.

6.2.6 Parity rates and survival estimation

Mosquitoes to be dissected were kept fresh (about 100 per day per collection method) or preserved in 70% ethanol for future staining for *W. bancrofti* larval stages identification using Mayer's acid haemalum technique before being individually dissected under a dissecting microscope (Laurence and Pester 1961). Female *Anopheles* were individually placed on a slide into a drop of saline and dissected using a dissecting needle to remove the ovaries from the abdomen. A stereomicroscope (X40) was used to observe the tracheole structure. Parity was determined by checking tracheole structure according to the method described by Detinova and Gillies (Detinova and Gillies 1964). Daily survival rates were calculated by Davidson's method based on the parity at the power of one divided by the duration of the gonotrophic cycle in days (Davidson 1954) and were equal to the cube root of the parity rate of the gonotrophic cycle (Davidson 1954). We used the gonotrophic cycle duration of three days observed

in our insectary at the Faculty of Medicine of Bamako for *Anopheles* females collected in the study villages and reared for other experimental purposes (unpublished data).

The freshly dissected mosquitoes in 2011 had the ovaries removed and examined for parity status according to Detinova's method (Detinova and Gillies 1964). For *W. bancrofti* larval stages recovery, the head, thorax and abdomen were examined separately in three drops of saline water using a stereomicroscope at X 200. The larval stages were identified according to the criteria of Nelson (Nelson 1959). The mosquitoes collected in 2012 were stored in pools of one to 20 females on an RNAlater® solution (Laney et al. 2010) before a processing with a PCR technique for parasite DNA identification as previously described by (Rao et al. 2014).

6.2.7 Fresh specimen and dissection techniques

Hemalum staining is a standardized mosquito staining procedure that involves a series of 30 min immersions of the mosquitoes in 70%, 55% and 25% alcohol solutions (Nelson 1958). Tubes containing approximately 20 mosquitoes are then stained in hemalum (Mayer's) stain (VWR, West Chester, PA) following a modification of Nelson (1958) for seven days before immersion in distilled water for three days (Nelson 1958). The stained mosquitoes were then stored in glycerol before dissection to identify larvae of *W. bancrofti*. The dissection was done using a dissecting microscope by macerating the head, thorax and abdomen of the individual mosquito on a slide and covering it with a coverslip for observation under a stereomicroscope (Service 1993).

6.2.8 Ethics statement

A collective village-wide oral consent was obtained from village elders, and all mosquito collectors signed an individual written consent. The study protocol and consent forms were approved by both the IRB of the LSTM (reference#10.88RS) and that of the Malian National Institute of Research in Public Health, Bamako, Mali (reference #9/11/CE-INRSP).

6.2.9 Data management and analysis

In the field, mosquito identification and dissection results were noted on specific data recording sheets. The recorded data were later entered into Microsoft Access dataset and analysed using SPSS version 14 (SPSS Inc., Chicago, IL) and GraphPad Prism software version 5 (GraphPad Software, La Jolla, CA). The collection methods were compared in terms of correlation mosquito yields using non-parametric Spearman correlation test and the number of mosquito collected per night per trap over the study period, while the parity rate and overall proportions of *An. gambiae s.l* were compared using their 95% confidence intervals. A simple Spearman linear regression was done using the yields of each individual collection round for each year and over the two-collection years to assess the relationship between the HLC yields and those of each of the two other mosquito collection methods.

A generalized linear mixed model, also called the random effects model (Boussari et al. 2012; Breslow and Clayton 1993), was used to assess the relative collection rates of the different collection methods as compared to the HLC. Village and trap type were included as fixed effects in the model and collection date was included as a random effect. A negative binomial model was fitted as there was evidence of over dispersion in the data. The confidence level was set at 95% for all statistical tests. For the vector

infection level assessment, the PoolScreen software version 2 was used to determine the maximum infection prevalence likelihood (MIPL) and its 95% confidence interval (Katholi and Unnasch 2006).

6.3 Results

6.3.1 Mosquito species composition and abundance by village, collection traps and time

In term of mean number of mosquito per person per night, in 2011, based on individual collection rounds yields, *Culex spp* had a significantly higher density in Boundioba (13 with 95% CI [5.24-20.85]) as compared to *Anopheles gambiae* complex (2 with 95% CI [0.82-2.99]); In Bougoula, a different scenario was observed with comaparable mean densities for the two species with 8 [5.05-10.6] versus 11 [5.89-16.73], respectively for *Culex spp* and *Anopheles gambiae* complex (Figure 6.4).

The percentage of *Anopheles gambiae* complex members from the total collected mosquitoes varied significantly by capture method. In 2011, *An. gambiae* complex mosquitoes represented 58.3% [55.92-60.55] of the total collected by HLC followed by the 40% [37.75-42.35] by ITTC and only 1.7% [1.18-2.41] by BGST. The same trend was observed in 2012 with 54.3% [51.71-56.89], 45.1% [42.48-47.66] and 0.6% [0.31-1.16] *Anopheles* captured by the HLC, the ITTC and the BGST, respectively. Overall, in the 2 villages, the BGST collected more *Culex spp* each year than the two other methods while, HLC collected more *Anopheles gambiae* complex than ITTC each year for the two villages combined) (Table 6.1). The vector density expressed in mean number of mosquito per person per night was higher for *Anopheles gambiae* complex members than Culex in Bougoula in 2011 and 2012 (Figure 6.4). The three collection methods showed comparable mean monthly numbers of mosquito per collection round given their overlapping 95% confidence intervals (Figure 6.5).

Table 6.1: Collected mosquitoes distribution per collection method in the two villages of the Kolondieba district in 2011 and 2012

Species collected		Total collected (%)	HLC %	[95% CI]	BGST %	[95% CI]	ITTC %	[95% CI]
Culex 2011	Bougoula	1,033 (37.50)	27 (2.6)	[1.76- 3.72]	917 (88.8)	[86.73- 90.59]	89 (8.6)	[7.02- 10.44]
	Boundioba	1,722 (62.50)	22 (1.3)	[0.82- 1.90]	1,664 (96.6)	[95.70- 97.41]	36 (2.1)	[1.49- 2.85]
	The 2 villages	2,755 (100)	49 (1.8)	[1.33- 2.32]	2,581 (93.7)	[92.73- 94.55]	125 (4.5)	[3.81- 5.36]
An. gambiae 2011	Bougoula	1,494 (85.57)	844 (56.6)	[53.97- 58.99]	18 (1.2)	[0.74- 1.86]	631 (42.2)	[39.75- 44.75]
	Boundioba	252 (14.43)	172 (68.3)	[62.31- 73.78]	12 (4.8)	[2.61- 7.95]	68 (27)	[21.78- 32.72]
	The 2 villages	1,746 (100)	1,017 (58.3)	[55.92- 60.55]	30 (1.7)	[1.18- 2.41]	699 (40)	[37.75- 42.35]
Culex 2012	Bougoula	2,464 (52.57)	463 (18.8)	[17.28- 20.37]	1,761 (71.5)	[69.66- 73.23]	240 (9.7)	[8.62- 10.96]
	Boundioba	2,223 (47.43)	114 (5.1)	[4.27- 6.11]	2,055 (92.5)	[91.29- 93.49]	54 (2.4)	[1.85-3.13]

	The 2 villages	4,687 (100)	577 (12.3)	[11.39- 13.27]	3,816 (81.4)	[80.28- 82.51]	294 (6.3)	[5.61- 6.99]
An. gambiae 2012	Bougoula	6,368 (81.81)	3,474 (54.6)	[53.33- 55.77]	35 (0.5)	[0.39- 0.76]	2,859 (44.9)	[43.68- 46.12]
	Boundioba	1,416 (18.19)	769 (54.3)	[51.71- 56.89]	9 (0.6)	[0.31- 1.16]	638 (45.1)	[42.48- 47.66]
	The 2 villages	7,784 (100)	4,243 (54.5)	[53.40- 56.61]	44 (0.6)	[0.42- 0.75]	3,497 (44.9)	[43.82- 46.03]

An. gambiae = Anopheles gambiae complex; *Culex= Culex spp;* HLC= Human landing catch; BGST= Biogents sentinel trap; ITTC= Ifakara tent trap type C

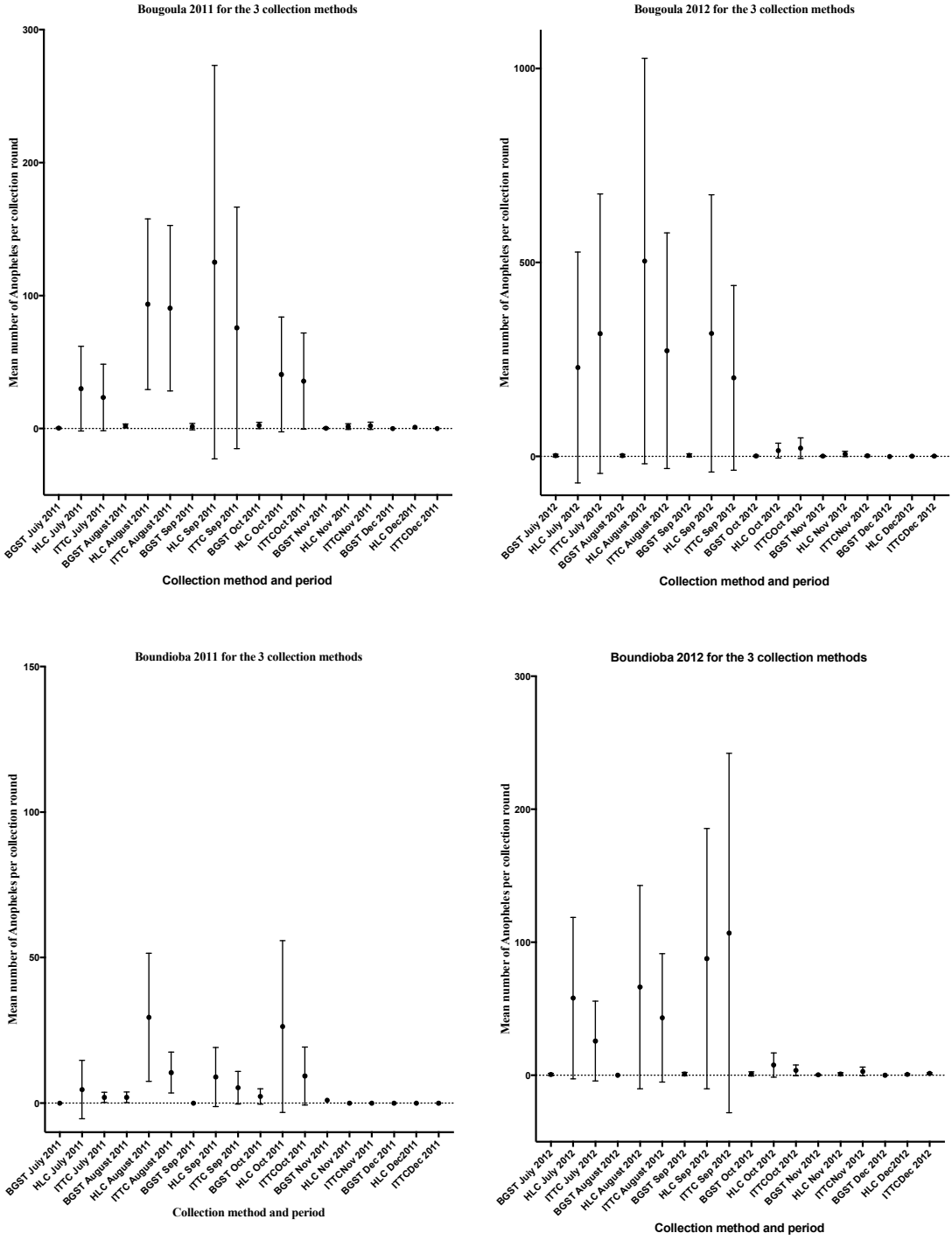


Figure 6.4 Variations in the monthly yields of *Anopheles gambiae s.l* in 2011 and 2012 using the three mosquito collection methods.

The bars represent the number of mosquito collected monthly using the indicated trapping method in 2011 (black bars) and in 2012 (white bars)

6.3.2 Comparison of the Mosquito Collection Traps

There was a strong and significant positive correlation between the HLC and ITTC yields of *An. gambiae s.l* members in both villages and over the two collection years. The correlation coefficients ranged from 66% to 84% and all p values were less than 0.007 (Table 6.2). The BGST yields were never significantly correlated with those of the HLC in the two villages over the two collection years with all coefficients less than or equal to 28% (Table 6.2). All of the *Anopheles* collected using the three collection methods were dissected, and none was found to be infected.

In 2011, the parity and daily survival rates were comparable for all three collection methods in both villages using their 95% confidence intervals. The parity and daily survival rates were also comparable between the two villages for each of the three methods using their 95% confidence intervals (Table 6.3). A significant difference was observed in the relative catch rates between villages (60% less for the village of Boundioba) and between the collection methods (20% and 98% less for the ITTC and BGST respectively as compared to the HLC) (Table 6.4). The Spearman linear regression using the combined data from the two villages over the two years showed that the HLC yield was equal to 28.15 plus the ITTC yield times 0.59 (Figure 6.6). This model had a slope significantly deviated from zero ($p<10^{-3}$) and $r^2=0.52$. The estimation has also been done for each village with relevant statistics (Figure 6.6).

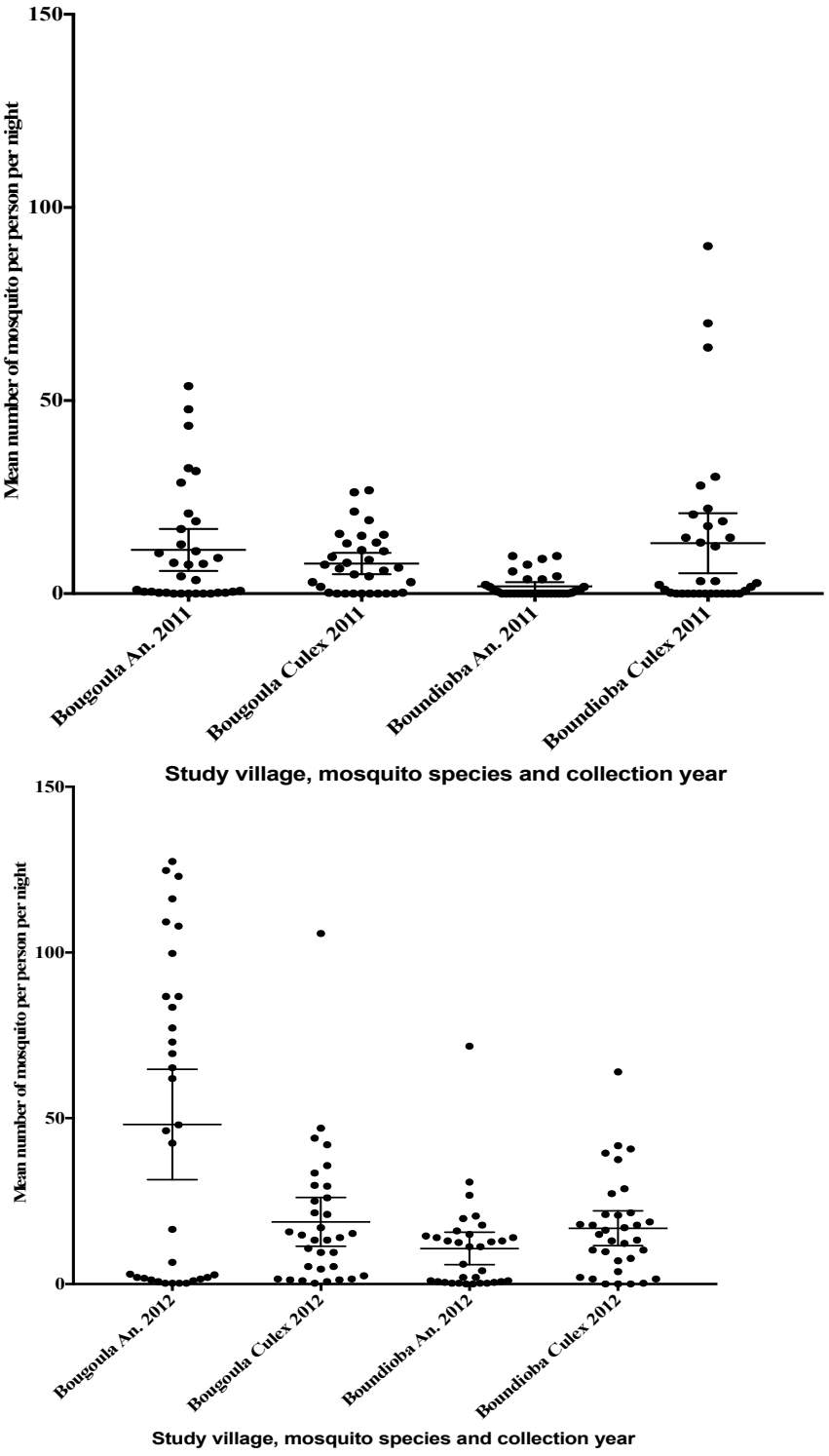


Figure 6.5 Variations in the mean mosquito density in the two study villages over the study period showing the 95% confidence intervals

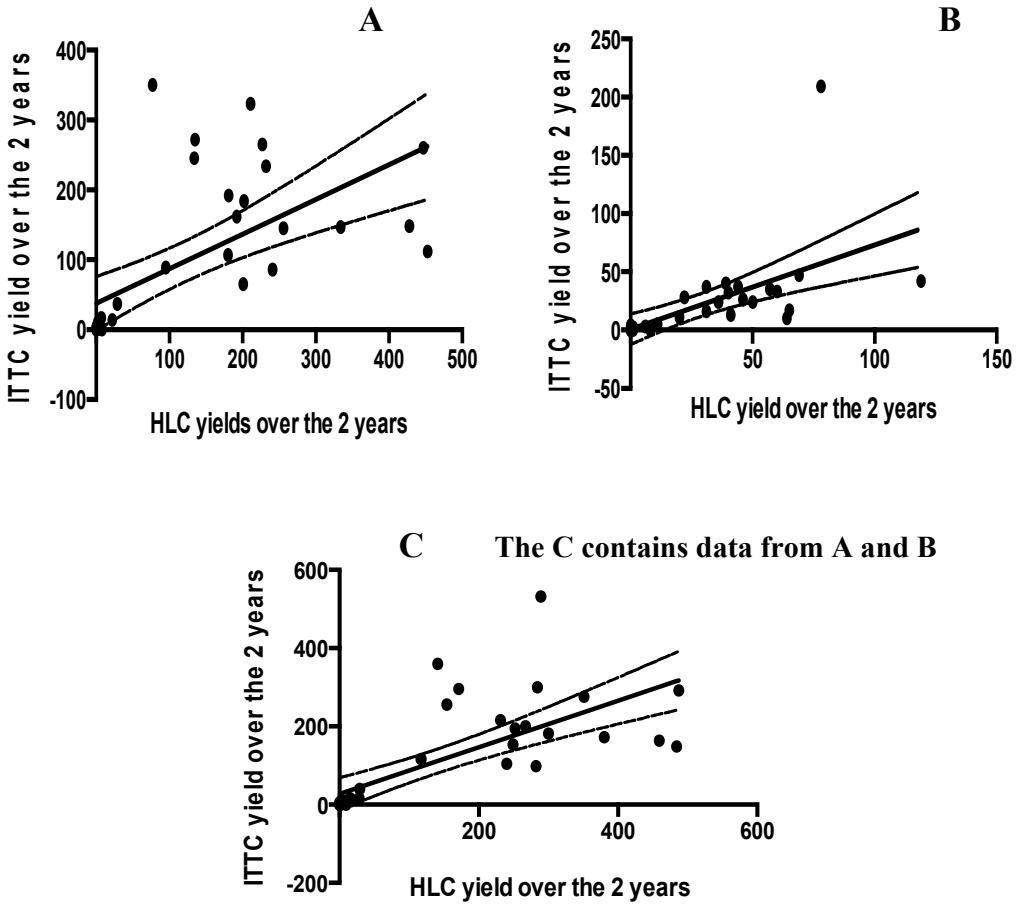


Figure 6.6 Variations in the correlation level between the monthly yields of *Anopheles gambiae s.l* from July to December in 2011 and 2012 using the three mosquito collection methods using a simple linear regression

Legend: Relationship between the HLC monthly yields of *An. gambiae s.l* and those of the ITTC in Bougoula (A) and in Boundioba (B) and in the villages combined (C). The black dots represent the monthly yields. The solid lines represent the linear regression line while the dashed lines represent the upper and lower limits of the 95 % confidence interval.

A: HLC yield for *An. gambiae s.l* in Bougoula = 0.50 x ITTC yield + 37.39

B: HLC yield for *An. gambiae s.l* in Boundioba = 0.72 x ITTC yield + 0.69

C: HLC yield for *An. gambiae s.l* (2 villages combined) = 28.15 + [0.59 * ITTC yield].

Table 6.2: Variations of the correlation level between the yields of the three methods per village and collection year

Villages and collection year	Tests	Correlation between the yields from the collection methods	
	Spearman correlation test	**HLC-ITTC**	**HLC-BGST**
Bougoula in 2011	R	0.74	0.28
	P	< 0.001	0.16
Bougoula in 2012	R	0.74	0.08
	P	<0.001	0.68
Bougoula over the 2 collection years	R	0.84	0.12
	P	<0.001	0.35
Boundioba 2011	R	0.66	0.23
	P	0.007	0.40
Boundioba 2012	R	0.74	0.10
	P	<0.001	0.58
Boundioba over the 2 collection years	R	0.77	0.07
	P	<0.001	0.65

HLC= Human landing catch; BGST= Biogents sentinel trap; ITTC= Ifakara tent trap type C

Table 6.3: Variations of the *Anopheles gambiae s.l* parity and survival rates per village in 2011

Collection methods	Villages	Parity rate n/N (%) [95% CI]	Survival rate (%) [95% CI]
BGST	**Bougoula**	17/18 (94.4) [72.71-99.86]	0.98 [0.90-1]
	Boundioba	11/12 (91.7) [61.52-99.79]	0.97 [0.85-1]
HLC	**Bougoula**	706/844 (83.6) [80.98-86.08]	0.92 [0.93-0.95]
	Boundioba	153/172 (89) [83.29-93.22]	0.95 [0.94-0.98]
ITTC	**Bougoula**	533/631(84.5) [81.4-87.21]	0.93 [0.93-0.96]
	Boundioba	54/68 (79.4) [67.88-88.26]	0.96 [0.88-0.97]

n/N, number parous divided by the total number dissected; HLC=Human landing catch; BGST= Biogents sentinel trap; ITTC= Ifakara tent trap type C

Table 6.4: Variation of the relative catch of the different collection methods yields according to the trap type and the village

Fixed effect	Relative catch	95% CI	p-value
Trap type			
HLC (reference type)	1		
BGST	0.017	[0.012, 0.023]	p<0.0001
ITTC	0.712	[0.593, 0.8551]	p=0.0003
Village			
Bougoula	1		
Boundioba	0.404	[0.1753, 0.9322]	p=0.0336

HLC= Human landing catch; BGST= Biogents sentinel trap;
ITTC= Ifakara tent trap type C

Table 6.5 shows that only one pool was found infected for *W. bancrofti* from both HLC and ITTC *Anopheles* yields in the village of Bougoula, with respectively 0.03% [0.0009% – 0.2%] and 0.04% [0.001% – 0.2%] as MIPL with 95% confidence intervals. The BGST collected *Anopheles* were not found infected but the 95% confidence intervals of the three collection methods yields' MIPL were largely overlapping indicating that they are comparable. No *W. bancrofti* infected pool was recovered in the village of Boundioba in 2012. From Table 6.6, it appears that *P. falciparum* was found in several pools from each study village in 2012 with comparable overall MIPL of 2% [95%CI (1.6% – 2.4%)] and 1.3% [95%CI (0.7% – 2.1%)] respectively in Bougoula and Boundioba. In Bougoula, a significantly higher MIPL was observed for the HLC collected *Anopheles* 3% [95%CI (2.3% – 3.8%)] as compared to that for ITTC, which was 1% [95%CI (0.9%-1.4%)]. In Boundioba, the HLC reported the highest MIPL but the three methods showed comparable 95% confidence intervals for *P. falciparum* MIPL (Table 6.6).

Table 6.5: Variation of the likelihood of *Anopheles gambiae s.l* infection prevalence likelihood with *Wuchereria bancrofti* in 2012 per collection method and per village

Bougoula 2012

Collection method	#Tested	# pools	pools size range	#positive pools	Wb infection prevalence likelihood* [95%CI]
HLC	3,460	185	[1-20]	1	0.03% [0.0009% – 0.2%]
ITTC	2,836	157	[1-20]	1	0.04% [0.001% – 0.2%]
BGST	33	10	[1-7]	0	0% [0% – 6%]
Total	**6,329**	**352**	[1-20]	**2**	**0.03% [0.004% – 0.1%]**

Boundioba 2012

Collection method	#Tested	# pools	pools size range	#positive pools	Wb infection prevalence likelihood* [95%CI]
HLC	718	49	[1-20]	0	0% [0% – 0.3%]
ITTC	637	47	[1-20]	0	0% [0% – 0.3%]
BGST	9	5	[1-3]	0	0% [0% – 19.2%]
Total	**1,364**	**101**	[1-20]	**0**	**0% [0% – 0.1%]**

HLC= human landing catch; ITTC= Ifakara tent trap type C, BGST= Biogents sentinel trap, Wb= *W. bancrofti,* CI= confidence interval; #= number

Table 6.6: Variation in the likelihood of *Anopheles gambiae s.l* infection prevalence likelihood with *Plasmodium falciparum* in 2012 per collection method and per village

Bougoula 2012

Collection method	#Tested	# pools	pools size range	#positive pools	Pf infection prevalence likelihood* [95%CI]
HLC	3,460	185	[1-20]	79	3% [2.3% – 3.8%]
ITTC	2,836	157	[1-20]	25	1% [0.9% – 1.4%]
BGST	33	10	[1-7]	1	3% [0.09% – 14.7%]
Total	**6,329**	**352**	[1-20]	**105**	**2% [1.6% – 2.4%]**

Boundioba 2012

Collection method	#Tested	# pools	pools size range	#positive pools	Pf infection prevalence likelihood* [95%CI]
HLC	718	49	[1-20]	11	2% [0.8% – 3.1%]
ITTC	637	47	[1-20]	5	1% [0.3% – 1.9%]
BGST	9	5	[1-3]	0	0% [0% – 19.2%]
Total	**1,355**	**101**	[1-20]	**16**	**1.3% [0.7% – 2.1%]**

HLC= human landing catch; ITTC= Ifakara tent trap type C, BGST= Biogents sentinel trap, Pf= *P. falciparum;*
CI= confidence interval; #= numbe

6.4 Discussion

6.4.1 Vector species composition and variation between the two villages

An. gambiae s.l were more frequent in the village of Bougoula in both collection years (Figure 6.5)), at each assessment point and using any collection method (data not shown). Such a dramatic difference in mosquito density between two villages separated by only 17 km in the same region could be due to several factors, including differences in the villages' ecological conditions, breeding site dispersal and features, housing characteristics, and the frequency and abundance of rain (Animut, Balkew, and Lindtjørn 2013; Munhenga et al. 2014). The level of education, behaviours and occupations (type of crops and agricultural methods used) of the population can also impact vector density, although these characteristics are very likely to be similar between the two study villages. Regardless of the reason for the observed differences in vector density, this type of variability requires further study as it may impact both the success of MDA and the implementation of surveillance strategies post-MDA.

Over the two years of the study, BGST yields were made of *Culex* complex members more frequently than the other two collection methods. Given the fact that *Culex* complex members are not vector of LF in West Africa, their role is not important in term of LF transmission assessment. Nonetheless, given the high number of *Culex spp* collected, even if they do not transmit LF, they may constitute a useful source for monitoring vector-human contact especially in areas where few *Anopheles* species exist (urban areas of most endemic African countries) and where several rounds of MDA have lowered both the LF infection and microfilaraemia rates. Finding *Culex spp*. infected with any stage of *W. bancrofti* DNA may presage an increase or re-emergence of LF transmission (Chadee, Williams, and Ottesen 2002).

6.4.2 Collection methods' comparison

The ability to follow the impact of entomological interventions or the re-emergence of an infection previously interrupted or dramatically reduced requires repeated assessments over a period of time. However, since vector density has important implications with respect to the determination of most transmission parameters, the use of different mosquito collection methods can make such comparisons difficult. Of the two trapping methods tested, the ITTC showed better correlation with the HLC than the BGST with respect to total yields, frequency trends and collected vector parity rates for *An. gambiae* complex over the transmission season. In fact, the BGST collected predominantly *Culex* complex members, which do not transmit LF in the study region.

Both the HLC and ITTC collected relatively old mosquitoes, which are more likely to participate in disease transmission, with a survival rate >92 and a parity rate of > 83%. The high parity rates of mosquitoes captured with these two methods indicate the suitability of the collected fauna for transmission assessment (Jensen et al. 1998; Lindsay et al. 1991). The proposed linear regression model provides an estimate of the vector density based on HLC using data from an ITTC, allowing comparisons between historical HLC data and new data acquired using an ethically more acceptable method and addressing the need to standardize operational vector sampling methods, as raised by Wang et al. (Wong et al. 2013).

In terms of infected mosquito identification, the HLC showed a higher MIPL for *P. falciparum* in Bougoula as compared to the ITTC. For *W. bancrofti* and in the village

of Boundioba, the collection methods were still comparable with respect to the infection MIPL. There seems to be an underestimation of *P. falciparum* when infection prevalence as well as vector densities are high. Such a scenario is likely to be more common for malaria as compared to LF due to the high impact of the MDA on LF endemicity levels in many endemic areas.

Overall, in each village, the three methods had comparable MIPL except in Bougoula where the HLC it had significantly higher one than ITTC. This may be due to the sample sizes that certainly may need to be higher to achieve statistical significance for the observed phenomenon especially in the village of Boundioba.

In most endemic areas, LF elimination programmes have been ongoing for several years and there is an increased need for surveillance prior to and after stopping MDA. Although the ideal package for surveillance has not yet been determined, it will likely be a combination of blood and vector surveillance on a regular basis with sustained community participation. The identification of the most cost-effective, safe and reliable vector surveillance method is, therefore, of high importance. Whereas the yield of *Anopheles* using HLC was twice that of the ITTC over the two years of the study, the ITTC uses one collector per collection point as compared to two for the HLC– one indoor and the other outdoor. Additionally, the cost of operation is higher for the HLC because of the need for training and expertise, especially in the setting of a community monitoring system that would be part of an integrated vector management system in endemic areas (Sikaala et al. 2014). Despite the initial cost of the tents which can pose a challenge, the ease of implementation, the possibility to use another type of bait in the tent (natural or artificial) (Jawara et al. 2009; Mukabana et al. 2012), the lack of

operator impact on the efficiency of the method, the capacity to collect both *Culex* and *Anopeles* complexes for xenomonitoring purposes, and the absence of ethical issues are also important factors in favour of the ITTC as compared to the HLC (Sikaala et al. 2014).

6.5 Conclusion

Anopheles collection using the ITTC provides data that are well correlated to those from the HLC, independent of the vector density. Consequently, ITTC provides an ethically acceptable alternative to HLC for use in monitoring mosquito vectors as part of entomological surveillance during and following MDA for LF. Furthermore, the relationship established between ITTC and HLC yields will allow the comparisons between new and historical data. Insights on the collected females' infection rate for the commonly transmitted parasitic diseases would be important.

Chapter 7

Objective 5. LF transmission intensity after six MDA rounds in two neighbouring villages with different *Anopheles* densities

7.1 Introduction

The MDA was instituted as early as 2005 in Mali starting with the region of Sikasso that was the most endemic of the country. Within Sikasso administrative region, the district of Kolondieba was one of the most heavily affected by LF since it has the highest number of LF chronic manifestations in the country according to the Malian NPELF (Dr Dembele, NPELF Manager, Personal communication January 2014).

This district is one of the two IUs that ever failed a pre-TAS in Mali. In 2010, the IU of Kolondieba failed with a microfilaraemia prevalence of 9.8% (49/500) for a threshold of < 1% within the > 5 year old in the sentinel and spot check sites of the implementation unit (IU) after five annual MDA rounds using ALB/IVER drugs combination. As recommended, two additional MDA rounds were done before a new assessment by the NPELF.

Ecological differences were observed between two villages of Kolondieba district with significantly different mosquito vectors frequencies, the village of Bougoula and that of Boundioba (chapter 6). None of these villages were a sentinel or spot check site when the IU (Kolondieba district) failed to the pre-TAS. Given the focal nature of LF, an IU that passes the pre-TAS can have some villages or other subdivisions that are still having relatively high microfilaraemia prevalences.

The WHO directions for such scenario were still not very detailed until the recent report of an ad hoc meeting that provided in December 2015 with more guidelines in case of TAS failure. A major component of these guidelines is the elucidation of the failure causes and the development of suitable alternative strategies (WHO 2016a).

In line with that, more evidence is needed in areas that fail to the pre-TAS/TAS or are having less than expected effect of MDA in order to build strong recommendations for these hotspots.

In order to investigate at a fine scale two settings with very different vector density and check if there is any added value to the use of the newly available antibody test for the Wb123 specific antigen of *W. bancrofti* L3 (Steel et al. 2012), a cross sectional study was conducted in the two villages of Kolondieba. The hypothesis of this chapter was that in villages with different vector densities, after six mass drug administration rounds, lymphatic filariasis endemicity level may be higher in the village with a higher vector density.

7.2 Methods

7.2.1 Study Sites

This study was conducted in Bougoula and Boundioba, two neighbouring villages described in the chapter 6 of the thesis.

The study villages were divided in 3 differents zones as described in chapter 6 of this thesis.

7.2.2 Study design

This was a longitudinal study with three visits in 2011, 2012 and 2014 for the parasitological aspect and a monthly visit from July to December in 2011 and 2012 for the entomological aspect. It was a community-based survey with blood testing in children >5 years old.

7.2.3 Parasitological and clinical data collection

7.2.3.1 Census

A complete census of the two villages' inhabitants was performed at the beginning of the study. All of the inhabitants were recorded with their name, surname, parental affiliation with the head of the family and their room coordinates using a GPS.

7.2.3.2 Blood samples collection and processing

All blood collections occurred at night (10 pm to 2 am). Eligible consenting volunteers for the blood collection underwent a finger prick to collect nocturnal peripheral blood for three thick smears of 20 microliters each (60 microliters) and a Whatman® paper filter (60 microliters). In 2014, only the ICTs (100 microliters) were used in one village (Boundioba) to check the LF infection trend. All of the parasitological survey targeted all volunteers > 5 years old as recommended for the follow-up survey after five rounds of effective MDA (WHO 2011b).

The blood collected on filter paper was left to dry in the field before being placed in a labelled envelope. The envelopes were stored in a hermetically-sealed plastic box with a desiccant (Silica gel) avoiding direct contact between the blood and the dessicant. The slides were stored in plastic boxes in a dry place. Slides were stained with a solution of Giemsa before observation using a stereomicroscope at X20 and confirmation with X40 or X100.

ICT results were marked on the card ten minutes after the blood sample application as recommended by the manufacturer. CFA detection using blood eluted from the filter spots was performed using the Og4C3 TropBio ELISA (TropBio, Townsville,

Australia) as previously described by Das et al (2012). Briefly, one dried blood spot on filter paper was cut and stored overnight at 4 °C in a solution of phosphate buffered saline (PBS) mixed with 0.05% Tween buffer. 100 µl of the eluted solution in 300 µl of sample diluent was boiled at 100 °C for five minutes and 50 µl of the boiled eluted solution was incubated overnight on a pre-coated plate. The following day, a washing buffer was used to wash the plate before adding 50 µl of rabbit anti-Onchocerca antibody in each well for an hour of incubation. After that, the plate was washed three times before reading with an ELISA reader machine. A spectrophotometer was used at 450 nm for the optical density determination. The value of 32,000 was used as the cut-off of positivity.

7.2.3.3 Medical examination

This assessment consisted of a brief examination of the patient in a discreet room based on a short interview about the subject's medical history. Concomitant abnormal conditions were treated or referred to the local nurse if found. If these conditions precluded a blood collection, the volunteer was treated and excluded from the blood collection component of the study.

7.2.3.4 Co endemic infection assessment

In the study area, 2 parasitic diseases are co endemic with LF, malaria and Mansonellosis. These 2 parasitic infection were studied due to several similarities they had with LF.

Malaria has the same vectors as LF in Mali, the *Anopheles gambiae and funestus* complexes. Additionally, while expecting relatively low infection and infectivity rates for *W. bancrofti* on collected *Anopheles* vectors due to several rounds of mass drug

administration, *P. falciparum* finding on these vectors were expected because of the high endemicity level of malaria in the study area. Malaria remains a major cause of death and morbidity in endemic areas (WHO 2016c), with infections by *P. falciparum* accounting for the majority of malaria mortality, though the less virulent *P. vivax*, and probably *P. ovale*, also contribute significantly to morbidity. These three plasmodium species exist in Mali (Williams et al. 2016). *Plasmodium* sporozoites injected by an infected female during a blood meal will migrate to the liver and the hepatic stage of the parasite life cycle starts by hepatocytes invasion and multiplication within them before the differentiation into schizonts containing thousands of hepatic merozoites. The obtained merozoites are later released into the blood where they initiate the erythrocytic stage characterized with the invasion and replication within red blood cells. Some of these asexual blood parasites differentiate into gametocytes that will ensure parasite transmission to the mosquito vector during another blood meal. A different life cycle within mammalian hosts is observed for *P. vivax* and *P. ovale*. Some sporozoites, once in the liver, do not develop immediately into schizonts, but remain at an uninucleate stage, in a quiescent form named hypnozoite, before resuming hepatic development on the impulse of still unknown factors. They can then cause relapses weeks, months or even years after the initial infection (Galinski, Meyer, and Barnwell 2013).

The vectors of *M. perstans* are biting midges (Diptera: *Ceratopogonidae*) belonging to the genus *Culicoides*. The females mainly bite around dawn and dusk although biting also occurs during day or night (Simonsen, Onapa, and Asio 2011). The mature mated female worms release live Mf that circulate without any specific periodicity (night or day time) in the blood often in high numbers. They measure about 190–200 by 4.0–4.5 μm. The mf are ingested by the vectors during blood meals and undergo a

maturation process. They migrate through the stomach wall to the thoracic muscles where further development takes place. They shorten and thicken to become 'sausage forms', but from the fifth day they increase considerably in length. Although not studied, *M. perstans* most likely has two moults in the vector like other filariae. The mature infective stage larvae (length 750–900 μm) may be seen in the proboscis 7–9 days after infection of the vector. The larvae escape from the proboscis by stretching and finally bursting the terminal membranous portion of the labrum (Sharp 1928).

7.2.4 Entomological data collection

Monthly mosquito collections were conducted from July to December in 2011 and 2012 as previously described in the chapter 6 of this thesis.

7.2.5 Mapping and spatial analysis

The geographical coordinates (latitude and longitude) of each individual/participant's household were determined using a GPS device. This was for assessing the magnitude and geographical distribution of LF vectors and infected humans and their households in the two study villages. Using the GIS software ArcGIS (ESRI 9.2, Redlands, CA), the data were extracted from the Garmin GPS.

7.2.6 Statistical analysis

As detailed in chapter 6 of this thesis, the same entomological analyses were used as needed. For the parasitological data, the same software was used for the proportions and continuous variables comparison between the two study villages. In 2012, some of the antigen and Mf positive subjects were not tested again but their data have been used for the 2012 data analysis since they participated in another study between the two surveys

that confirmed their status. The 95% confidence intervals were used for statistical comparisons between point estimates.

7.3 Results

7.3.1 Study population description

A total of 958 subjects were included in the parasitological aspect of the study with 532 from the village of Boundioba and 426 form that of Bougoula (Table 7.1). There were more females in both villages, with 70.1% and 66.9% respectively in Bougoula and Boundioba. The sample median age was 26 years (Table 7.1).

Table 7.1: Study population characteristics in 2012

	Villages	
Characteristics	**Bougoula (N=532)**	**Boundioba (N=426)**
Sex		
Male	159 (29.9%)	141 (33.1%)
Female	373 (70.1%)	284 (66.9%)
Age group		
Median age for the study population	26 years	26 years
6-25 years	240 (45.1%)	231 (54.2%)
26 years and above	292 (54.9%)	195 (45.8%)

N=Number of subjects sampled

7.3.2 Parasitological and clinical results in the two villages

An overall *W. bancrofti* microfilaraemia prevalence of 0.8% [0.39-1.58] (8/958) was observed in the study population. Seven of the eight microfilaria-positive subjects were from zone B of the village of Boundioba, and one was from zone C. Five of the eight were ≥26 years old and two were in the younger age group (Table 7.2). One 9-year-old male with microfilaraemia was diagnosed in the village of Boundioba. He was living in a family where no infected adult was identified. Six of the eight microfilariae positive

subjects were females aged 20 to 43 years old with microfilarial loads ranging from 17 to 383 mf per ml of blood. No microfilaria-positive subject was identified in the village of Bougoula (Table 7.2).

The village of Boundioba had a significantly higher *W. bancrofti* Mf prevalence 1.9% [0.88-3.53] as compared to Bougoula where no Mf positive subject was identified 0% [0-0.56] (Table 7.2). The same scenario was observed for *W. bancrofti* antigen prevalence with 0.8% [0.24-1.80] (4/532) in Bougoula and 3.5% [2.06-5.61] (15/426) in Boundioba (Table 7.3).

Table 7.2: *Wuchereria bancrofti* microfilaraemia prevalence rate variations within the > 5 years old per village, zone, and age group in 2012

VILLAGE	Zone	Age group	Total sampled	Wb Mf positive		[95% CI]
				N	%	
Bougoula	A	6-25 years	94	0	0	[0-3.14]
		≥26 years	87	0	0	[0-3.39]
	Total A		181	0	0	[0-1.64]
	B	6-25 years	88	0	0	[0-3.35]
		≥26 years	98	0	0	[0-3.01]
	Total B		186	0	0	[0-1.60]
	C	6-25 years	58	0	0	[0-5.03]
		≥26 years	107	0	0	[0-2.76]
	Total C		165	0	0	[0-1.80]
Bougoula Total			**532**	**0**	**0**	[0-0.56]
Boundioba	A	6-25 years	50	0	0	[0-5.82]
		≥26 years	26	0	0	[0-10.88]
	Total A		76	0	0	[0-3.87]
	B	6-25 years	147	2	1.4	[0.23-4.42]
		≥26 years	128	5	3.9	[1.45-8.44]
	Total B		275	7	2.5	[1.12-4.97]
	C	6-25 years	34	1	2.9	[0.15-13.66]
		≥26 years	41	0	0	[0-7.05]
	Total C		75	1	1.3	[0.07-6.40]
Boundioba Total			**426**	**8**	**1.9**	[0.88- 3.53]
2 villages Total			**958**	**8**	**0.8**	**[0.39-1.58]**

Wb= *W. bancrofti*; N= number of subjects

Table 7.3: *Wuchereria bancrofti* antigen prevalence rate variations within the > 5 years old per village, zone, and age group in 2012

VILLAGE	Zone	Age group	Total sampled	Wb antigen positive		[95% CI]
				N	%	
Bougoula	A	6-25 years	94	1	1.1	[0.05-5.13]
		≥26 years	87	0	0	[0-3.39]
	Total A		181	1	0.6	[0.03-2.69]
	B	6-25 years	88	0	0	[0-3.35]
		≥26 years	98	3	3.1	[0.78- 8.10]
	Total B		186	3	1.6	[0.41-4.33]
	C	6-25 years	58	0	0	[0-5.03]
		≥26 years	107	0	0	[0-2.76]
	Total C		165	0	0	[0-1.80]
Bougoula Total			**532**	**4**	**0.8**	[0.24-1.80]
Boundioba	A	6-25 years	50	0	0	[0-5.82]
		≥26 years	26	1	3.8	[0.19-17.54]
	Total A		76	1	1.3	[0.07-6.32]
	B	6-25 years	147	3	2	[0.52-5.45]
		≥26 years	128	10	7.8	[4.04-13.48]
	Total B		275	13	4.7	[2.65-7.75]
	C	6-25 years	34	1	2.9	[0.15-13.66]
		≥26 years	41	0	0	[0-7.05]
	Total C		75	1	1.3	[0.07- 6.40]
Boundioba Total			**426**	**15**	**3.5**	[2.06- 5.61]
Total for the 2 villages			**958**	**19**	**2**	**[1.23- 3.02]**

Wb= *W. bancrofti*; N= number of subjects

The village of Boundioba had a filarial antigen prevalence rate of 3.5% [2.06- 5.61] (15/426) significantly higher than the one in Bougoula with 0.8% [0.24-1.80] (4/532). In Bougoula, three infected subjects were from the zone B and the fourth from Zone A. In terms of age, three were in the ≥26 year age group and the fourth in the younger age group (Table 7.3). In Boundioba, 13 of the 15 infected individuals were from Zone B and one from each of the two other Zones. Additionally, 10 of the 15 infected individuals were from the ≥26 year age group and the three remaining from the younger age group. No statistically significant difference was observed between the 3 zones in Bougoula nor in Boundioba regarding *W. bancrofti* antigen prevalence even if the Zone B that corresponds to the middle of the village with the highest population desnity seems to have higher numbers of infected people (Table 7.3).

In 2014, 367 volunteers were tested using the ICT in the village of Boundioba where microfilaria positive subjects were observed in 2012. Their average age was 19 years varying between 11 and 73 years old (data not shown). The mean infection rate was 7.6% [5.23-10.69] (28/367) with comparable prevalences between the three zones in the village (1.5% [0.07-7.14], 8.8% [5.36-13.39] and 9.4% [4.89-16.17]) respectively for the zones A, B and C (Table 7.4). While the *M. perstans* infection was comparable between the three zones in each village, Boundioba had a significantly higher prevalence of 11.5% [8.73-14.80] (49/426) as compared to Bougoula 0.6% [0.14-1.53] (3/532) (Table 7.5).

Clinically, two and four cases of elephantiasis were observed in Bougoula and Boundioba, respectively (data not shown). The recorded data for hydrocele prevalence were not reliable since some volunteers did not report obvious hydroceles that could be perceived without the subject removing his clothes.

Table 7.4: *Wuchereria bancrofti* antigen prevalence rate within the > 5 years old subjects in the village of Boundioba in 2014

		Age in years			ICT Positive		
Collection point	**Total sampled**	**Median**	**Min**	**Max**	**N**	**%**	**[95% CI]**
Zone A	67	33	14	61	1	1.5	[0.07-7.14]
Zone B	194	32	11	70	17	8.8	[5.36-13.39]
Zone C	106	31	12	73	10	9.4	[4.89-16.17]
Total	367	32	11	73	28	7.6	[5.23-10.69]

Table 7.5: *Manonella perstans* microfilaraemia prevalence rate variations within the > 5 years old per village, zone, and age group in 2012

VILLAGE	Zone	Age group	Total sampled	Mp positive		[95% CI]
				N	%	
Bougoula	A	6-25 years	94	0	0	[0-3.14]
		≥26 years	87	0	0	[0-3.39]
	Total A		181	0	0	[0-1.64]
	B	6-25 years	88	0	0	[0-3.35]
		≥26 years	98	2	2	[0.34-6.58]
	Total B		186	2	1.1	[0.18-3.51]
	C	6-25 years	58	0	0	[0-5.03]
		≥26 years	107	1	0.9	[0.05-4.52]
	Total C		165	1	0.6	[0.03-2.95]
Bougoula Total			**532**	**3**	**0.6**	[0.14-1.53]
Boundioba	A	6-25 years	50	6	12	[5.01-23.29]
		≥26 years	26	7	26.9	[12.61-46.14]
	Total A		76	13	17.1	[9.86-26.82]
	B	6-25 years	147	7	4.8	[2.11-9.19]
		≥26 years	128	21	16.4	[10.73-23.59]
	Total B		275	28	10.2	[7.01-14.19]
	C	6-25 years	34	2	5.9	[0.99-18.10]
		≥26 years	41	6	14.6	[6.15-27.97]
	Total C		75	8	10.7	[5.08-19.25]
Boundioba Total			**426**	**49**	**11.5**	[8.73-14.80]
2 villages Total			**958**	**52**	**5.4**	**[4.12-7]**

7.3.3 Entomological results in the two villages

Monthly variation of *An. gambiae s.l* proportions, densities and infection rates by village

With the exception of the month of December 2012, when three mosquitoes were collected in both villages, the village of Bougoula had a higher *Anopheles* mosquito proportion within the yields of each collection month as compared to the village of Boundioba (Table 7.6). When considering all the individual collection rounds yields over the study period, a statistically significant difference was observed each year between the two villages in term of overall annual *Anopheles* desnity (number of *Anopheles* per person per night) using all collection methods yields. In 2011, an overall annual density recorded in Bougoula was 13.34 [95% CI: 7.22-19.46] versus 3.5 [95% CI: 1.78-5.21] in Boundioba. The same scenario was observed in 2012 with 48.16 [95% CI: 31.54-64.78] in Bougoula and 11.42 [95% CI: 6.25-16.58] in Boundioba.

In Bougoula, *Culex* species mean density per person per night doesn't show any significant monthly variations in 2011. The same scenario was observed in 2012 (Figure 7.1).

In Boundioba, *Culex* mean number per person per night doesn't show any significant monthly changes in 2011 between the month of July and that of November (which were the lowest as compared to the months of August, September and October). In contrast, in 2012, only in December the density was significantly lower than the other months that were still having comparable monthly densities (Figure 7.2).

For *An. gambiae* complex members, in 2011, the monthly desnities in Boundioba were comparables while in Bougoula, a significant monthly changes of the densities was observed between the months of July, September, October and November and the month of August when the the highest density was observed (Figures 7.2 and 7.3).

In Boundioba, there was no significant difference between the 3 zones as related to the ABR while in Bougoula, the Zone B showed a significantly higher ABR of 242 bites per person per year with a 95% CI [203-280] as compared to the two other zones of the village (Table7.7). For *Culex* species, the ABR were comparable between the three zones in both villages (Table7.7). In Bougoula, *An. gambiae* complex members had sigificantly higher number of bites per year per person in each of the three zones as compared to any of the three zones of Boundioba. For *Culex* species, in 2011, the 2 villages zones had comparable ABR in the three collection zones (Table 7.7).

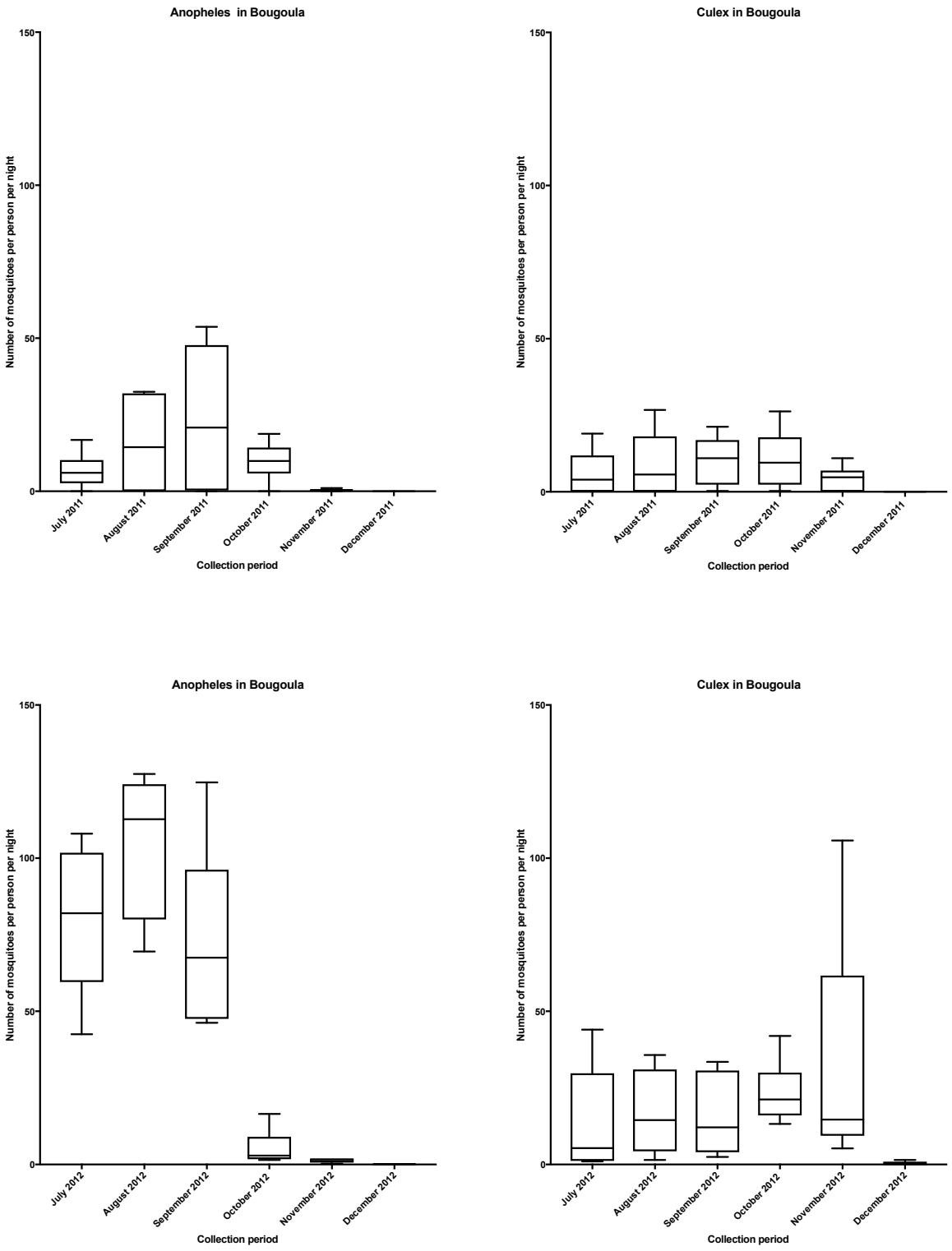


Figure 7.1 Monthly variations of the mean mosquitoes density in the village of Bougoula with 95% confidence intervals

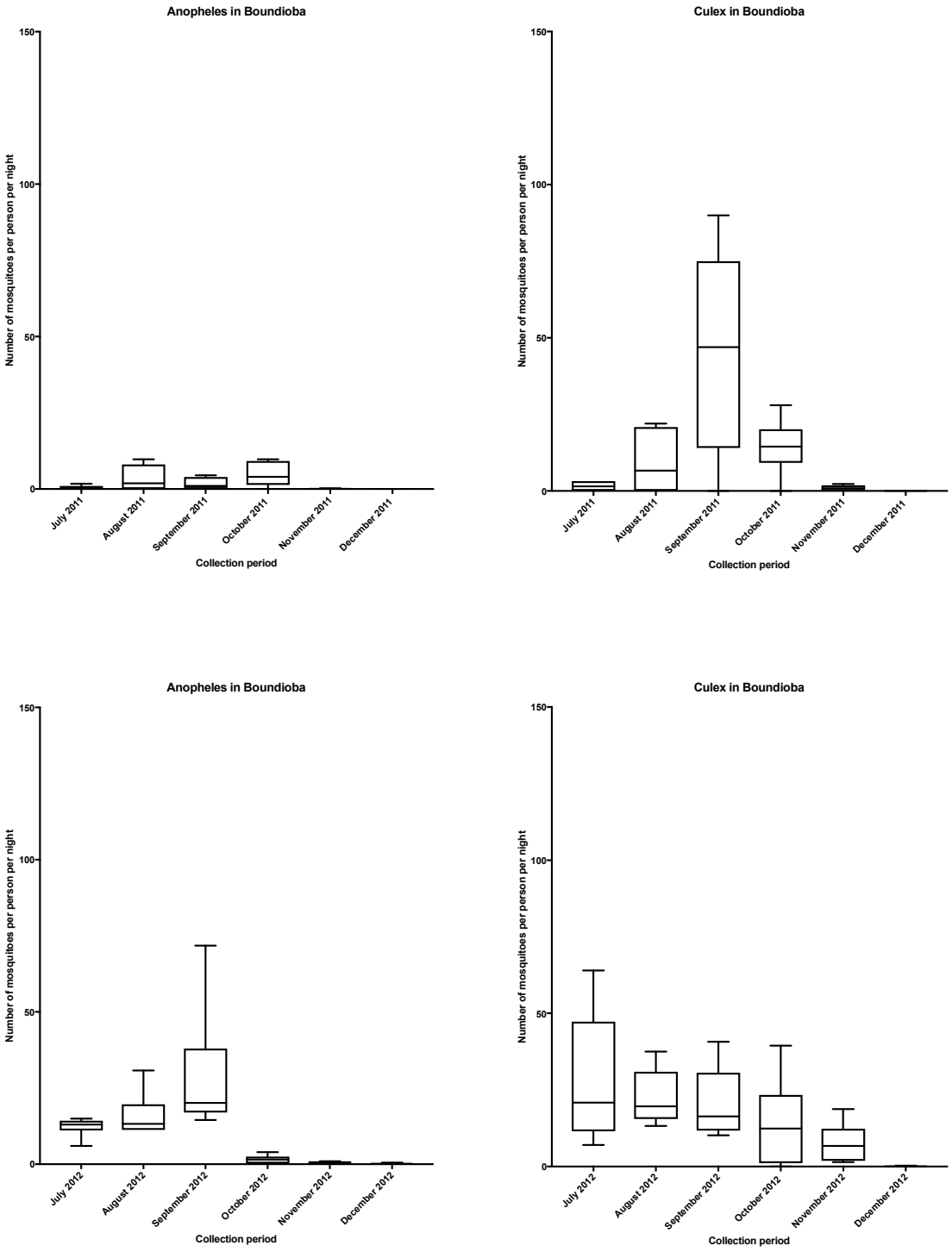


Figure 7.2 Monthly variations of the mean mosquitoes density in the village of Boundioba with 95% confidence intervals

Table 7.6: Monthly variation of *Anopheles gambiae* complex proportions in the two villages of the district of Kolondieba in 2011 and 2012 using the three mosquito collection methods

Years	Month	July		August		September		October		November		December		
	Village	N	% 95% CI	N	% 95% CI	N	% 95% CI	N	% 95% CI	N	% 95% CI	N	% 95% CI	Total
2011	Bougoula	161	10.78 [9.28-12.43]	372	24.9 [22.76-27.14]	708	47.39 [44.86-49.93]	236	15.81 [14.01-17.71]	15	1 [0.58-1.61]	2	0.13 [0.02-0.44]	1,494
	Boundioba	10	3.97 [2.03-6.96]	84	33.33 [27.72-39.33]	43	17.06 [12,79-22.09]	114	45.24 [39.16-51.42]	1	0.4 [0.02-1.94]	0	0 [0-1.18]	252
	Total	**171**	**9.79 [8.47-11.26]**	**456**	**26.12 [24.10-28.22]**	**751**	**43.01 [40.70-45.35]**	**350**	**20.05 [18.22-21.97]**	**16**	**0.92 [0.54-1.45]**	**2**	**0.11 [0.02-0.38]**	**1,746**
2012	Bougoula	1,918	30.12 [29-31.26]	2,516	39.51 [38.31-40.72]	1,774	27.86 [26.77-28.97]	128	2.01 [1.69-2.38]	29	0.46 [0.31-0.64]	3	0.05 [0.01-0.13]	6,368
	Boundioba	295	20.83 [18.78-23.01]	383	27.05 [24.78-29.41]	684	48.31 [45.71-50.91]	37	2.61 [1.87-3.55]	14	0.99 [0.56-1.62]	3	0.21 [0.05-0.58]	1,416
	Total	**2,213**	**28.43 [27.44-29.44]**	**2,899**	**37.24 [36.17-38.32]**	**2,458**	**31.58 [30.55-32.62]**	**165**	**2.12 [1.82-2.46]**	**43**	**0.55 [0.40-0.72]**	**6**	**0.08 [0.03-0.16]**	**7,784**

N= Number of *An. gambiae* complex; % =percentage

Table 7.7: Mosquitoes density and annual biting rate variation per Zones using the HLC in the two villages in 2011

Village	Species	Zone A			Zone B			Zone C		
		N	ABR	95 % CI	N	ABR	95 % CI	N	ABR	95 % CI
Bougoula	*Anopheles*	182	98	[79-117]	451	242	[203-280]	212	114	[93-135]
	Culex spp	8	4	[-6-14]	13	7	[-3-17]	6	3	[-5-11]
Boundioba	*Anopheles*	70	38	[20-56]	43	23	[4-42]	59	32	[10-54]
	Culex spp	3	2	[-13-17]	8	4	[-6-14]	11	6	[-3-15]

ABR= annual biting rate; *Anopheles* = *Anopheles gambiae* complex ; *Culex spp*= *Culex* species

In Bougoula, *An. gambiae s.l* were found infected with *W. bancrofti* in two pools out of the 104 tested in July with a MIPL of 0.1% [95%CI (0.01%-0.4%)]. This corresponds to an overall MIPL of 0.03% [95%CI (0.004% - 0.1%)] for the 352 pools tested from July to December. Over the same period, 105 pools were positive for *P. falciparum* out of 352 pools tested, with an overall MIPL of 2% [95%CI (1.6%-2.4%)] (Table 7.8). *P. falciparum* infected pools were recovered every month except in December. The highest infection rate was observed in November when 16.2% of the tested *Anopheles* pools were positive for *P. falciparum*. The same scenario was observed in Boundioba where 16 pools overall were found positive out of 101 pools tested with a MIPL of 1.3% [95% CI (0.7%-2.1%)] (Table 7.9). In this village, the highest MIPL were recorded in July and November (6.3% and 7.4% respectively). One of the two pools infected with *W. bancrofti* was also positive for *P. falciparum* (data not shown). The infection rates were not analysed at the zone level but only at the village level.

In the village of Boundioba, *An. gambiae s.l* was not found infected with *W. bancrofti* (Table 7.9). In Bougoula, the overall *P. falciparum* MIPL was comparable to that of Boundioba with 2% [95%CI (1.5% – 2.4%)] and 1.2% [95%CI (0.6% – 2.1%)], respectively (Table 7.8; Table 7.9). The same scenario was observed for *W. bancrofti* with 0.03% [95%CI (0.004% – 0.1%)] and 0% [95%CI (0% – 0.1%)] respectively for Bougoula and Boundioba (Table 7.8; Table 7.9).

Table 7.8: Monthly variation of *Anopheles gambiae s.l* infection rate with *Wuchereria bancrofti* or *Plasmodium falciparum* in 2012 in Bougoula

Bougoula 2012							
Month	**#Tested**	**# pools**	**pools size range**	**# Wb positive pools**	**Wb infection prevalence likelihood* with [95%CI]**	**# Pf positive pools**	**Pf infection prevalence likelihood* with [95%CI]**
July	1,910	104	1-20	2	0.1% [0.01%-0.4%]	43	2.9% [1.9% – 3.9%]
Aug.	2,492	130	3-20	0	0% [0% – 0.08%]	45	2.2% [1.6% – 3%]
Sept.	1,767	96	1-20	0	0% [0% – 0.1%]	11	0.7% [0.3% – 1.2%]
Oct.	128	9	5-20	0	0% [0% – 1.5%]	2	1.8% [0.2% – 6.2%]
Nov.	29	11	1-8	0	0% [0% – 6.4%]	4	16.2% [4.3% – 37.2%]
Dec.	3	2	1-2	0	0% [0% – 0.5%]	0	0% [0% – 0.5%]
Total	**6,329**	**352**	1-20	**2**	**0.03% [0.004% – 0.1%]**	**105**	**2% [1.6% – 2.4%]**

ND=Not Done, #=number, CI =Confidence interval; Wb= *W. bancrofti*; Pf= *P. falciparum,* *= Maximum likelihood from the PoolScreen software (Katholi and Unnasch 2006)

Table 7.9: Monthly variation in *Anopheles gambiae s.l* infection rate with *Wuchereria bancrofti* or *Plasmodium falciparum* in 2012 in Boundioba

Boundioba 2012

Month	#Tested	#pools	pools size range	# Wb positive pools	Wb infection prevalence likelihood* with [95%CI]	# Pf positive pools	Pf infection prevalence likelihood* with [95%CI]
Jul.	293	21	2-20	0	0% [0% – 0.7%]	12	6.3% [3% – 11.4%]
Aug.	382	25	1-20	0	0% [0% – 0.5%]	0	0% [0% – 0.5%]
Sept.	635	39	1-20	0	0% [0% – 0.3%]	2	0.3% [0.04% – 1.1%]
Oct.	37	4	1-18	0	0% [0% – 5%]	1	2,9% [0.09% – 14.6%]
Nov.	14	10	1-2	0	0% [0% – 12.8%]	1	7.4% [0.2% – 32.9%]
Dec.	3	2	1-2	0	0% [0% – 47.3%]	0	0% [0% – 47.3%]
Total	**1,364**	**101**	**1-20**	**0**	**0% [0%– 0.1%]**	**16**	**1.3% [0.7% – 2.1%]**

ND=Not Done, #=number, CI =Confidence interval; Wb= *W. bancrofti*; Pf= *P. falciparum,*

*= Maximum likelihood from the PoolScreen software (Katholi and Unnasch 2006)

Spatial distribution of the parasitological data over the two study villages

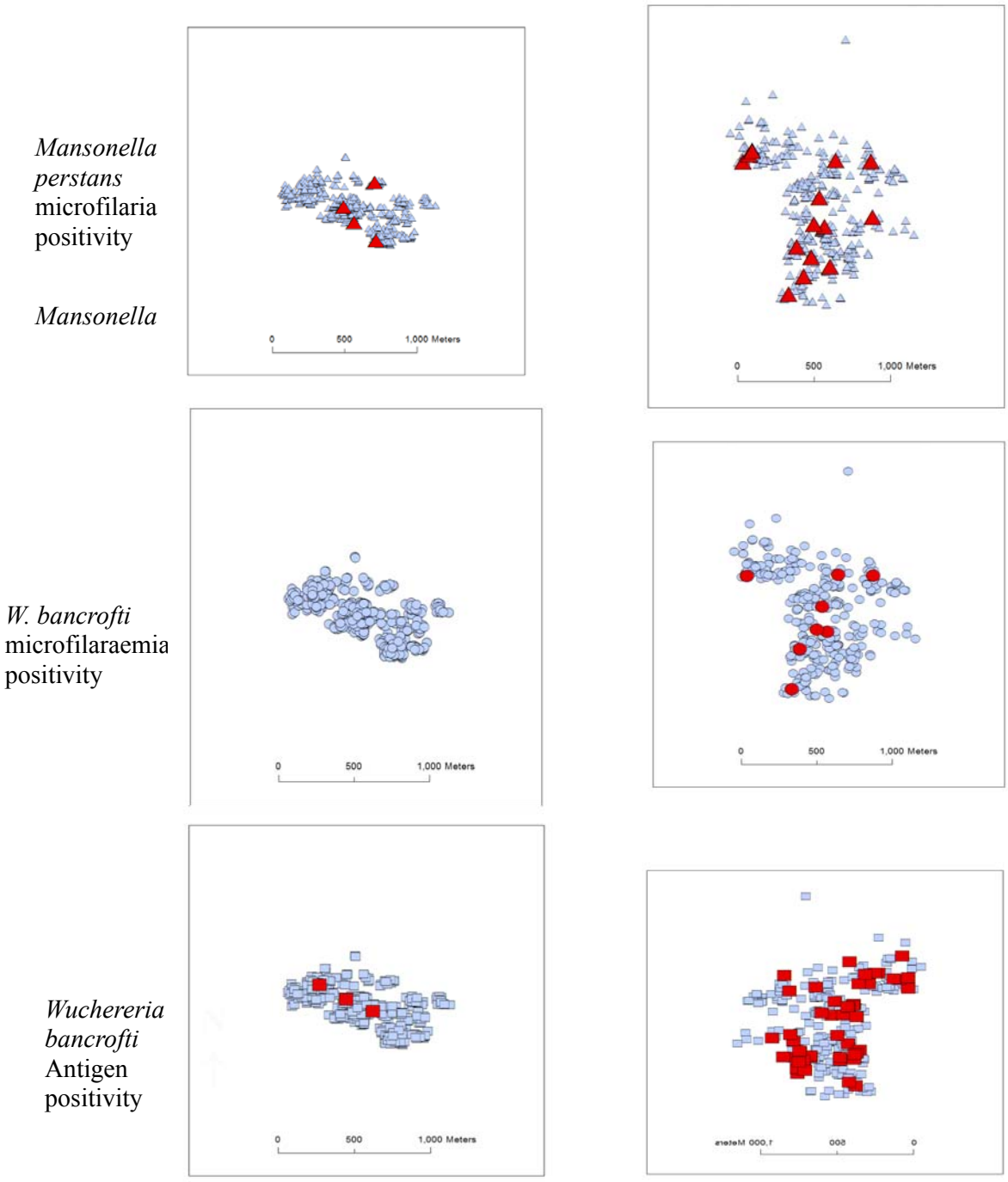


Figure 7.3 Villages of Bougoula and Boundioba with *Wuchereria bancrofti* and *Mansonella perstans* infected subjects' location

Legend:

W. bancrofti antigen positive

W. bancrofti antigen negative

W. bancrofti microfilariae positive

W. bancrofti microfilariae Negative

M. perstans microfilariae positive

M. perstans microfilariae negative

7.4 Discussion

7.4.1 Study population

From 2011 to 2012, volunteers from the villages of Bougoula (n=532) and Boundioba (n=426) participated in this study to assess the impact of six to seven rounds of MDA on LF transmission and its focal nature in two neighbouring villages with significantly different densities of *Anopheles* vectors. After informed consent for all the phases of the study, the majority of the volunteers were female in both villages, reflecting the demographic profile of Malian society.

7.4.2 Residual *W. bancrofti* microfilaraemia carriers

This follow-up survey after six MDA rounds identified a total of eight *W. bancrofti* microfilarial carriers in Boundioba, highlighting the presence of a residual reservoir of this parasite in this village. In Bougoula, no *W. bancrofti* microfilarial carriers were found. More importantly, the six infected adults were from the same area of Boundioba, Zone B. The three zones in each village were virtually selected before the start of the study by geographical location and the associated type of housing (density of houses mainly) that are affecting the number and nature of the potential mosquito breeding sites. Despite the relatively young age of two of the microfilaria-positive subjects (the youngest was nine years old), a lack of MDA drug use by these eight people likely explains their persistent microfilaraemia. Issues related to MDA coverage are due to the lack of adhesion of certain members of endemic communities and to the lack of motivation of the community-based drug distributors. A more detailed assessment is needed, but from our discussions with the microfilaria-positive subjects, it became apparent that none of them participated in all of the MDA campaigns against LF in their community for diverse reasons. Infection was not imported, since they were residents.

Systematic non-compliance with MDA against LF is a real threat (Dicko et al. 2014) even in areas where the 65% coverage is achieved (Chapter 5) and there is a need to correctly assess and address it as suggested by Alexander in several Indian settings (Alexander 2015).

7.4.3 Public health implication of *Mansonella perstans* infection

Despite the non incrimination of *M. perstans* as a human pathogen, several cases of infection (49/426) (Table 7.5) were observed in Boundioba, the village with the observed *W. bancrofti* microfilarial carriers (8/426) (Tables 7.2). Furthermore, the coincidental higher frequency of the infected subjects in Zone B where six of the *W. bancrofti* microfilarial carriers were located may be a sign of less individual protection measure use in that specific area. Both *M. perstans* and *W. bancrofti* are vector borne diseases and although *Culicoides spp* midges are much smaller than mosquitoes, an effective insecticide netting system could help reduce human-vector contact. Given the high prevalence and spread of this parasite in Mali as well as in West Africa in general (Bassene et al. 2015; Coulibaly et al. 2009; Simonsen et al. 2011; Stensgaard et al. 2016), it would be useful to better assess the pathogenicity of this parasite because of the associated high microfilarial loads and their potential immunological implications.

7.4.4 Disparities in *An. gambiae s.l* densities between the villages

The density of *An. gambiae s.l* was significantly higher in Bougoula as compared to Boundioba by all the collection methods. Such differences in vector densities between relatively close villages have been reported in many settings in Mali from entomological surveys (Baber et al. 2010). This may be due to the rainfall abundance and pattern, the temperature and the number and nature of the mosquitoes breeding sites in different

villages. Based on infection status and vectors density, microfilarial carriers should have been more frequent in the village where the *Anopheles* density was higher. Yet, no *W. bancrofti* microfilarial carriers were identified in Bougoula. It is possible that microfilarial carriers do exist, but with low loads that could not be detected with 60 µl of blood or with the current sample size. It is also possible that higher transmission level occurred in Boundioba with lower vector density because of the greater availability of the microfilarial reservoir. Nonetheless, there is a real threat to the success of the NPELF in this area that needs careful follow-up of microfilarial carriers and a tailored sensitization campaign to encourage behavioural change towards better compliance with interventions such as the annual MDA.

7.4.5 *An. gambiae s.l* infection with *W. bancrofti* and/or *P. falciparum*

By comparing the vector infection status with *W. bancrofti* in Boundioba and Bougoula, it appeared that the infection was nil in the first while the second showed two infected pools of *Anopheles* in July 2012. This scenario was opposite to that of human infection in the two villages. Assuming that the transmission level is higher where mosquito density is high, the higher human infection rate in Boundioba is consistent with the hypothesis that the observed microfilarial carriers were MDA non-compliant subjects infected before or early after the MDA initiation. Conversely, the absence of microfilarial carriers in the village of Bougoula where the *Anopheles* density is higher may reflect insufficient sampling. This is based on the fact that *Anopheles gambiae* is a vector that requires a high parasite load to get infected (Boakye et al. 2004; Pichon 2002). These complex scenarios suggest that it is important to take into account entomological parameters such as vector density when deciding to stop MDA in previously hyper endemic areas (Bockarie, Pedersen, et al. 2009; WHO 2013a).

The presence of malaria parasite *P. falciparum* in all month with a significant difference only in August with a higher MIPL in Bougoula. For malaria transmission pattern, there seems to do not be a difference between these two neighbouring villages. Such a difference would be expected when LF transmission was still occurring at a relatively higher rate since the two parasites are transmitted by the same vectors. The actual impact of the co infection with *W. bancrofti* and *P. falciparum* is still not well known because of scarce data. Nonetheless, in co endemic areas, filarial worms could reduce *Plasmodium* infectivity in mosquitoes (Matthew T. Aliota et al. 2011) and vectors' infection rates could most likely be reflecting infection levels with these parasites in the human population in the area (Derua Yahya et al. 2015).

7.4.6 Implications of the geographical distribution of residual infection of lymphatic filariasis

Given the locations of infected individuals and microfilarial carriers in the two villages (Figure 7.3) and the ABR (Table 7.7) at the Zone level, there was no significant difference observed. *Anopheles* density and ABR do not appear to be the main parameters driving the endemicity level of the disease at the village scale. The generated data prevent to speculate about the between zones levels variations that rely on very small numbers. At village level, Boudioba showed a higher infection prevalence for W. bancrofti and a lower vector density.

Mitjà et al (2011) in PNG reached similar conclusions about the lack of a significant relationship between *Anopheles* vector density and the LF endemicity level (Mitjà et al. 2011). Better designs are difficult given the current MDA strategy, but are required for a strong conclusion because the current study as well as the one of Mitjà et al (2011)

in PNG are ecological studies that compare two neighbouring villages (Mitjà et al. 2011). The limits associated with this type of design are that it only allows hypothesis generation (Morgenstern 1995).

A fine scale evaluation of LF endemicity may be misleading if several parameters are not assessed at the same time. It can especially be a concern when an entire health district or EU is assessed for LF transmission by sampling in only one village as a sentinel site. The recommended accompanying control site use (WHO 2011b) is very important in order to report a more representative endemicity level for the EU given the high endemicity level differences between neighbouring villages.

The potential threat of infection transmission after five to six MDA rounds raised by these data, especially in Boundioba where detectable microfilarial carriers were observed, was confirmed by the evidence of recent transmission in this village as demonstrated by the *W. bancrofti* CFA prevalence of 7.6% in children >5 years old using ICT. According to the WHO guidelines (WHO 2011b), this antigen prevalence after the 5th MDA round should be <2% to even start planning the last MDA round and the TAS thereafter in the 6-7 year old children. Specific and adapted elimination measures are needed for this area that still need to be checked with other LF diagnosis tools since ICT has been reported to overestimate the infection prevalence (Dorkenoo et al. 2015). Furthermore, the relatively high reported coverages for the MDA in this area (all > 65%) have never been specifically checked by the national programme and its partners through a coverage monitoring survey.

As previously discussed in this thesis, isolated persistence of LF infection in a limited area may not be a big threat if *An. gambiae s.l* is the main vector. Since these villages are part of the district of Kolondieba, TAS will be conducted by the national programme the EU that is made with the district of Kolondieba plus another district called Kadiolo to check the frequency of the phenomenon in the district and decide whether or not to continue MDA.

7.5 Conclusion

In light of these results, in villages under MDA, the vector density may not necessarily be associated with a higher endemicity level or risk of MDA failure to follow-up assessment in a village undergoing MDA in previously hyper endemic areas where *An. gambiae s.l* are the main vectors of LF. Vector density alone may be misleading because even though it is rapidly perceived due to the nuisance associated to the frequent bites, it may have lost some of its epidemiological importance because of the relatively high coverage with insecticide treated materials in rural endemic areas. Villages with several other risk factors such as Mf positive subjects during the follow-up survey, history of low MDA coverage and baseline high infection rate among others deserve great attention when implementing MDA follow-up and post MDA surveillance strategies.

Chapter 8

Objective 6. Transmission of Lymphatic Filariasis in urban settings

8.1 Introduction

Of the five genera of mosquitoes transmitting LF, *Culex* is the most common worldwide and represents through the *Cx. pipiens* complex (especially *Cx. quinquefasciatus*) the principal vector of nocturnal periodic *W. bancrofti* in urban areas of Asia, Africa, the West Indies, South America, and Micronesia. In Sub-sharan Africa, its area of transmission is limited to the Eastern part of the continent (Bockarie, Pedersen, et al. 2009). The high density of *Cx. quinquefasciatus* in urban areas is due to the high frequency of the specific types of breeding sites this species prefers, such as different types of stagnant water more or less polluted.

In West Africa, *Anopheline* species that are less abundant in urban areas than *Culex spp.* are the main vectors of LF (de Souza et al. 2014). In non-rural areas (urban), e.g cities and towns, communities are characterized by relatively high human population densities and high standards of living in comparison to villages and hamlets in rural areas.

Culex species in Africa have been found not susceptible to local strains of *W. bancrofti* by several authors (Bockarie, Pedersen, et al. 2009; de Souza et al. 2014; WHO 2013a), but experimentally, they have been found susceptible to *W. bancrofti* from India (Kuhlow and Zielke 1978), Sri Lanka (Jayasekera et al. 1980) and Tanzania (Curtis, Kihamia, and Ramji 1981). Based solely on the vector-parasite relationship, *Culex* species are potentially more competent for LF transmission than *Anopheline* mosquitoes, especially when the microfilariae load is low as usually a result of the MDA (Bockarie, Pedersen, et al. 2009; WHO 2013a). MDA is currently the main intervention of the GPELF, for LF elimination by 2020 (WHO 2016a).

Guidelines and thresholds for monitoring the elimination process have been provided.

In many African countries, especially in West Africa, people move frequently from rural areas to urban areas, either temporarily for seasonal work or permanently for economic or security reasons (i.e. lack of employment after the rainy season, civil wars) (de Souza et al. 2014). In Mali, LF elimination activities started in 2005 in the most endemic areas and scaled up to attain 100% geographic coverage in 2009 (Dembélé, Bamani, Dembélé, M. O. N. O. Traoré, et al. 2012). Bamako, the capital city has been found to have an LF antigen prevalence of 1%. Indeed, among the total of 599 people tested (about 100 persons per locality in six localities) using the ICT, nine were positive with 0 positive in two localities, one positive in two localities, and five and two positive in each of the two remaining localities for an overall prevalence of 1.5% (Table 8.1). The localities with positive subjects were in the peripheral areas of the city. Thus, MDA was initiated in the city in 2008.

These localities are also called "quartiers". The quartiers represent the smallest administrative unit in Bamako. A group of geographically closed quartiers constitute a commune. The district of Bamako has a total of six communes adminstered by mayors. Given the frequent movement of the population from rural endemic areas to African cities, the high density of *Culex* species, the very limit antigen prevalence in this area and the three rounds of annual MDA, the likelihood of ongoing transmission in this urban area, where *Anopheline* mosquitoes are rare, is low. The present chapter is designed to determine if *Culex* species are incriminated in *W. bancrofti* transmission in this urban area and to assess the current endemicity level of this neglected tropical disease (NTD).

8.2 Methods

8.2.1 Study site

This study was conducted in Bamako, the capital city of Mali in West Africa. It is the most populated of the 63 districts of the country with an estimated population of 1 810 000 as of 2009. It covers an area of 1420 km^2 and is the place for the main economic activities. There are regular buses and planes to all the neighbouring countries as well as the rural and sub-urban areas of the country. The Niger River forms a very large water body at the middle of the city. The city is located in the Sahel zone and has a tropical wet and dry climate with average high temperature of over 30°C.

The eight selected quartiers are the six NPELF sentinel sites plus two other sites to assess the presence of lymphedema cases (Faladie and Bozola). More details on the eight localities are provided in Appendix 1.

An overall LF prevalence of 1.5% was reported for the district of Bamako by the mapping survey in 2004 using ICT (Table 8.1). The MDA coverage rates in the sentinel sites of Bamako varied from 39% in Sabalibougou in 2008 to 100% in Sokonafing in 2009. With the exception of the first year (2008), all coverage rates were higher than 65% (NPELF, unpublished data) (Appendix 3). Vector collection was performed in only seven of the eight quartiers where human subjects were tested for LF infection because of logistical constraints.

Table 8.1: LF prevalence in the National Programme for Elimination of Lymphatic Filariasis sentinel sites of Bamako for mapping purpose in 2004

Name of the Communes of Bamako	Selected quartiers in the commune	Number tested	Number of positive	Prevalence using ICT in %	[95% CI]
Commune I	Dianguinebougou	100	0	0	[0-2.95]
Commune II	Bakaribougou	100	5	5	[1.85-10.73]
Commune III	Sokonafing	100	0	0	[0-2.95]
Commune IV	Taliko	99	2	2	[0.34-6.51]
Commune V	Sabalibougou	100	1	1	[0.05-4.83]
Commune VI	Niamakoro	100	1	1	[0.05-4.83]
Total		**599**	**9**	**1.5**	[0.74-2.74]

Source: NPELF, appendix of the Mapping survey report

8.2.2 Study design

This was a cross sectional study conducted in 2011 in eight quartiers of Bamako, the capital city of Mali. It comprises an entomological assessment as well as a parasitological assessment of LF in the selected localities of Bamako. The entomological survey was conducted in October to increase the likelihood of capturing more *Anopheles* specimens while *Culex* density is still high in this urban area.

8.2.3 Data collection

8.2.3.1 Entomological data

In Bamako, the collections were performed in October 2011 for seven consecutive days with a day per locality. During the day of collection in each locality, six CDC

light traps (indoor) and six CDC gravid traps (outdoor) were used from 6 pm to 6 am. Each light trap was in a volunteer's room and operated after removing all the other source of light. It was suspendend at about 1.3 meter above the floor of the room, close to the occupant who was using a bednet sometimes. The selected localities are administrative sub-divisions of Bamako, the capital city. At each of the six collection sites, one light trap and one gravid trap were operated 100 meters from each other. The sites were selected in each quartier according to ecological factors such as water bodies, specific breeding sites, and presence of LF clinical signs in local people.

8.2.3.2 Collected mosquitoes processing

The collected mosquitoes were sorted by morphology into distinct species (*Culex spp, An. gambiae s.l, An. funestus,* other *An.* species and *Aedes spp*) and stored in pools of one to 30 according to the collection method and the quartier in 1ml Nunc® Tubes containing absolute alcohol. The next day, they were stored at room temperature in the laboratory before the PCR to detect *W. bancrofti* infection. The PCR technique used was previously described by (Rao et al. 2006) and used in Chapter 6.

8.2.3.3 Parasitological data

From March 21, 2011 to April 27, 2011, blood samples for thick smears were collected from volunteers from eight quartiers of the district of Bamako (Bakaribougou, Bozola, Dialakorodji, Faladiè, Niamakoro, Sabalibougou, Sirakoro dounfing and Taliko). The head of each quartier as well as the local notables were met to obtain community consent for both the entomological and parasitological components of the current study. The research team worked with the local health workers through the whole process. The proposal was part of the NPELF surveillance activities.

Volunteers aged 14 years and above residing in the selected localities were invited to participate to the study. The volunteers, after signing an informed consent form if aged 18 and above or an assent form if < 18 years in adition to the consent form signed by a tutor, underwent a brief health history interview focused on LF. Depending on the pathology detected by the physician, advice was provided as well as assistance or free medicines if needed and available with the research team.

Blood samples were collected by finger prick on site for making three calibrated 20 microliters blood films on three different glass slides between 10 pm and 2 am as well as three blood spots of 20 microliters each on Whatman® filter papers. The following day, the slides were dried on site and sent to the laboratory for 5% Giemsa staining and reading by experienced stereomicroscopists at X40.

The dried blood spots were stored in individual envelopes with a desiccant (silica gel) before their use for *W. bancrofti* CFA detection using the Og4C3 TropBio ELISA (TropBio, Townsville, Australia) as previously described by Gass et al (2012) (Gass et al. 2012). This ELISA method has previously been described in the Chapter 6.

8.2.4 Data management and analysis

Vector infection likelihood and the related 95% confidence intervals were estimated using Poolscreen v2.0 (Katholi and Unnasch 2006). Collected data were analyzed using SPSS version 14 (SPSS Inc., Chicago, IL) and GraphPad Prism software version 5 (GraphPad Software, La Jolla, CA). For proportions comparisons, the Chi^2 test or the Fisher's exact test was used as appropriate.

8.3 Results

The number of subjects enrolled in the eight localities ranged from 81 (Faladie) to 207 (Dialakorodji). Overall, women made up the majority of the sample with 66.27% overall of the 1,002 volunteers enrolled (Table 8.2). Subjects aged 14 to 24 years were more common overall at 42% and the locality of Dialakorodji had the largest sample of 207 volunteers while the smallest one was from the locality of Faladie with 81 volunteers (Table 8.3).

Table 8.2: Distribution of study subjects by gender in the eight study localities of Bamako

Localities	Total enrolled	Women (%)	Men (%)
Bakaribougou	149	63.09	36.91
Bozola	141	85.11	14.89
Dialakorodji	207	70.05	29.95
Faladie	81	43.21	56.79
Niamakoro	88	80.68	19.32
Sabalibougou	100	54	46
Sirakoro dounfing	142	68.31	31.69
Taliko	94	51.06	48.94
Total	**1,002**	**66.27**	**33.73**

Table 8.3: Distribution of the study population by age group in the eight study localities of Bamako

Localities	Total enrolled	Proportion in each age group (in %)					
		14-24	25-34	35-44	45-54	55-64	≥ 65
Bakaribougou	149	56	22	9.4	6	2.7	4
Bozola	141	57	16	11	8.5	3.6	4.3
Dialakorodji	207	38	17	17	14	8.7	5.3
Faladie	**81**	41	19	17	11	9.9	2.5
Niamakoro	88	49	14	23	8	4.6	2.3
Sabalibougou	100	35	23	20	12	6	4
Sirakoro dounfing	142	25	17	11	21	13	13
Taliko	94	33	20	13	11	11	13
Total	**1,002**	**42**	**18**	**15**	**12**	**7.3**	**6.2**

A total of 6,174 *Culex spp* (85.2%), 16 *An. gambiae s.l* (0.2%), 26 *Aedes spp* (0.4%), 858 *Ceratopogonidea* (11.8%) and 170 other insects not identified (2.3%) were collected in October 2011 in the seven study localities of Bamako visited for mosquito collection (Table 8.4). The 6,174 *Culex spp* were pooled into 1 to 30 specimens per pool to make the 252 pools that were tested. The 2 additional pools made with the 16 *Anopheles gambiae s.l* and the 26 *Aedes spp* were also tested. No infected pool was identified using the PCR technique (Table 8.5).

The night time blood thick smear was negative for the 1,002 volunteers tested in the eight selected localities for parasitological studies (Table 8.6). The overall prevalence from the TropBio Og4C3 ELISA was 81.2% among the 1,002 subjects tested. A prevalence of 90% or higher was recorded in all tested localities except Dialakorodji and Bozola that showed 31.4% and 75.2%, respectively (Table 8.7). *M. perstans* was detectable in women (0.75%) and men (0.30%) (Table 8.8). It was observed only in

the subjects older than 24 years with 1.09%, 2.04% and 1.37% respectively in the 25-34 years, 35-44 years and 55-64 years old age groups (Table 8.9).

Table 8.4: Number of flying insects collected per species in the seven visited localities of Bamako in October 2011

Locality	*Culex spp*		*An. gambiae s.l*		*An. funestus*		*Aedes spp*		*Ceratopogonidea*		Other		Total
	N	% [95% CI]	N	% [95% CI]	N	%	N	% [95% CI]	N	% [95% CI]	N	% [95% CI]	N
Faladie	1318	95.6 [94.5-96.6]	2	0.1 [0.02-0.5]	0	0	4	0.3 [0.09-0.7]	34	2.5 [1.7-3.4]	20	1.5 [0.9-2.2]	1,378
Bakaribougou	1235	95.1 [93.9- 96.2]	2	0.2 [0.03-0.5]	0	0	0	0 [0.0-0.2]	42	3.2 [2.4-4.3]	19	1.5 [0.9-2.2]	1,298
Dialakorodji	572	91.1 [88.7-93.1]	4	0.7 [0.2-1.7]	0	0	7	1.1 [0.5-2.2]	16	2.5 [1.5-4.02]	29	4.6 [3.8-6.5]	628
Niamakoro	710	85.7 [83.2-88.1]	1	0.1 [0.0-0.6]	0	0	6	0.7 [0.3-1.5]	90	10.9 [8.9-13.1]	21	2.5 [1.6-3.8]	828
Sabalibougou	1309	75.9 [73.8-77.9]	0	0 [0.0-0.17]	0	0	2	0.1 [0.02-0.4]	400	23.2 [21.2-25.2]	14	0.8 [0.5-1.3]	1,725
Sirakoro	274	47.2 [43.1-51.2]	6	1 [0.42-2.14]	0	0	4	0.7 [0.2-1.7]	254	43.7 [39.7-47.8]	43	7.4 [5.5-9.8]	581
Taliko	756	93.8 [91.9-95.3]	1	0.1 [0.0-0.61]	0	0	3	0.4 [0.09-1.01]	22	2.7 [1.8-4.04]	24	3 [1.96-4.3]	806
Total	**6,174**	**85.2 [84.4-86.03]**	**16**	**0.2 [0.13-0.35]**	**0**	**0**	**26**	**0.4 [0.24-0.52]**	**858**	**11.8 [11.1-12.6]**	**170**	**2.3 [2.02-2.7]**	**7,244**

Sirakoro= Sirakoro dounfing; *spp*= Species

Table 8.5: *Culex spp* infection rates in the seven study localities of Bamako in October 2011

Localities	Total Number collected	Pools size range	Number of pools tested	Number of Positive pools	Maximum likelihood of infection prevalence (95%CI)
Bakaribougou	1,318	1 to 30	53	0	[0%-0.002%]
Dialakorodji	1,235	2 to 30	48	0	[0%-0.002%]
Faladie	572	9 to 30	24	0	[0%-0.003%]
Niamakoro	710	1 to 30	31	0	[0%-0.003%]
Sabalibougou	1,309	1 to 30	49	0	[0%-0.002%]
Sirakoro dounfing	274	2 to 30	16	0	[0%-0.007%]
Taliko	756	1 to 30	31	0	[0%-0.003%]
Total	**6,174**	**1 to 30**	**252**	**0**	**[0%-0.003%]**

Table 8.6: Prevalence of *Wuchereria bancrofti* microfilariae carriage according to the gender in the eight visited localities of Bamako

Localities/quartiers	Total Women sampled	% Women positive	Total Men sampled	% Men positive
Bakaribougou	94	0	55	0
Bozola	120	0	21	0
Dialakorodji	145	0	62	0
Faladie	35	0	46	0
Niamakoro	71	0	17	0
Sabalibougou	54	0	46	0
Sirakoro dounfing	97	0	45	0
Taliko	48	0	46	0
Total	**664**	**0**	**338**	**0**

Table 8.7: *Wuchereria bancrofti infection* prevalence variations in the eight study localities of Bamako using the TropBio Og4C3 ELISA on filter paper dried blood sample

Localities	Total enrolled	Number Tested	Positive		[95% CI]
			Number	(%)	
Bakaribougou	149	147	142	96.6	[90.93-97.97]
Bozola	141	137	103	75.2	[67.44-8187]
Dialakorodji	207	169	53	31.4	[24.70-38.65]
Faladie	81	81	81	100	[96.37-100]
Niamakoro	88	88	85	96.6	[91-99.13]
Sabalibougou	100	95	90	94.7	[88.72-98.04]
Sirakoro dounfing	142	142	135	95.1	[90.49-97.82]
Taliko	94	94	85	90.4	[83.16-95.23]
Total	**1,002**	**953**	**774**	**81.2**	**[78.64-83.60]**

Table 8.8: *Mansonella perstans* infection prevalence variations according to the gender in the eight study localities of Bamako

Localities	Total Women sampled	% Women positive	[95% CI]	Total Men sampled	% Men positive	[95% CI]
Bakaribougou	94	0	[0-3.14]	55	0	[0-5.30]
Bozola	120	0	[0-2.47]	21	0	[0-13.29]
Dialakorodji	145	2.07	[0.53-5.53]	62	1.61	[0.08-7.70]
Faladie	35	0	[0-8.2]	46	0	[0-6.31]
Niamakoro	71	0	[0-4.13]	17	0	[0-16.16]
Sabalibougou	54	0	[0-5.40]	45	0	[0-6.44]
Sirakoro dounfing	97	1.03	[0.05-4.98]	45	0	[0-6.44]
Taliko	48	2.08	[0.10-9.85]	46	0	[0-6.31]
Total	**664**	**0.75**	**[0.28-1.66]**	**337**	**0.3**	**[0.01-1.45]**

Table 8.9: *Mansonella perstans* infection prevalence variations according to the age groups in the different localities of Bamako

Age groups/Years	14-24		25-34		35-44		45-54		55-64		≥65	
Localities	**N**	**%pos**	**N**	**%pos**	**N**	**%pos**	**N**	**%pos**	**N**	**%pos**	**N**	**%pos**
Bakaribougou	83	0	33	0	14	0	9	0	4	0	6	0
Bozola	80	0	23	0	15	0	12	0	5	0	6	0
Dialakorodji	79	0	35	2.86	36	5.56	28	0	18	5.56	11	0
Faladie	33	0	15	0	14	0	9	0	8	0	2	0
Niamakoro	43	0	12	0	20	0	7	0	4	0	2	0
Sabalibougou	34	0	23	0	20	0	12	0	6	0	4	0
Sirakoro dounfing	35	0	24	0	16	6.25	30	0	18	0	19	0
Taliko	31	0	19	5.26	12	0	10	0	10	0	12	0
Total	**418**	**0**	**184**	**1.09**	**147**	**2.04**	**117**	**0**	**73**	**1.37**	**62**	**0**

N= number of volunteers ; %pos= % positive

8.4 Discussion

In Mali, as part of the LF elimination process, MDA was instituted in 2005 starting in the region of Sikasso that had the highest overall infection rate of 18.6% in 2004 (Dembélé, Bamani, Dembélé, M. O. N. O. Traoré, et al. 2012). According to the mapping survey data, all the eight administrative regions of the country needed the ALB/IVER combination for interrupting LF transmission.

LF transmission is mainly rural in Mali and in the other West African countries. This is primarily due to the abundance of the main LF vector – *An. gambiae s.l* in those areas as well as an important reservoir of parasite constituted by the infected and microfilarial carriers in rural areas.

In Mali, these Mf carriers move to the big cities and rice cultivation areas of the country for seasonal work since the main activities in the rural areas are agricultural based on maize, millet and cotton. These crops are grown during the rainy season that extends from July to December. From January to June, most young people leave their villages for the cities to find temporary jobs. These rural people's movements are directed to the peripheral areas where appartments' renting is cheaper, opportunities for room sharing and the likelihood of finding people from one's original village higher. These factors place the peripheral localities at higher risk of transmission since the newly moved rural people may bring more reservoirs for the local vectors. Another observation in line with this rural exodus is the *M. perstans* infection rate in some of the localities where people come mainly from the district of Kolokani for seasonal work (personal communications with the chief health officer of Dialakorodji, October 2011). Midges of *Culicoides* genus transmit this parasite especially in rural areas

(Bassene et al. 2015; Noireau, Itoua, and Carme 1990). No *Culicoides* were collected during our study in Bamako despite a high density captured using the same traps in Kolokani, the site of an ongoing PhD thesis study on these vectors (Diallo A, unpublished data). Furthermore, the fact that there are very few infected individuals and only in two localities, corroborates the imported origin of *M. perstans* infections in Bamako.

Our data provides no evidence for LF transmission in Bamako, given the rarity of the microfilarial carriers (0 positive over more than 1000 subjects of 14 years old and above tested) and the failure to detect any parasite DNA in 252 pools of *Culex* processed. MDA started in Bamako in 2008 and by 2011, three rounds had already occurred with coverage rates >65% every treatment year except the first year (Appendix 2). Whether the current lack of microfilarial carriers is due to MDA or because transmission was absent even before MDA is not possible to determine from our current data or from the national programme's baseline assessment data that were generated using ICT alone. It remains clear that the disease does not seem to be a public health problem in Bamako and that transmission is not occurring given the low frequency of the *An. gambiae* and *funestus* complexes, the only known vectors of LF in Mali (Toure 1979), and the non-infection status of *Culex spp*. The observed very high prevalence with the ELISA tests are higher than all the prevalence reported in Mali even before the MDA initiation (Dembélé, Bamani, Dembélé, M. O. Traoré, et al. 2012). These rates close to 100% infection rate were due to the storage condition or a contamination of our samples since a second batch of tests were used on the same dried bllod spots and similar results were obtained. It should be noted that these Og4C3 kits were successfully used on the samples of the chapter 7 of this thesis.

An. gambiae s.l abundance was relatively low in the study sites but the total numbers of mosquitoes collected was substantial and would allow detection of infection rates as low as one infected female/1000 tested with a 63% probability when approximately a total of 1000 mosquitoes are tested (Katholi and Unnasch 2006). We actually processed 6,174 *Culex spp*. Such a low *W. bancrofti* microfilarial prevalence could be expected after three MDA in endemic areas if initial prevalence was low (Farid et al. 2007; Goodman et al. 2003).

In Bamako, given the limitation pattern that characterizes the *Culex spp* and increases its ability to take up microfilariae and bring them to the infective stage even if the microfilarial load is very low, such number of vectors is strongly evocative of a lack of transmission. This is due to the lack or very rare infected mosquito-human host contact as demonstrated by the current xenomonitoring findings. It has been reported that even mosquitoes that feed on a microfilarial carrier can be detected as positive because of *W. bancrofti* DNA in the ingested blood (Rao et al. 2006). Moreover, the absence of microfilariae carriers in the tested samples highlights the non-availability or rarity of an infection reservoir for the local mosquitoes to sustain transmission in Bamako.

In terms of vector control, it is very important to identify and characterize the vector involved in LF transmission in all endemic areas and to implement, if required, a tailor-made vector control strategy based on the generated information. *Culex spp* is the most widely distributed LF vector but, in Africa, its transmission area seems to be limited to the Eastern part of the continent (Bøgh et al. 1998; Maxwell et al. 1990).

Clinical signs of LF, especially the different stages of lymphedema were checked among the subjects involved in the blood collection. A total of three cases of elephantiasis were observed in one family in the locality of Faladie. This, motivated the inclusion of other families in the vicinity of that family into the mosquito collection sites as well as the sensitization for parasitological assessment. The remaining localities were the ones that the NPELF is using as sentinel sites (Dembele 2004, unpublished data). In that family located in Faladie, the father, a 68-year-old man originally from the region of Koulikoro was infected in his native village. When he moved to Bamako for professional reasons, he already had early stage lymphedema, according to the discussion with him. About 15 years later, his two daughters, but not his son or his wife, started to have the same signs that had reached the stage of elephantiasis with dermatological complications when the current study was taking place. This suggests the possibility of very focal urban transmission of LF in Bamako.

The use of the dried blood spots was problematic in obtaining valid results with the TropBio ELISA for *W. bancrofti* CFA Og4C3 (TropBio, Townsville, Australia). The malfunction was reported to the manufacturer, who replaced the kits. When the tests were repeated with the new batch of tests, the same trend of all samples being positive was observed. Since the testing was performed in our laboratory with good quality control (Das et al. 2012), we assume that the failure may be due to the quality of the kits or to the sample storage quality between the collection and processing periods. This needs to be further investigated. Until these issues are clarified, we suggest that dried blood spot use for CFA detection with the TropBio kits (TropBio, Townsville, Australia) be done with caution.

After three rounds of MDA, undertaking a TAS with the RPRG's authorization in this urban area may be advisable, especially if funding constraints exists, so that money can be reallocated to other areas where transmission is still on-going. Even if funds are reallocated, surveillance should be continued as in any endemic or at risk of transmission area in order to detect early resurgence or appearance of LF.

8.5 Conclusion

There is no evidence from the collected data of active LF transmission or an endemicity level that may require an intervention in Bamako. The few *M. perstans* microfilarial carriers identified in two of the eight visited localities provide information about the endemic areas that people living in Bamako have come from.

Chapter 9

General discussion and recommendations

9.1 Principal findings and novelty of data

The main findings of this thesis was the details on the short and long term capacity of West African LF vectors (*An. gambiae s.l)* to transmit LF in various conditions linked with the current MDA in hyper endemic areas. It also assessed the focal nature of LF transmission at the intra village level and compared the commonly used HLC to two alternative mosquito collection methods.

MDA is the main pillar of the LF elimination strategy for the GPELF and is on-going or about to start in many endemic countries. The elimination process is underway but many gaps need to be filled to end up with the best tools and strategies for its acceleration and sustainability. This work generated data on the validity of the current surveillance strategy that still needs more accurate and standardized tools and endpoints. This thesis reports for the first time the outcome of up to five years post-MDA annual assessment of *W. bancrofti* transmission using both entomological and parasitological data in an *Anopheles* transmission area where ALB/IVER is the recommended drug regimen and *An. gambiae s.l* the main vector for LF and malaria. These features are found mainly in the Western part of Africa. The long term impact of the MDA was more assessed in *Culex* transmission areas in LF endemic areas.

In the Sikasso region, constratsting pattern were observed in the two study areas that were in 2 different districts, as related to the MDA efficacy. In the sentinel area made up of six neighbouring villages, seven MDA rounds with the ALB/IVER regimen were successful not only in stopping LF transmission but also in sustaining it for up to five years after the last MDA. In contrast, another hyper endemic area (two neighbouring

villages treated by the NPELF) in a neighbouring district of Kolondieba failed at the MDA follow-up survey (pre-TAS) after the sixth and seventh MDA rounds.

The reasons for these different efficacies of MDA in two hyper endemic areas were discussed in this thesis. The six sentinel villages were treated as part of a research study with close supervision and observation of the different activities while the two villages were treated and supervised as part of the NPELF activities that are less closely followed due to the extent of the programme intervention areas. In Mali, all eight administrative regions are endemic for LF and need MDA. Of note, the failure in the two villages has been detected using the ICT only which has been found to overestimate the infection rate in children. Dorkenoo et al (2015) found that the 13 ICT positive subjects were negative for nocturnal microfilaraemia and Og4C3 ELISA (Dorkenoo et al. 2015). More recently, in 2015, Wanji et al made a similar observation (Wanji et al. 2015). Thus, the ICT results should be checked and taken with caution by always thinking about the overestimation it may bring when it comes to take public health decisions. For instance, decision to continue mass drug administration (TAS failure) or to stop it (TAS passing successfully) after five or more treatment rounds would greatly benefit from the results of a set of available tests plus the experts' opinions until one test is accepted by all the stakeholders as the suitable one for TAS based on its results.

Additionally, the research study settings are usually different from the public health intervention areas such as evaluation or implementation units. These differences can be in terms of population size and geographically covered areas, population overall awareness about the issue of interest, the differences between the implementors of the same intervention such as the mass drug administration and the way the data are

collected. Any comparison especially if it is aimed at influencing the policy and behaviour of the concerned population should extensively take in account these potential differences for sound decision making.

To confirm the ICT overestimation in children, the Wb123 and the Og4C3 ELISA were used in the six sentinel villages. The same tests are needed to assess areas where ICT finds >2% infection rate in 6-7 years old. Such overestimated prevalence may lead to further epidemiological assessment in the vicinity of the positive children's houses as well as two additional MDA rounds before a new TAS. The burden of these additional studies in terms of funds, logistical and health staff time consumption is high enough to push stakeholders to foster the validation of new field adapted POC usable diagnosis methods. In *Anopheles* transmission areas, it has been observed that focal transmission can still exist without being a real threat for a transmission re-emergence due to the vector's lower capacity to transmit when parasite density is low and facilitation is the transmission pattern.

Currently, the lack of recommendations for assessment of the adult microfilaraemia rate during the surveillance period seems to be a weakness, since it has been observed here that only adults were microfilarial carriers and may provide the mosquito vectors with a reservoir of parasites for transmission (chapter 5). This group could be added to the TAS sample to improve the quality of the collected information since the prevalence and geographic distribution of microfilarial carriers in the village or area can be insightful in term of transmission risk and the need for additional urgent measures. This is especially relevant in areas that were previously hyper endemic for LF with environmental conditions suitable for mosquito vectors. A recent ad hoc

meeting report of WHO experts in 2016 provided with insights regarding the management of localized hotspot that could threaten the elimination process when the EU passes the TAS. Since most NTD funding agencies only support activities that are in line with WHO guidelines, clear guidelines and detailed directions accoding to different scenarios are needed to sustainably tackle the threat represented by by microfilaremiae positive adults in areas that had a high baseline LF prevalence.

Additionally, this thesis showed very promising results for the ITTC (chapter 6), a human baited trap that had strongly correlated *Anopheles* yields with the HLC overall as well as monthly in two villages with significantly different *Anopheles* densities. The two methods produced comparable infection rates for *P. falciparum* in collected *An. gambiae s.l.* This human baited trap is not associated with any ethical concerns in term of potential human-vector contact. Its yields for *An. gambiae s.l* are less than the HLC ones but that can be overcome by increasing the number of traps. The other challenges of this trap are the cost of acquisition and its bulkiness that make it difficult to use in different places over the collection areas. These two issues could also be compensated by the modification of the tent using locally available materials and weather adapted lighter weight fabrics. The possibility of using the tent over a relatively longer period and the possibility to use it as a community-based mosquito collection method make it more cost effective than the HLC (Sikaala et al. 2014). It has no associated collector specific potential impact (dexterity, alertness, odour, and rapidity among other) (Wong et al. 2013).

Finally, the generated data in chapter 7 allow to confidently consider the focal nature of the LF transmission foci after > five MDA rounds. The GIS tools were useful in that

matter and should be increasingly used by LF elimination programmes' officers since the increase of LF infection in children can sometimes be in one or two families and the operational attitude should be different than in the case of a non-clustered spread of the infection.

9.2 Limitations and proposed method of improvement

As part of the post MDA surveillance, the most informative method for mosquito pool screening for filarial infection and or infectivity, the RT-PCR assay developed by Laney et al in 2010. LF vectors' infection rate is a sign of the presence of one or more larvae of *W. bancrofti* at the stage L1 to L2 (non infective stages) while the infectivity rate is epidemiologically more important since it says that the pool had at least one infective stage *W. bancrofti* larve (L3). The L3 carriage can be considered as a risk of transmission and also a sign of vector ability to pick up and bring the ingested microfilariae to the infective stage. has been used for the processing of additional mosquito collections done by PSC in 2012. Unfortunately, this technique could not be used to process the samples collected in the Kolondieba district for the collection methods comparison and the LF indices assessment in the two study villages in that district because of the expected absence or very low *W. bancrofti* infection rate and the fact that extracting the RNA would impede any assessment of the malaria parasite *P. falciparum* infection and co infection rates. Collecting more mosquitoes and doing both the DNA extraction for the malaria assessment in vector as well as the RNA extraction for determining the infectivity level of the mosquito pools seemed a better option. This is why we initially used RNALater solution for mosquito samples storage.

The number of mosquito pools infected with *W. bancrofti* was so low that many deductions cannot be reasonably made from it (2 pools over 350 made with 6,329 female *Anopheles* tested overall). One of the two pools found positive in July was co infected with *P. falciparum*, which was quite commonly infecting the pools of 1-30 females with monthly infection rates in 2011 ranging from 0% in August to 6.9% in November (Chapter 6). This raises another reason for control in this area because with the high vector density, such infection rates for *P. falciparum* can cause a high malaria transmission rate if no intervention is done. An assessment of how a good integration can be done between the LF and malaria control programme will be useful for the fight against these two parasitic diseases transmitted by the same vectors.

Additionally, instead of pooling the mosquitoes per collection method in the two villages of Kolondieba, they could be pooled per village zone (each of the two villages was actually divided into three zones at the beginning) and per collection method. This was not part of the study objectives but could have provided information on the zone with the infected mosquitoes. Nonetheless, this would have unexpected financial costs for processing more pools which would then be of much smaller sizes.

A study in a larger previously hyper endemic area that underwent ≥ 5 MDA rounds would be insightful about the mosquito infection/infectivity distribution and how it relates to infected human distribution across the area and finally allow an assessment of the risk for the neighbouring villages.

The same study could be conducted in areas where LF endemicity is found higher than the threshold after ≥ five MDA using ICT and the other available LF diagnosis

methods to better quantify the overestimation of the ICT and eventually identify the best method to recommend for the TAS. In such settings, the importance of the migration (number of people and frequency) between the concerned area and surrounding areas, the MDA coverage rates and drug distributors' motivation level would also be important aspects to investigate using a mixed quantitative and qualitative approach.

It is also crucial to reassess at least the infection and microfilarial prevalence in neighbouring villages within *Anopheles* flight range (~2 km according to Baber et al (2010) in Mali) (Baber et al. 2010) to see extent of the transmission resurgence and plan the needed MDA accordingly in the concerned area. A study on vector specific species capacity to transmit should be initiated to investigate *An. gambiae s.l* capacity to transmit according to the limitation model. This has previously been reported in Ghana (Amuzu et al. 2010). If confirmed in many areas, this would oblige review of all of the GPELF's previous estimations in term of thresholds and elimination strategies in *Anopheles* transmission areas.

Finally, another concern is the fact that after five years without any MDA, in case of transmission re-emergence, the population compliance with MDA is unknown, although the adverse events frequencies have been shown to be significantly lower at that time. Those questions need urgent responses and actions should be taken to avoid a failure of the whole process after important efforts by the WHO and its partners.

9.3 Recommendations from these data

(i) Further studies on Wb123 should be conducted in other endemic areas at different stages of the LF elimination process to ascertain its suitability for TAS in order to determine the best test or group of tests that can more reliably be used in order to detect early a resurgence of LF transmission;

(ii) if an EU fails the TAS, a follow-up survey should be initiated to assess the of the positive children's villages and neighbourhood using not only the ICT test but also other LF diagnosis methods such as the Og4C3 and the Wb123 antibody test;

(iii) Reference laboratory for LF surveillance after MDA stopping should be instituted in endemic countries and also for group of countries in the same sub-region in order to insure the long term follow up that would be required to verify elimination;

(iv) A safe and sustainable xenomonitoring should be planned and undertaken based on locally acceptable and community-based human baited method for mosquito collection; this could be done using a more user friendly format of the Ifakara tent trap type C (after more standardization and validation studies);

(v) MDA usefulness should be established in African big cities where there is lack of evidence of transmission using a criteria based on not only a prevalence of antigenemia ≥ 1% but also on vector fauna and other environmental characteristics. A standardized method specifically for high-density of humans population should be developed to identify potential hotspots that should be targeted as risk areas to help save resources and funds.

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Publications

During my PhD research activities and writing up, I published or contributed to 20 peer reviewed publications.

The following three of them were from my thesis data. A copy of each of them is inserted in the appendices section of the thesis:

1. **Related to Chapter 5**

   Coulibaly YI, Coulibaly SY, Dolo H, Konate S, Diallo AA, Doumbia SS, Soumaoro L, Coulibaly ME, Dicko I, Sangare MB, Dembele B, Sangare M, Dembele M, Touré YT, Kelly-Hope L, Polman K, Kyelem D, Traore SF, Bockarie MJ, Klion AD and Nutman TB. Dynamics of antigenemia and transmission intensity of Wuchereria bancrofti following cessation of mass drug administration in a formerly highly endemic region of Mali. Parasit Vectors. 2016 Dec 3; 9(1):628.

2. **Related to Chapter 4**

   Coulibaly YI, Dembele B, Diallo AA, Konaté S, Dolo H, Coulibaly SY, Doumbia SS, Soumaoro L, Coulibaly ME, Bockarie MJ, Molyneux D, Nutman TB, Klion AD, Toure YT, Traore SF. The Impact of Six Annual Rounds of Mass Drug Administration on Wuchereria bancrofti Infections in Humans and in Mosquitoes in Mali. Am J Trop Med Hyg. 2015 Aug 5;93(2):356-60.

3. **Related to Chapter 3**

   Coulibaly YI, Dembele B, Diallo AA, Kristensen S, Konate S, Dolo H, Dicko I, Sangare MB, Keita F, Boatin BA, Traore AK, Nutman TB, Klion AD, Touré YT, Traore SF. Wuchereria bancrofti transmission pattern in southern Mali prior to and following the institution of mass drug administration. Parasit Vectors. 2013 Aug 28; 6(1): 247.

The others are:

4. Traore B, Oliveira F, Faye O, Dicko A, Coulibaly CA, Sissoko IM, Sibiry S, Sogoba N, Sangare MB, **Coulibaly YI**, Traore P, Traore SF, Anderson JM, Keita S,Valenzuela JG, Kamhawi S, Doumbia S. Prevalence of Cutaneous Leishmaniasis in Districts of High and Low Endemicity in Mali. PLoS Negl Trop Dis. 2016 Nov 29;10(11):e0005141. doi: 10.1371/journal.pntd.0005141.
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Appendices

Appendix 1. Brief description of the study localities of Bamako

Communes	Selected localities	Brief description of the locality	Reason of the locality choice
II	Bakaribougou and Bozola	The Communes II, bounded on the east by the backwater of Korofina, on the west by the hill of Point G, on the north by the northern limit of the District of Bamako and on the south by the bed of the River Niger, it covers 16.81 km² and has a population of 160 680 inhabitants. The town has eleven localities including Bakaribougou (N = 12° 39'204'' and W = 007° 58' 466'') and Bozola (N = 12° 38'443'' and W = 007° 59' 393'').	Bakaribougou is a sentinel site for the LF elimination programme while Bozola is at the border of River Niger and has more *Culex* breeding sites than any other locality of Bamako
III	Sirakoro dounfing	The town III is limited to the north by the Kati, east by the People of the boulevard that separates it from the Commune II, to the south by the portion of the Niger River and to the west by the backwater of Farako Lido, it covers an area of 23 km². Its population is 119 287 inhabitants. Twenty localities comprise this Commune including the recently added villages of Koulouninko and Sirakoro dounfing (N = 12°41'360''and W = 008° 03' 145'').	Sirakoro dounfing is a rural area recently added to the District because of its proximity and the quickly expanding population
IV	Taliko	The Commune IV is bounded on the east by the Commune III, north and west by Kati another district of the country and south by the left bank of the Niger River. It covers an area of 36,768 hectares with a population of more 200 000 inhabitants in 2001. The town IV consists of eight localities including Taliko (N = 12°37'808'' and W = 008° 03' 209'').	Taliko is a sentinel site for the LF elimination programme

V	Sabalibougou	The Commune V covers an area of 41 square kilometers and has 249,727 inhabitants. It is bounded to the north by the Niger River, the south by the airport area and the town of Kalanban-Coro, on the east by the Commune VI and Niger. It consists of eight districts including Sabalibougou (N = 12^{o} 35'596'' and W = 008^{o} 00' 159'').	Sabalibougou is a sentinel site for the LF elimination programme
VI	Faladie and Niamakoro	Commune VI with an area of 8,882 hectares is the largest district of Bamako. Its population is about 600 000 inhabitants. It consists of ten localities including Faladie (N = 12^{o} 35'565'' W = 007^{o} 57' 498'') and Niamakoro (N = 12^{o} 35'119'' and W = 007^{o} 58' 399'').	Niamakoro is a sentinel site for the LF elimination programme while three cases of lymphedema were observed
Kati	Dialakorodji	The district of Kati is the closest to that of Bamako and Dialakorodji (N = 12^{o}43'202'' and W = 007^{o} 58' 053'') is bordering sub-urban area where people from Kolokani an endemic area are very common. Kolokani and Kati are both from the Koulikoro region.	Dialakorodji is a sentinel site for the LF elimination programme and is the favorite destination for internal migrants from the district of Kolokani

Appendix 2. Lymphatic filariasis mass drug administration coverage rates in the sentinel sites of Bamako from 2008 to 2011

Communes	Locality	2008	2009	2010	2011
Commune I	Dianguinebougou	61	110	93	77
Commune II	Bakarybougou	47	100	110	89
Commune III	Sokonafing	83	**117**	99	82
Commune IV	Talico	54	103	96	78
Commune V	Sabalibougou	**39**	91	97	80
Commune VI	Niamakoro	77	112	107	89

Source: National Lymphatic filariasis elimination programme's annual MDA reports

Appendix 3. Chapter 3 related published paper in Parasites and Vectors

Wuchereria bancrofti transmission pattern in southern Mali prior to and following the institution of mass drug administration

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Abstract

Background

The Global Programme to Eliminate Lymphatic Filariasis (GPELF) was launched in 2000 with the goal of stopping transmission of lymphatic filariasis (LF) through yearly mass drug administration (MDA). Although preliminary surveys of the human population in Mali suggested that *Wuchereria bancrofti* infection was highly endemic in the Sikasso district, baseline entomological data were required to confirm high levels of transmission prior to the selection of villages in this region for a study of the impact of MDA on transmission of LF by anopheline vectors.

Methods

W. bancrofti transmission was assessed in 2001 (pre-MDA) and 2002 (post-MDA) in the Central District of Sikasso in southern Mali by dissection of *Anopheles* mosquitoes caught using the human landing catch (HLC) method. The relative frequencies and molecular forms of *An. gambiae* complex were determined.

Results

The majority (86%) of the anopheline vectors captured were identified as *An. gambiae* complex, and these accounted for >90% of the entomological inoculation rate (EIR)

during both years of the study. There was a dramatic decrease in the number of *An. gambiae* complex mosquitoes captured and in the *An. gambiae* complex infectivity rates following MDA, accounting for the observed decrease in EIR in 2002 (from 12.55 to 3.79 infective bites per person during the transmission season). *An. funestus* complex mosquitoes were responsible for a low level of transmission, which was similar during both years of the study (1.2 infective bites per person during the transmission season in 2001 and 1.03 in 2002).

Conclusions

Based on the entomological data from this study, the district of Sikasso was confirmed as an area of high *W. bancrofti* transmission. This led to the selection of this area for a multi-national study on the effects of MDA on LF transmission by anopheline vectors. Comparison of vector transmission parameters prior to and immediately following the first round of MDA demonstrated a significant decrease in overall transmission. Importantly, the dramatic variability in EIR over the transmission season suggests that the efficacy of MDA can be maximized by delivering drug at the beginning of the rainy season (just prior to the peak of transmission).

Background

Lymphatic filariasis (LF) is a chronic debilitating infection caused by the mosquito-borne filarial nematodes, *Wuchereria bancrofti* (*W. bancrofti*), *Brugia malayi* and *Brugia timori*. Worldwide, more than one billion people are at risk of infection, among which 120 million are already infected, the majority of whom are in India, with an estimated 49.2% of the infection burden, followed by sub-Saharan Africa with 34.1% [1]. *W. bancrofti* is responsible for approximately 90% of LF cases worldwide and all of the cases in sub-Saharan Africa, where the most common vectors are *An. gambiae*

and *An. funestus* complexes [2]. Forty-three million people are officially recognized as being disabled due to LF and millions more suffer from social and psychological problems [3]. Nevertheless, the socio-economic burden of LF is underestimated in many endemic areas that are among the poorest of the world [4].

In Mali, although the public health importance of LF was noted as early as the 1970′s [2], the prevalence and distribution were not studied again until 2002, when the National Lymphatic Filariasis Elimination Program (NLFEP) provided the first countrywide LF map. Based on antigen testing using ICT cards, all 8 administrative regions of Mali were shown to be endemic for LF with an overall prevalence of 7.07%, ranging from 1% in the north to 18.6% in the south. In anticipation of the launch of NLFEP activities in Mali, a pilot study of the impact of mass drug administration (MDA) with albendazole and ivermectin on *W. bancrofti* transmission was initiated in collaboration with the World Health Organization (WHO) in Ghana, Mali, and Nigeria. The present study was designed to provide baseline data on vector transmission in this highly endemic region of Mali and to assess the effects of the first round of MDA.

Study site identification and characterization

The study was initiated in the Sikasso district in southern Mali prior to the introduction of MDA for the elimination of LF. This area was historically known to be endemic for *W. bancrofti,* and mapping surveys performed by the National Program for the Elimination of Lymphatic Filariasis confirmed a high prevalence of circulating filarial antigen (CFA) positivity (as assessed by ICT card testing of 50-100 individuals/village) in the village of Dozanso and a neighboring village in 2001 (unpublished data). Additional ICT card surveys were subsequently carried out in the

larger villages surrounding Dozanso in 2001 and led to the selection of 6 high prevalence villages (Dozanso, Gondaga Missasso, N'Torla, Niantansso and Zanadougou) for baseline entomological studies.

The study villages are comparable in terms of socio-cultural indicators, health care seeking behavior and disease perception. The distance between the villages and the community health care center of Kolokoba ranges from 6 to 15 km (mean 9.5 km), occupied by cotton fields, backwaters, and trees typical of the dense Sudan Savannah vegetation. Rainfall in this region ranges between 1200 and 1500 mm per year, with a rainy season that extends from July to December. Due to the high levels of transmission documented during the first year of the study, yearly MDA was instituted in the 6 study villages in June 2002, one month prior to the second entomological survey.

Study population

A complete census, including the name, age, sex and profession of each inhabitant, was performed in all 6 villages. All dwellings were recorded and assigned an identification number. A global positioning system device (GPS) was used to produce basic maps of the locations of the 6 villages within the Central District of Sikasso. The total population of the 6 study villages was 3,681 in 2001, consisting primarily of farmers, whose main occupations are agriculture (cotton, maize, millet and peanut) and domestic animal breeding. The ICT card surveys carried out in 2001 revealed CFA prevalences varying from 81.8% in Niantanso (165/202) to 24.6% in Zanadougou (50/202) (Table 1). The prevalence of microfilaremia was assessed in 2002 (prior to the initiation of MDA) by examination of 3 slides of 20 µl of night blood/subject, and ranged from 40% in Dozanso (48/120) to 13.8% in N'Torla (27/196).

Table 1
Characteristics of the study population prior to MDA

Villages	Total tested	Male	Female	Mf positive	CAg positive
		%	%	%	%
Dozanso	120	54.2	45.8	40	61.7
Missasso	207	35.3	64.7	20.3	36.9
Gondaga	212	45.8	54.2	15.1	43.4
Niantanso	202	42.1	57.9	29.7	81.8
N'Torla	196	50.5	49.5	13.8	40.3
Zanadougou	202	30.7	69.3	17.3	24.6

Mf Microfilaremia, *CAg* Circulating filarial antigen.

A collective village-wide oral consent was obtained from the villages' elders, and all study participants signed individual written informed consent. The study protocol and consent forms were approved by both the Institutional Review Board (IRB) of the World Health Organization/Tropical Diseases Research (WHO/TDR) and the ethics committee of the Faculty of Medicine, University of Bamako, Mali.

Study design

This was a longitudinal study during which monthly entomological surveys were performed in 6 study villages from July to December in 2001 (prior to initation of MDA with albendazole and ivermectin) and in 2002 (one month after the first MDA).

Laboratory analysis

Entomological surveys were performed 12 days per month (2 days/village/month) by the same team. Mosquitoes were collected by two trained field personnel in each room of four different houses in each village using the HLC method. One collection team worked from 6:00 pm to midnight and the second from midnight to 6:00 am.

Mosquitoes were captured using a Colluzi and Petrarca type mouth aspirator connected to a paper cup as the storage container. A supervisor retrieved the containers at two-hour intervals. The captured mosquitoes were kept overnight at ambient temperature in a paper cup under a damp cloth and dissected the following morning.

Mosquitoes were sorted morphologically for species identification (*An. gambiae* and *An. funestus* complexes). Some *An. gambiae* complex specimens were processed by polymerase chain reaction (PCR) method to distinguish between the 2 members of the complex (*An. arabiensis* and *An. gambiae ss*). The *An. gambiae ss* were further processed by PCR to identify the molecular forms, M and S, as described by Favia *et al.*[5,6]. The head, thorax and abdomen were dissected separately for each mosquito and recovered parasite larvae were categorized into L1, L2 or L3 stages.

Entomologic parameters assessed included infection rate, infectivity rate, human biting rate (HBR) and entomological inoculation rate (EIR) and were calculated as previously described [5,7]:

- Infection rate: proportion of mosquitoes found infected after dissection with any *W. bancrofti* larval stage (L1–L3).
- Infectivity rate: proportion of mosquitoes found infected with one or more infective larvae (L3).
- Human biting rate (HBR): number of mosquitoes caught during the HLC × 30/(total number of collectors used per collection × number of collections in the month).
- Entomological inoculation rate (EIR): HBR × infectivity rate. The results of the monthly HBR (from all night HLC) multiplied by the *W. bancrofti* infectivity rate for

a given species give an estimate of the number of infective bites of *W. bancrofti* received per human per month.

Data management and analysis

Data were analyzed using SPSS version 14 (Statistical Package for Social Sciences) (SPSS Inc., Chicago, IL) and Prism V5.0 (GraphPad Software). The Chi square test or the Fisher's exact test was used as appropriate to compare proportions. The confidence level was set at 95% for all statistical tests.

Results

Monthly variations in vector densities

A total of 23,265 and 12,986 mosquitoes were collected in the 6 villages of the district of Sikasso from July to December in 2001 and 2002, respectively. Overall, *An. gambiae* complex (20,957 in 2001 and 11,190 in 2002) were more frequently captured than *An. funestus* complex (2,308 in 2001 and 1,796 in 2002) among the active vector fauna. At the beginning of the transmission season, *An. gambiae* complex was collected more frequently than *An. funestus* complex (158 fold more in July 2001 and 138 fold more in August 2002). This trend diminished towards the end of the transmission season with equal collection of both species in December 2001 (Table 2) and only a two-fold increase in collection of *An. gambiae* complex in November and December 2002 (Table 3).

Table 2
Monthly variation of the entomological parameters for the transmission of lymphatic filariasis in six villages of the District of Sikasso in 2001

***Anopheles funestus* complex**						
Month	**Collected**	**Dissected**	**Infected (%)**	**Infective (%)**	**HBR**	**EIR**
July	25	25	0 (0)	0 (0)	4	0
Aug	33	33	0 (0)	0 (0)	5	0
Sep	278	148	4 (2.7)	2 (1.4)	43	0.6
Oct	1402	789	51 (6.5)	15 (1.9)	219	4.2
Nov	514	432	17 (3.9)	13 (3)	80	2.4
Dec	56	44	0 (0)	0 (0)	9	0
Total	**2,308**	**1471**	**72 (4.9)**	**30 (2)**	**60**	**1.2**
***Anopheles gambiae* complex**						
Month	**Collected**	**Dissected**	**Infected (%)**	**Infective (%)**	**HBR**	**EIR**
July	3960	3959	123 (3.1)	88 (2.2)	618.75	13.75
Aug	4971	4948	137 (2.8)	91 (1.8)	776.72	14.28
Sep	9096	4708	211 (4.5)	120 (2.5)	1421.25	35.53
Oct	2320	2005	137 (6.8)	61 (3)	362.5	10.9
Nov	544	544	36 (6.6)	12 (2.2)	85	1.88
Dec	66	66	2 (3)	0 (0)	10.31	0
Total	20,957	16,230	646 (4)	**372 (2.3)**	545.76	12.55

N Number, *%* Percent, *HBR* Monthly biting rate, *EIR* Entomological inoculation rate.

Table 3
Monthly variation in the entomological parameters related to the transmission of lymphatic filariasis in six villages of the District of Sikasso in 2002

***Anopheles funestus* complex**						
Month	**Collected**	**Dissected**	**Infected (%)**	**Infective (%)**	**HBR**	**EIR**
July	14	14	2 (14.3)	0 (0)	2.2	0
Aug	18	18	2 (11.1)	0 (0)	2.8	0
Sep	342	342	22 (6.4)	4 (1.2)	53.4	0.64
Oct	786	786	38 (4.8)	16 (2)	122.8	2.46
Nov	600	600	26 (4.3)	20 (3.3)	93.8	3.1
Dec	36	36	2 (5.6)	0 (0)	5.6	0
Total	**1,796**	**1,796**	**92 (5.1)**	**40 (2.2)**	**46.8**	**1.03**
***Anopheles gambiae* complex**						
Month	**Collected**	**Dissected**	**Infected (%)**	**Infective (%)**	**HBR**	**EIR**
July	1,646	1,646	18 (1.1)	2 (0.1)	257.2	0.26
Aug	2,488	2,488	37 (1.5)	5 (0.2)	388.8	0.78
Sep	2,846	2,846	244 (8.6)	40 (1.4)	444.7	6.23
Oct	3,214	3,214	160 (5)	70 (2.2)	502.2	11.05
Nov	924	924	34 (3.7)	22 (2.4)	144.4	3.46
Dec	72	72	12 (16.7)	2 (2.8)	11.3	0.31
Total	**11,190**	**11,190**	**505 (4.5)**	**141 (1.3)**	**291.4**	**3.79**

N Number, *%* Percent, *HBR* Monthly biting rate, *EIR* Entomological inoculation rate.

Relative frequencies of An. gambiae complex members and An. gambiae s.s. molecular forms.

Among the 15,869 *An. gambiae* complex members examined by PCR for specific species identification, 99.02% (15,713/15,869) were *An. gambiae s.s.* and 0.98% (156/15,869) was *An. arabiensis*. The frequency of *An. gambiae s.s.* decreased towards the end of the rainy season (December) while that of *An. arabiensis* increased slightly

(Trends Chi square = 90.57; $p < 10^{-4}$) (Table 4). Significant monthly variation in the relative frequencies of the two species was observed ($p < 10^{-6}$). The overwhelming majority (95.09%; 14,942/15,713) of the *An. gambiae s.s.* collected in 2001 were the S molecular form (Table 4). This high frequency of the S molecular form was observed in all of the study villages (data not shown).

Table 4

Monthly variation in the relative frequencies of *Anopheles gambiae complex members* and the molecular forms of *Anopheles gambiae sensu stricto* in 2001

	***An. gambiae* complex members**				***An. gambiae ss* molecular forms**			
	An. gambiae ss		***An. arabiensis***		**M Form**		**S Form**	
Months	N	(%)	N	(%)	N	(%)	N	(%)
July	3,895	99.7	12	0.3	195	5	3,700	95
August	4,859	99.5	24	0.5	233	4.8	4,626	95.2
September	4,480	98.6	63	1.4	211	4.7	4,269	95.3
October	1,895	98.4	31	1.6	80	4.2	1,815	95.8
November	521	95.8	23	4.2	31	6	490	94
December	63	95.5	3	4.5	21	33.3	42	66.7
Total	15,713	99.02	156	0.98	771	4.91	14,942	95.09

S Form = *An. gambiae* Form Bamako or Savannah; M Form = *An. gambiae* Form Mopti.

Vector infection rates and transmission pattern

Both *An. gambiae* and *An. funestus* complexes were found to be harboring infective larvae during the two years of study (2001 and 2002). In July and August, 100% of the infective *W. bancrofti* larvae were recovered from *An. gambiae* complex (Figure 1).

An. funestus complex harbored infective larvae with increasing rates from September to November. No infective mosquito was recovered in December 2001 (Table 2). In both 2001 and 2002, the *An. funestus* complex became increasingly more important in LF transmission from September through November (Table 2 and and 3).

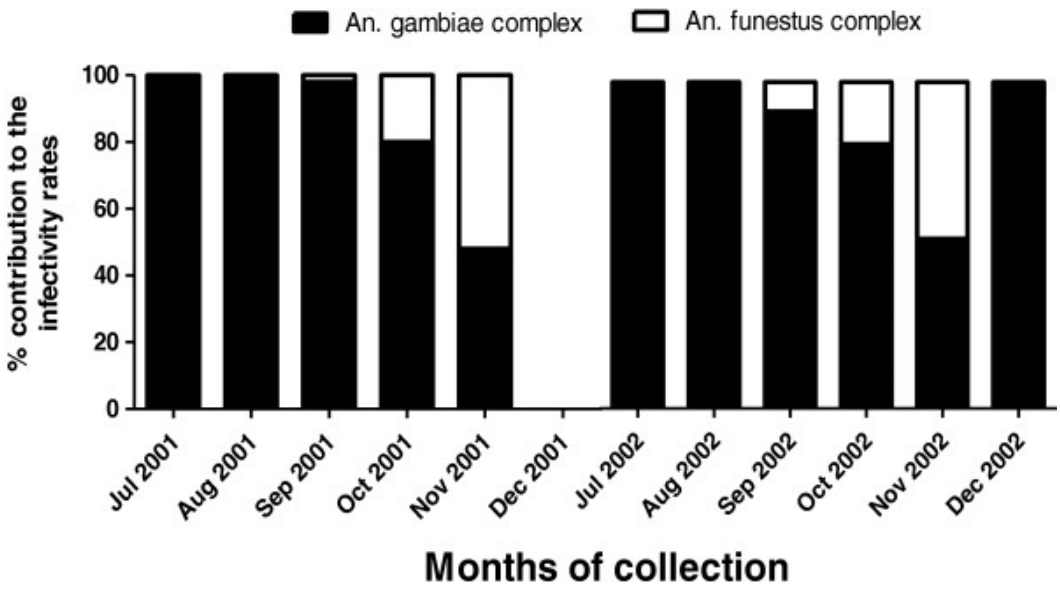

Figure 1

Species contribution to the overall monthly infectivity rates in 2001 and 2002. The black represents the contribution of *Anopheles gambiae* complex and the white the contribution of *Anopheles funestus* complex.

Overall, infection rates were comparable between *An. gambiae* and *An. funestus* complexes with 4% versus 4.9% (Chi2 = 2.90; p = 0.09) in 2001 (Table 2) and 4.5% versus 5.1% (Chi2 = 1.31; p = 0.25) in 2002 (Table 3). In 2001, *An. funestus* complex was found to be carrying *W. bancrofti* larvae from September to November with monthly infection rates ranging from 2.7% to 6.5% while infected *An. gambiae*

complex was recovered each month from July to December with rates ranging from 2.8% to 6.8% (Table 2). In 2002, both *An. funestus* and *An. gambiae* complexes were found infected from July to December with monthly rates ranging respectively from 4.3% to 14.3% and 1.1% to 16.7% (Table 3). Whereas infection and infectivity rates were similar in 2001 and 2002 for *An. funestus* complex (4.9% versus 5.1% infection; Chi2 = 0.04, p = 0.82 and 2% versus 2.2% infectivity; Chi2 = 0.06, p = 0.80), the infectivity rate for *An. gambiae* complex significantly decreased in 2002 following MDA (1.3% versus 2.3% infectivity in 2001; Chi2 = 37.86, p < 10-3), despite a small increase in infection rate (4.5% versus 4.0% infection in 2001; Chi2 = 4.54, p = 0.03).

In 2001, the overall monthly EIR was more than 10 fold higher for *An. gambiae* complex than *An. funestus* complex. The *An. gambiae* complex was responsible for 0 to 35.53 infective bites per human per month. The *An. funestus* complex was responsible of 0 to 4.2 infective bites per human per month. In November 2001, the *An. funestus* complex was responsible for more infective bites than the *An. gambiae* complex (2.4 versus 1.8) but no infective bite was recorded in December (Table 2). In 2002, there was a dramatic decrease in the overall EIR for *An. gambiae* complex as compared to 2001 (from 12.55 to 3.79 infective bites per person during the transmission season). In contrast, for *An. funestus* complex, the overall EIR in 2001 (1.2 infective bite/person) (Table 2) was similar to that recorded in 2002 (1.03 infective bite/person) (Table 3).

Discussions

The baseline entomological data collected in this longitudinal study confirmed measurable transmission of *W. bancrofti* in the 6 study villages in Sikasso prior to the initiation of MDA. As had been reported previously in Mali, *An. gambiae* and *An.*

funestus complexes were the predominant vectors [2,8]. In keeping with the high prevalence of human infection in these villages, the recorded vector densities were higher, especially for *An. gambiae* complex, than those reported in Banambani (Sudan savannah area) in Mali, where *W. bancrofti* is endemic but the prevalence of infection is lower [8]. Other *Anopheles* species (*An. pharaoensis*, *An. nili*, *An. rufipes*) were collected but not systematically processed during this study because of their very low relative frequencies, precluding an epidemiologically significant role in the transmission of LF, and the fact that they have not been recognized as vectors of *W. bancrofti* in Mali and other neighboring West African countries [2,9].

Among the *An. gambiae* complex members examined by PCR for specific species identification, the S form of *Anopheles gambiae s.s*. was predominant. A predominance of the S form of *An. gambiae s.s* among vectors of LF has also been observed in Ghana [10]. Although the PCR identification of *An. gambiae* complex species and *An. gambiae s.s.* molecular forms was not performed on all the collected mosquitoes for logistical reasons, at least 76% of the mosquitoes collected each month were dissected to ensure that the samples tested were temporally and geographically representative. Mosquitoes were sent for PCR analyses without identity numbers that could link them to the dissection results precluding the determination of infection rates for the different molecular forms.

Overall, the highest monthly vector relative frequencies for *An. gambiae* complex were found in July and August (at least 99% of the vectors collected in the month), while those of *An. funestus* complex were observed in November and December (at least 33% of the vectors collected in the month). Similar variations in the relative frequencies of the two vectors were reported in Banambani [8] and are related to

differences in environmental conditions during the transmission season and the breeding preferences of each species (*An. funestus* complex prefers shadowed and vegetated breeding sites while *An. gambiae* complex prefers sunny breeding sites with limited vegetation) [2,8]. The frequencies of *An. gambiae s.s.* and *An. arabiensis*, two members of the *An. gambiae* complex, also showed differing patterns during the transmission season.

Due to the low infection and infectivity rates, processing of pools of *Anopheles* vectors for *W. bancrofti* infection is the recommended strategy for following vector transmission rates during MDA [11,12]. A recently developed L3 specific RT-PCR allows infective pools to be distinguished from infected pools and provides a more accurate determination of the transmission potential for *W. bancrofti*[9,13]. In the present study, the infection and infectivity profiles of the two morphologically distinct *Anopheles* species complexes (*An. gambiae s.l.* and *An. funestus*) were quite different, suggesting that the two species complexes should be processed for PCR in separate pools if detailed information regarding their relative contributions to monthly transmission is desired. Nonetheless, in the setting of post MDA assessment, where human-vector contact is the main factor of interest, *An. gambiae* and *An. funestus* complexes can be processed in the same pool [11,13].

In 2002 (post MDA), the number of mosquitoes caught was approximately half that in 2001 (before MDA). This effect was most dramatic for *An. gambiae* complex where the number captured decreased by almost 50%. Potential reasons for this decrease in mosquito numbers include changes in climate, increased awareness of the study area population with respect to the role of mosquitoes in disease transmission (resulting in less breeding sites and increased use of insecticide treated nets), and the effect of

ivermectin on mosquito survivorship. Examination of the rainfall, temperature and humidity records for the region did not show any major differences between 2001 and 2002, suggesting that climate did not play a major role in the decreased number of mosquitoes. Although decreases in mosquito numbers following the initiation of MDA [14] and an effect of ivermectin on mosquito survivorship [15,16] have both been described, these factors were not directly addressed in the present study.

Whereas the decreased vector numbers in 2002 (post-MDA) clearly contributed to the overall decrease in EIR observed for the *An. gambiae* complex, infectivity also declined significantly in 2002, suggesting that multiple factors may have played a role in the observed decrease in transmission including the decrease of the mf prevalence and loads consecutive to the MDA [14]. The fact that a similar decrease in EIR was not seen for *An. funestus* complex may have been due to the low overall numbers of *An. funestus* complex mosquitoes captured, although a higher degree of facilitation by *An. funestus* complex as compared to *An. gambiae* complex cannot be excluded. Unfortunately, the study was not designed to address this issue, and published data comparing facilitation between the two species are limited [17,18].

Despite the fact that the overall mosquito infection rates were relatively stable during the six months of collection in each of the two transmission seasons, the EIR for *W. bancrofti* varied considerably over the course of the seasons as a result of the large differences in vector densities and HBR [11]. This has important implications for the timing of MDA for LF in this region, since drug administration conducted at the beginning of the rainy season would be predicted to be most effective in decreasing transmission due to maximal reduction in mf prevalence and loads at the precise time that vector density and biting rates are beginning to rise.

Ethical approval for this study was obtained from WHO and University of Bamako. At the time that the study was performed, Human Landing Catch was considered an ethically acceptable method of mosquito collection. The collectors in this study were adult village residents normally exposed to mosquito bites. The collectors were not given antimalarial prophylaxis, but were provided access to a health practitioner (nurse) during the study in the event of malaria infection as recommended for adult subjects living in malaria endemic area. Since the goal of HLC is to collect the mosquito before it bites, the risk of infective bite is actually quite low. Although HLC is still used in some settings, research is actively ongoing in our center and others to find a comparable method that does not involve human bait [19-21].

Conclusion

In conclusion, the entomological data from the present study confirmed the district of Sikasso as an area of high *W. bancrofti* transmission. This led to the selection of this area as the site of a multi-national study on the effects of MDA on LF transmission by anopheline vectors and as the first region in Mali for implementation of MDA with ivermectin and albendazole to eliminate transmission of LF. Comparison of the vector transmission parameters prior to and immediately following the first round of MDA demonstrated a significant decrease in overall transmission after institution of MDA. Importantly, the dramatic variability in EIR over the transmission season suggests that the efficacy of MDA can be maximized by delivering drug at the beginning of the rainy season (just prior to the peak of transmission).

Competing interest

The authors declare that they have no competing interests.

Authors' contributions

YIC, SFT, YTT designed and conceived the study; TBN, ADK, BAB, YTT approved final version of the manuscript and helped with the analysis and drafting of the manuscript; YIC, BD, AAD, SKo, FK, AKT collected and processed the samples and drafted the manuscript; YIC, BD, HD, ID, MBS, SK managed the data, did the statistical analysis and helped to draft the manuscript. All the authors read and approved the final manuscript.

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Appendix 4. Chapter 4 related published paper in American Journal of Tropical Medicine and Hygiene

The Impact of Six Annual Rounds of Mass Drug Administration on *Wuchereria bancrofti* Infections in Humans and in Mosquitoes in Mali

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Abstract

Wuchereria bancrofti prevalence and transmission were assessed in six endemic villages in Sikasso, Mali prior to and yearly during mass drug administration (MDA) with albendazole and ivermectin from 2002 to 2007. Microfilaremia was determined by calibrated thick smear of night blood in adult volunteers and circulating filarial antigen was measured using immunochromatographic card test in children < 5 years of age. Mosquitoes were collected by human landing catch from July to December. None of the 686 subjects tested were microfilaremic 12 months after the sixth MDA round. More importantly, circulating antigen was not detected in any of the 120 children tested, as compared with 53% (103/194) before the institution of MDA. The number of infective bites/human/year decreased from 4.8 in 2002 to 0.04 in 2007, and only one mosquito containing a single infective larva was observed 12 months after the final MDA round. Whether this dramatic reduction in transmission will be sustained following cessation of MDA remains to be seen.

Introduction

Lymphatic filariasis (LF) caused by *Wuchereria bancrofti* is endemic throughout West Africa,[1,2] where the predominant vectors are species of the *Anopheles gambiae* and *Anopheles funestus* complexes.[1] One of the key questions that has emerged as the Global Program for the Elimination of Lymphatic Filariasis (GPELF) has grown globally since 2000[3–5] is the impact that annual mass drug administration (MDA) of albendazole and ivermectin (Mectizan®) has had on the transmission of the *W. bancrofti* infection in different epidemiological settings in Africa.

Whereas the long-term impact of combination albendazole/diethylcarbamazine (ALB/DEC) on transmission of LF has been demonstrated in multiple epidemiologic settings,[6–8] only a few studies have examined the impact of repeated annual administration of albendazole/ivermectin (ALB/IVER), the regimen used throughout West Africa because of the overlapping geographic distributions of LF and onchocerciasis.[9–11] To evaluate the effect of ALB/IVER on *W. bancrofti* transmission in varied epidemiologic settings in Africa, a multi-country study was initiated in 2001 in Mali and Ghana (West Africa) and Kenya (East Africa). Regular assessments of prevalence in the human population (circulating filarial antigen and microfilarial levels), and in the *Anopheles* vector population (annual transmission potential [ATP]) were conducted prior to and during six annual MDA using ALB/IVER. We report here the results of the portion of the study conducted in a highly endemic area of Mali.

Methods

Study sites.

The study was undertaken in six villages of the district of Sikasso: Dozanso, Gondaga, Missasso, N'Torla, Niantanso, and Zanadougou. The total population of the study area was 5,120 in 2008, according to demographic information available from the Sikasso

Regional Directorate responsible for planning and statistics. The administrative region of Sikasso covers 76,480 km^2 in the southern Sudan savannah area. The region has a total population of 2.45 million, the highest population density in the country with 32 inhabitants per km^2 in 2008. Prior to this study, there had been no MDA implemented in this area. The study villages have previously been described.[12] The mean distance between the villages is approximately 15 km occupied by cotton fields, backwaters, and typical Sudan savanna vegetation. During the study period, the rainfall ranged from 1,000 to 1 500 mm/year with the rainy season extending from July to December.

Study design.

To assess the impact of six consecutive annual MDA rounds on *W. bancrofti*infection and transmission in these six villages of Sikasso District, a monthly cross-sectional entomological survey was undertaken from July to December each year, as well as a parasitological assessment in July each year just prior to the MDA and the entomological survey, from 2002 to 2008. All six villages received MDA for 6 years. During the seventh year (2008), ALB/IVER was not distributed in two villages with no evidence of ongoing transmission to provide preliminary data in anticipation of stopping MDA in the remaining villages the following year.

Study population.

A complete census, including the name, age, sex, and profession of every inhabitant, was performed in the study villages every year before the parasitological assessment. All subjects ≥ 2 years of age who presented for evaluation were included in the study.

Parasitological and clinical assessment.

Each year, before starting the mosquito collection, a parasitological assessment was performed. Sixty microliters of night blood were obtained by fingerprick from adult volunteers (15 years and above) for preparation of three thick smears. The slides were stained with Giemsa for identification and quantification of *W. bancrofti* microfilariae. The adult volunteers as well as children ≤ 5 years of age were tested for *W. bancrofti* circulating antigen using immunochromatographic card test during the first year (at baseline) and after the sixth MDA. The clinical assessment consisted of a brief interview and physical exam focusing on characteristic manifestations of LF, namely lymphedema and hydrocele. Any clinical stage of lymphedema (from reversible pitting edema to elephantiasis) or hydrocele (small, big, unilateral, or bilateral) was considered as a case and recorded without additional information.

Mass drug administration.

ALB/IVER was administered to all eligible subjects (not pregnant or breastfeeding within a week of delivery, taller than 90 cm, and aged 5 years and above) in collaboration with the district and community health care staff using the health workers as drug distributors. MDA coverage rates were calculated based on the number of eligible subjects.

Entomological studies.

Villagers were trained to collect mosquitoes from 6:00 pm to 6:00 am using the human landing catch (HLC) method. A 12-day monthly entomological survey was carried out concomitantly by different teams in each of the six villages to determine village-wide *W. bancrofti* transmission potential. The parameters assessed included the human biting rate (HBR), infection rate, infectivity rate, ATP, and the entomological

inoculation rate (EIR) during the study period. From July to December each year, mosquitoes were collected by two collectors per room in four different rooms in each village at night. The first collection team worked from 6:00 pm to midnight and the second from midnight to 6:00 am in each room. The collector caught the mosquitoes as they tried to land using a mouth aspirator connected to a paper cup as the storage container, as developed by Coluzzi and Petrarca. The mosquitoes that were collected during night were kept in ideal conditions (temperature, relative humidity using wet wipes) and dissected early the following morning.

Laboratory analysis.

Mosquito samples were sorted by species (*An. gambiae* s.l. and *An. funestus*) on the basis of morphology.[13] Members of the *An. gambiae* complex were identified in a sample of dissected mosquitoes by polymerase chain reaction (PCR) assays in 2002 (at baseline) as described by Favia and others.[14]

During the dissection, the head and thorax were dissected separately for each mosquito, and recovered parasite larval stages were categorized into L1, L2, and L3. Parity status was determined by dissecting the ovaries and observing the tracheal coils.[15]

Multiple entomological parameters were calculated as previously described[12,13,16]:

1) Infection rate: proportion of mosquitoes found infected after dissection with any *W. bancrofti* larval stage (L_1–L_3)

2) Infectivity rate: proportion of mosquitoes found infected with one or more infective larvae (L3)

3) HBR: number of mosquitoes caught during the HLC × 30/(total number of collectors used per collection × number of collection in the month)

4) EIR: HBR × infectivity rate. The results of the monthly HBR (from all night HLC) multiplied by the *W. bancrofti* infectivity rate for a given species give an estimate of the number of infective bites of *W. bancrofti* received per human per month.

5) ATP: the sum of the monthly EIR over the year.

Data management and analysis.

All data were recorded on standard data sheets and entered into the computer using SPSS version 12 (Statistical Package for Social Sciences, SPSS Inc., Chicago, IL) and GraphPad Prism version 5. The χ^2 test or Fisher's exact test was used as appropriate for the comparison of proportions. The confidence level was set at 95% for all statistical tests.

A collective village-wide oral consent was obtained from the villages' elders as well as a signed individual written consent form from all study participants and/or guardians. Both the Institutional Review Board of the World Health Organization/Tropical Diseases Research and the Ethics committee of the Faculty of Medicine of Bamako in Mali approved the protocol and the consent forms.

Results

Clinical, parasitological, and MDA.

A total of 1,333 subjects from six villages aged 2 years and above have been included in the analysis. Females represented 57.8% of this population. Lymphedema and hydrocele had prevalences of 0.3 and 2.8%, respectively (Table 1). Cross-sectional assessment of the human microfilaremia prevalence rate showed a dramatic decrease in the prevalence of *W. bancrofti* microfilaremia over the course of the study ($P < 10^{-4}$)

from 21.4% (244/1139) in 2002 (Table 1) before the first MDA to 0.2% (2/856) in 2007 and 0.0% (0/760) in 2008 after the sixth MDA round (data not shown). The geometric mean microfilaria (mf) densities in microfilaria-positive individuals also decreased from 103 mf/mL in 2002 to 63 mf/mL in 2006, 17 mf/mL in 2007 and to 0 mf/mL in 2008 (data not shown). Subjects aged 45 and above had the highest *W. bancrofti* microfilaremia prevalence (41.6%) and geometric mean microfilaria density (137 mf/mL) (Figure 1). The antigen carriage rate in children also decreased during this period from 53.09% (103/194) in 2002 to 0.0% (0/120) in 2008 ($P < 10^{-4}$) (Table 2).

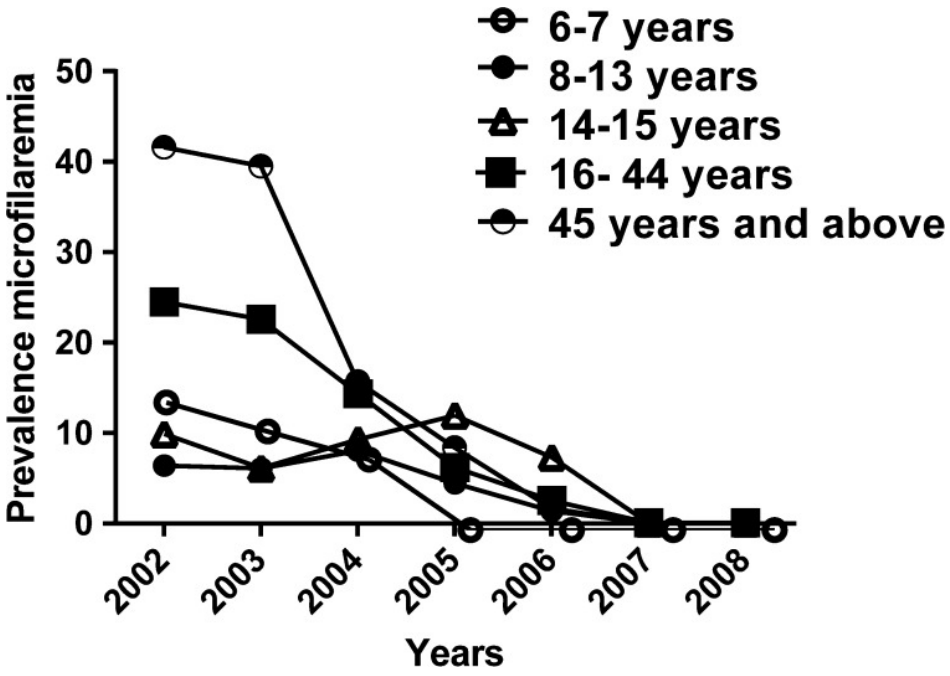

Figure 1.
Geometric mean prevalence of *Wuchereria bancrofti* and microfilaremia prevalence by age over the six consecutive mass drug administration rounds in the study area.

Table 1

Baseline characteristics of the study population

	Total	Male %	Female %	Lymphedema %	Hydrocele %	Mf+ %	Cag+ %
Age group 2–5 years	219	47.9	52.1	0	0	ND	2.2
6–7 years	81	56.8	43.2	0	0	4.5	7.1
8–13 years	231	47.2	52.8	0	0	6.1	20.3
14–15 years	50	60	40	0	0	2	4.4
16– 44 years	596	33.9	66.1	0.2	1.7	60.2	52.3
45 years and above	156	53.2	46.8	1.3	14.1	26.2	13.7
Total	1,333	42.2	57.8	0.3	2.8	21.4	46.6
Villages*							
Dozanso	120	54.2	45.8	1.7	7.7	40	61.7
Missasso	207	35.3	64.7	0	11	20.3	36.9
Gondaga	212	45.8	54.2	0	6.2	15.1	43.4
Niantanso	202	42.1	57.9	0.5	7.1	29.7	81.8
N'Torla	196	50.5	49.5	0	3	13.8	40.3
Zanadougou	202	30.7	69.3	0	6.5	17.3	24.6

Cag+= circulating filarial antigen; Mf+= microfilaremia, ND =not done.

Table 2

Variation in the *Wuchereria bancrofti* circulating antigen carriage rate among individuals of 5 years and older in 2002 and 2008

	Baseline (2002)			Post 6 MDA (2008)		
Localities	No. tested	Pos	%	No. tested	Pos	%
Dozanso	120	48	40	86	0	0
Missasso	207	42	20.2	138	0	0
Gondaga	212	32	15.1	167	0	0
Niantanso	202	60	24.8	129	0	0
N'Torla	196	27	13.7	141	0	0
Zanadougou	202	35	17.3	99	0	0
Total	1139	244	21.4	760	0	0

The coverage rate for the eligible population varied from 67% to 78% during the first five MDA. In the four villages treated in 2008, coverage remained high at 89.6% (3201/3574). Mild adverse events were reported by 0.6% (13/2135) of the subjects in 2002 and the frequency decreased over time with only a few cases of mild headache reported in 2008 (data not shown). No severe adverse events were recorded during the study.

Entomology patterns.

An. gambiae complex members represented 90.1% of the vector fauna at baseline. Their mean infectivity rate was 2.3 (372/16,230) whereas *An. funestus* complex members had an infectivity rate of 2.3% (372/16,230). The annual transmission potential was 76 and 8 infective bites per human per year, respectively, for *An. funestus* and *An. gambiae* complexes (Table 3). Among the 15,869 *An. gambiae*complex members examined by PCR for specific species identification, 99.02% (15,713/15,869) were *An. gambiae* s.s. and 0.98% (156/15,869) were *Anopheles arabiensis*. The most common sibling species of *An. gambiae* s.s. in all localities were the Bamako/Savannah molecular form (S form), which comprised 95.09% (14,942/15,713) of the mosquitoes examined, followed by the Mopti molecular form (M form), which accounted for 3.8% (data not shown).

Table 3

Baseline vector characteristics in the study area prior to the MDA

Species	No. of collected	No. of dissected	No. of infected (%)	No. of infective (%)	HBR	EIR	ATP
Anopheles gambiae s.l.	20957 (90.1)	16230	646 (4.0)	372 (2.3)	545.8	12.6	76
Anopheles funestus	2308 (9.9)	1471	72 (4.9)	30 (2.0)	60.1	1.2	8
Overall	23265	17701	718 (4.1)	402 (2.3)	605.9	13.9	84

The annual vector HBR decreased over time from 605.9 bites per human per year in 2001 (Table 3) to 203.96 bites per human per year in 2007 (Table 4). The vector infection rate (*An. gambiae* s.l. and *An. funestus*) also decreased dramatically (by more than 98.11%) from 4.1% (718/17701) in 2001 (Table 3) to 0.04% (2/4680) in 2007, 12 months after the sixth MDA (Table 4). Of the two infected *An. gambiae*complex mosquitoes, one harboured a single infective L3 larva and the second one, a single non-infective L2 larva. Thus, the mosquito infectivity rate in 2007 was 0.02% (1/4,680) (Table 4). Due to the combination of a decrease in mosquito biting rates and lower numbers of infective mosquitoes, the EIR (number of infective bites per human per year) decreased by 99.7% from 4.8 in 2001 to 0.05 in 2006 and to 0.04 in 2007, 12 months after the sixth MDA (Table 4).

A similar effect was noted on the ATP, which decreased by 99.95% (Table 4). For *An. funestus* complex, the EIR (1.2 infective bites per person per month) and the ATP (seven infective bites per person per year) decreased by 100% by the end of sixth MDA evaluation while for *An. gambiae* complex, the EIR (12.6 infective bites per person per month) and the ATP (75.3 infective bites per person per year) decreased, respectively, to 0.04 and 0.2 (Supplemental Tables 1 and 2).

Table 4

Annual variation of the *Anopheles gambiae* and *Anopheles funestus* LF transmission level over the six MDA rounds

Years	Number of mosquito collected	Number of mosquito	HBR	Infection rate (L1/L2 pos) % (positive/ N)	Infectivity rate (L3 pos) % (positive/N)	EIR	ATP
Before (2001	23265	17701	605.9	4.1 (718/17701)	2.3 (402/17701)	13.9	84
MDA 1 (2002	12986	12986	338.2	4.6 (597/12986)	1.4 (181/12986)	4.8	28.1
MDA 2 (2003	18394	18394	479	1.2 (222/18394)	0.2 (44/18394)	1.1	6.9
MDA 3 (2004	13021	13021	339	1.1 (143/13021)	0.1 (16/13021)	0.4	2.5
MDA 4 (2005	10622	9578	276.61	0.17 (16/9578)	0.05 (5/9578)	0.14	0.9
MDA 5 (2006	10604	10604	276.1	0.06 (6/10604)	0.02 (2/10604)	0.05	0.3
MDA 6 (2007	7832	4680	203.96	0.04 (2/4680)	0.02 (1/4680)	0.04	0.3

Of note, the two infected mosquitoes were found in two different villages, Dozanso and Niatanso, resulting in average infection rates of 0.11% ($N = 916$) and 0.28% ($N = 362$), respectively. Thus, despite an average EIR of 0.04 infective bites per person during the study period, the EIR in 2007 was 0 in all of the villages except Dozanso, where it was 0.28 (data not shown).

Discussion

Consistent with the data from other studies,[9–11] six rounds of MDA with albendazole and ivermectin were extremely effective in reducing the prevalence of *W. bancrofti* microfilaremia in residents of a highly endemic area of Mali. Although testing for microfilaremia was limited to 53.02% (604/1139) of the total population eligible for MDA in the six villages, it is unlikely that the infection rate in the remaining population was substantially higher than that in the tested subjects. Thus, the observed impact of MDA on *W. bancrofti* microfilaremia in the present study is compatible with the long-term objective of the GPELF to interrupt transmission using MDA alone.

As previously reported in the baseline study,12 the dominant vector, *An. gambiae* s.l., continued to account for more than 90% of the mosquito vectors collected in this area over the 7 years of the study, followed by *An. funestus* (data not shown). The overall trend in any given year was characterized by a high frequency of *An. gambiae* s.l. early in the rainy season followed by a gradual decrease in *An. gambiae* s.l. and a gradual increase in the abundance of *An. funestus* toward the end of the rainy season.12 These changes are related to the climatic conditions over the year and not a result of MDA.13

In addition to seasonal variation, the vector density, and consequently the HBR, showed significant yearly variation from 2001 to 2008. The dramatic decrease in HBR following the first MDA in the study area has previously been reported and was most likely due to increased awareness of the study area population with respect to the role of mosquitoes in disease transmission (resulting in less breeding sites and increased use of insecticide-treated nets) and the effect of ivermectin on mosquito survivorship.12 Long-lasting insecticide-treated nets (LLITNs) were provided for free only to mothers just after delivery at the community health center from 2002 to 2004. Beginning in 2005, LLITN availability in the six villages increased because of the free distribution campaigns for vector control related to malaria prevention. Although yearly variations in HBR are not unusual in Mali and have been observed in the neighboring sites of Pimperena (unpublished data), this would not be expected to have a significant effect on EIR without a concomitant change in the vector infectivity rate.

Despite high levels of transmission prior to the institution of MDA, the vector infection and infectivity rates decreased to a very low, but detectable, level in 2007. Only two captured mosquitoes were infected with *W. bancrofti*, of which only one was infective, representing a more than 99% reduction in the infectivity rate. No differences were apparent between the two villages that continued to have infected mosquitoes and the four other villages with respect to overall compliance with the program or distance to non-MDA villages.

The mean EIR and ATP were also reduced by more than 99% after six MDA rounds. Although persistence of transmission despite low levels of microfilaremia in the human population has been reported with *Culex*species that exhibit limitation (decreasing yield of infective larvae per mf as the number of ingested mf increases), *An. gambiae* and *An. funestus* complexes demonstrate facilitation

(increasing yield of infective larvae per mf as the number of ingested mf increases).17,18 Consequently, the dramatic reduction in transmission intensity (only one infective larvae recovered and an infectivity rate of the anopheles vectors of 0.02% (the cutoff of 0.03% has been proposed) is likely sufficient to interrupt transmission in this rural area of Sikasso district19 as sexual reproduction is required in the human host to produce microfilaria.

Nevertheless, caution should be exercised in stopping MDA as there be might be variation in the efficiency of the different sibling species within the *An. gambiae* group of mosquitoes; thus continued close surveillance for resurgence of transmission will be essential. In this regard, a staggered approach to stopping, as undertaken in this study, may be most prudent.

In summary, the data to date suggest that six rounds of MDA with albendazole and ivermectin may be sufficient to interrupt transmission in a highly endemic region of Mali where Anopheles is the main vector. Annual evaluation of the human and vector populations for evidence of *W. bancrofti* infection continues in the study villages following cessation of MDA and will be essential to validate this conclusion. Although the effects of vector control measures, such as impregnated mosquito bed nets, were not assessed in this study, such interventions may provide additional benefit, particularly in the maintenance of transmission interruption after MDA is stopped, and should be explored in future studies.

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Footnotes

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Supplemental Table 1

Annual variation of the *Anopheles gambiae* LF transmission level over the six MDA rounds

Years	Number of mosquito collected	Number of mosquito dissected	HBR	% (positive/*N*)	% (positive/*N*)	EIR	ATP
Before	20957	16230	545.8	4 (646/16230)	2.3 (372/16230)	12.6	75.3
MDA 1	11190	11190	291.4	4.5 (505/11190)	1.3 (141/11190)	3.8	22.7
MDA2	17825	17825	464.2	1.2 (213/17825)	0.2 (42/17825)	0.9	5.6
MDA 3	11818	11818	307.8	1.1 (128/11818)	0.1 (15/11818)	0.3	1.8
MDA 4	10072	9080	262.29	0.15 (14/9080)	0.04 (4/9080)	0.1	0.6
MDA 5	10514	10514	273.8	0.06 (6/10514)	0.02 (2/10514)	0.05	0.3
MDA 6	7755	4624	201.95	0.04 (2/4624)	0.02 (1/4624)	0.04	0.2

ATP = annual transmission potential; EIR = entomological inoculation rate; HBR = human biting rate; LF = lymphatic filariasis; MDA = mass drug administration; pos = positive.

Supplemental Table 2

Annual variation of the *Anopheles funestus* LF transmission level over the six MDA rounds

Years	Number of mosquito collected	Number of mosquito dissected	HBR	Infection rate (L1/L2 pos) % (positive/ N)	Infectivity rate (L3 pos) % (positive/N)	EIR	ATP
Before	2308	1471	6 0.1	4.9 (72/1471)	2 (30/1471)	10.2	7
MDA 1	1796	1796	4 6.8	5.1 (92/1796)	2.2 (40/1796)	10.03	6.2
MDA 2	569	569	1 4	1.6 (9/569)	0.4 (2/569)	00.06	0.3
MDA 3	1203	1203	3 1.3	1.2 (15/1203)	0.1 (1/1203)	00.03	0.2
MDA 4	550	498	1 4.32	0.4 (2/498)	0.2 (1/498)	00.03	0.2
MDA 5	90	90	2.3	0 (0/90)	0 (0/90)	0	0
MDA 6	77	56	2.01	0 (0/56)	0 (0/56)	0	0

ATP = annual transmission potential; EIR = entomological inoculation rate; HBR = human biting rate (monthly); LF = lymphatic filariasis; MDA = mass drug administration; pos = positive.

Appendix 5. Chapter 5 related published paper in Parasites and Vectors

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Parasites & Vectors

Dynamics of antigenemia and transmission intensity of Wuchereria bancrofti following cessation of mass drug administration in a formerly highly endemic region of Mali

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Abstract

Background: After seven annual rounds of mass drug administration (MDA) in six Malian villages highly endemic for Wuchereria bancrofti (overall prevalence rate of 42.7%), treatment was discontinued in 2008. Surveillance was performed over the ensuing 5 years to detect recrudescence.

Methods: Circulating filarial antigen (CFA) was measured using immunochromatographic card tests (ICT) and Og4C3 ELISA in 6–7 year-olds. Antibody to the W. bancrofti infective larval stage (L3) antigen, Wb123, was tested in the same population in 2012. Microfilaraemia was assessed in ICT-positive subjects. Anopheles gambiae complex specimens were collected monthly using human landing catch (HLC) and pyrethrum spray catch (PSC). Anopheles gambiae complex infection with W. bancrofti was determined by dissection and reverse transcriptase polymerase chain reaction (RT-PCR) of mosquito pools.

Results: Annual CFA prevalence rates using ICT in children increased over time from 0% (0/289) in 2009 to 2. 7% (8/301) in 2011, 3.9% (11/285) in 2012 and 4.5% (14/309) in 2013 (trend $\chi^2 = 11.85$, df =3, P = 0.0006). Wb123 antibody positivity rates in 2013 were similar to the CFA prevalence by ELISA (5/285). Although two W. bancrofti-infected Anopheles were observed by dissection among 12,951 mosquitoes collected by HLC, none had L3 larvae when tested by L3-specific RT-PCR. No positive pools were detected among the mosquitoes collected by pyrethrum spray catch. Whereas ICT in 6–7 year-olds was the major surveillance tool, ICT positivity was also assessed in older children and adults (8–65 years old). CFA prevalence decreased in this group from 4.9% (39/800) to 3.5% (28/795) and 2.8% (50/1,812) in 2009, 2011 and 2012, respectively (trend $\chi^2 = 7.361$, df =2, P = 0.0067). Some ICT-positive individuals were microfilaraemic in 2009 [2.6% (1/39)] and 2011 [8.3% (3/36)], but none were positive in 2012 or 2013.

Conclusion: Although ICT rates in children increased over the 5-year surveillance period, the decrease in ICT prevalence in the older group suggests a reduction in transmission intensity. This was consistent with the failure to detect infective mosquitoes or microfilaraemia. The threshold of ICT positivity in children may need to be re-assessed and other adjunct surveillance tools considered.

Keywords: Wuchereria bancrofti, Transmission assessment survey, Anopheles gambiae complex, Mass drug administration, Post-MDA surveillance

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Background

Lymphatic filariasis (LF) is a public health problem in 71 countries and is associated administration (MDA) to all eligible residents of the endemic communities and morbidity management [2]. MDA is aimed at interrupting LF transmission through

clearing of peripheral blood microfilariae that prevent human-to-human vector-borne transmission [2].

As Bancroftian filariasis was found to be endemic in all eight administrative districts of Mali, ranging from 1% in Timbuktu (northern part of Mali) to > 18% in Sikasso (southern part of the country) [3], annual MDA using ivermectin and albendazole was initiated sequentially starting from the most highly endemic district in the country [3]. Sentinel sites were established in Sikasso as part of a multi-country initiative to assess LF transmission during and after stopping MDA. The baseline data and the impact of six rounds of MDA on human infection and potential transmission in this sentinel site have been previously reported [4].

The current study reports data collected to assess transmission after MDA was stopped in 2007 (after seven rounds of MDA). Although this study was initiated prior to the formal WHO recommendations for transmission assessment surveys (TAS), which require demonstration of an infection rate of < 1% in > 400 children aged 6–7 years using the immunochromatographic card test (ICT) to document interruption of transmission [5], a similar approach was taken using ICT testing of children aged 6–7 years. ICT testing of a cohort of children ≥ 8 years old and adults and entomological assessment of LF transmission were performed. Finally, the use of several additional methods (Og4C3 ELISA; Polymerase Chain Reaction (PCR) targeting *Wuchereria bancrofti* DNA; and *W. bancrofti* infective larval stage specific antigen Wb123-based IgG4 immunoassays) to assess transmission interruption in this previously highly LF-endemic region (Sikasso) of Mali was explored. Our data support an integrated approach to surveillance.

Methods

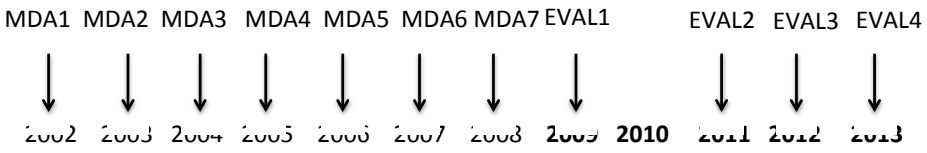
Study sites

The study area comprised 6 villages in Sikasso district: Gondaga, Dozanso, Missasso, N'torla, Niatanso and Zanadougou. These villages are located in the rural commune of Kolokoba that is located 332 km southeast of Bamako the capital city. *Wuchereria bancrofti* infection prevalence as assessed by the detection of CFA using ICT prior to MDA was 46% [4]. This area is also endemic for *Mansonella perstans*, but not *Onchocerca volvulus* infection. Based on 2012 undergone 7 annual rounds of MDA prior to its cessation in 2008, at which time the CFA prevalence had decreased to 0/760 children tested and the *Anopheles gambiae* complex mosquitoes showed an infection rate of 0.04% and an infectivity rate of 0.02% that were felt to be incompatible with active LF transmission [6]. There was a mean programmatic coverage rate based on the total population of 75.6% that varied from 67 to 78% [6]. A year after cessation of MDA (in 2009), no infected 6–7 year-old children were found among the 120 tested in the 6 villages.

Study design

As post-MDA surveillance, a yearly cross-sectional parasitological assessment of all children 6–7 years of age and all eligible older volunteers aged 8 years and above was performed in July from 2009 to 2012. In addition, a monthly entomological assessment of LF transmission (from July to December) was conducted in the six study villages in 2009, 2011 and 2013. In 2013, only children aged 6–7 years were tested with the ICT, along with a thick smear from night blood. Infective stage *W. bancrofti* larvae (L3) were assessed in mosquitoes using an L3-specific reverse transcriptase PCR (RT-PCR) technique as previously described [7]. The study design is illustrated in Fig. 1. EVAL

refers to the ensemble of surveillance testing performed in any given year.



ICT	+	+	+	+
CFA ELISA	+		+	
MF	+	+	+	+
Wb blood PCR	+			
WB123 Antibody			+	
HLC Vector Collection/Dissection	+	+	+	+
PSC/RT-PCR			+	

Figure 1: Time line of treatment, EVAL, and monitoring. The years in which MDA and EVAL surveys were performed are shown by the arrows and times at which monitoring tools in support of EVAL are shown by the + sign .

Parasitological and serological assessments

Infection status was assessed using the ICT card test for the detection of circulating *W. bancrofti* antigen (Allere, Portland, ME, USA). Dried blood spots were collected for additional laboratory analyses. Microfilaraemia was assessed by finger prick at night (between 22:00 and 02:00 h) among ICT-positive volunteers using a calibrated thick smear. Yearly parasitological studies were conducted in July, at the beginning of the transmission period, except in 2009 when, for logistical reasons, this assessment was performed in October. Because of the concern of potential transmission, additional diagnostic tests were performed on eluted blood spots from the 6–7 year-old children in 2012, namely the Og4C3 ELISA (Tropbio Townsville, Australia) and ELISA testing for antibodies to Wb123 as previously described [8].

Entomological assessment

Each month, a 12-day entomological survey was conducted in the six villages to assess the village wide *W. bancrofti* transmission patterns during the LF transmission period in Mali from July to December [4]. Two human landing catch (HLC) sessions were organized per month and per village. Two collectors worked inside each of four collection rooms per session.

Because *An. gambiae* is endophilic, collections were performed indoors to maximize yield. A total of 72 collection rounds were undertaken with the HLC. The collection was done from 18:00 to 06:00 h, and for ethical reasons, the collectors were replaced at midnight at each collection site.

All *An. gambiae* and *An. funestus* complexes collected were freshly dissected for parity status based on techniques previously described [9, 10] and for infection (any larval stage) and infectivity (L3 stage) status by individual mosquito dissection as previously described [11].

In 2012, the PSC (Pyrethrum Spray Catch) was used to collect mosquito vectors in addition to the HLC using Premium®, a pyrethrinoid-based insecticide, in 30 randomly selected rooms per village in each of the six collection months. During each of the 36 PSC collection rounds, the number of persons sleeping in each visited room was recorded on the mosquito collection sheet. The collected mosquitoes during the PSC were pooled (1 to 20 mosquitoes) in the field and stored in tubes containing RNA later and sent to Smith College for *W. bancrofti* RNA detection by RT-PCR as previously described [7].

For PSC, the monthly biting rate was determined by dividing the number of fed and

half-gravid female *Anopheles* collected in a room by the number of sleepers in the room the night before the collection multiplied by 30 [12]. The annual biting rate (ABR) was the sum of all the monthly biting rates calculated over the year [12]. From HLC-collected *Anopheles*, the parameters were determined as previously reported [12, 13].

Sampling

The present study predated the official WHO guidelines for TAS [5]. Because the evaluation unit was small (<300 children aged 6-7 years), all of the available eligible children were screened.

Data analysis

The collected data were entered using Microsoft Access 2007 and analysed using Graph Pad prism version 5 and Statistical Package for Social Sciences (SPSS) version 20. To compare the infection prevalences between villages or mosquito species, we used the Pearson χ^2 or the Fisher's exact test, if necessary. The trend χ^2 was used to test the statistical significance of any frequency or proportion's trend over time.

Results

Study demographics

We assessed 289 children aged 6–7 years in 2009, 301 in 2011, 285 in 2012 and 309 in 2013. Concomitantly, available older children and adults were assessed in 2009 (n = 800), 2011 (n = 795), and 2012 (n = 1,812) (Tables 1, 2). In 2013, testing of older

children and adults was restricted to those who were positive by ICT in 2012 (n = 50). Although the sizes of the 6 study villages differed, the study populations within the villages were quite well balanced in terms of gender within both the 6 to 7 year-old children and the ≥ 8 year-olds throughout the study period (Table 2).

CFA and Wb123 antibody prevalence assessment over the surveillance period

The CFA prevalence in 6–7 year-old children increased significantly over the surveillance period, from 0% (0/289) in 2009 to 2.7% (8/301) in 2011 and 4.5% (14/309) in 2013 (Trend χ^2 = 12.80, df = 3, P = 0.0003) (Table 3). In contrast, there was a significant decrease in CFA positivity over the study period in the ≥ 8 year-olds, from 4.9% (39/800) in 2009 to 3.5% (28/795) in 2011, to 2.8% (50/1,812) in 2012 (Trend χ^2 = 697.8, df = 2, P = 0.0001). Whereas none of the ICT-positive 6-7 year-olds had detectable microfilaraemia, 1 of 39 (2.6%) individuals in the older group was microfilaraemic in 2009, and 3/36 (8.3%) were microfilaraemic in 2011. In 2012, none of the 50 ICT-positive older subjects was microfilaraemic (Table 3). Forty-four of the previously ICT-positive older subjects, as well as 6 of the 6-7 year-olds who were ICT-positive and 8 years old at the time of the 2013 survey, were reassessed in 2013. None of the 28 subjects who remained ICT-positive in 2013 had detectable microfilaraemia (data not shown). Positivity rates for both the Og4C3 ELISA for CFA and testing for antibodies to the *W. bancrofti*-specific antigen, Wb 123, were similar to the results obtained using the ICT tests (χ^2=3.52, *df = 2, P* = 0.173).

Entomological assessment

The number of mosquitoes collected using the HLC over the study period is detailed

in Table 4. The highest ABR using the HLC was 374 bites per person in 2012 and the lowest was in 2011 with 155 bites per person. Among the dissected mosquitoes, the parity rates were significantly different between the 3 yearly entomological surveys with 84% (3,675/4,380) in 2009, 84% (2,406/2,853) in 2011 and 88% (5,032/5,718) in 2012 ($\chi^2 = 40.76$, $df = 2$, $P < 10^{-4}$). In 2009, two (0.05%) filaria-infected *Anopheles* females were detected (Table 4) without any infective larval stage recovered. In 2011 and 2012, no *W. bancrofti* larvae were found in the dissected mosquitoes (data not shown). With the PSC method during the 6 months of collection in 2012, 1,907 mosquitoes were collected and the ABR was 100 bites per person per year. The number of mosquitoes collected with the PSC technique was 3.75 times less than that collected with the HLC in 2012. Moreover, both the infection and infectivity of the PSC-collected mosquitoes were 0 (Table 4). Of note, *An. gambiae* complex was the most frequent vector comprising more than 99 % of the active vector fauna each year as compared to *An. funestus* complex (data not shown).

We observed the highest vector density (12 mosquitoes per person per night) in 2012 with 7,165 mosquitoes collected by 576 collectors. This density was 2.4 times higher than that in 2011 (2,962 mosquitoes) and 1.6 times more than that in 2009 (4,448 mosquitoes). Of the 2,962 and 7,165 mosquitoes collected in 2011 and 2012, respectively, the frequencies of *An. pharaoensis* varied from 1.31% in 2011 to 0.32% in 2012 while the frequencies of *An. rufipes* varied from 0.30% in 2011 to 0.01% in 2012. These species were very rare during the previous collection years in this area and were never found to be infected with *W. bancrofti* (Table 4).

Discussion

The current study investigated the LF transmission patterns following cessation of

MDA during the surveillance period from 2009 to 2013 in six neighbouring previously highly LF endemic villages in the Sikasso region in Mali. In 2008, after seven rounds of MDA, the *W. bancrofti* microfilaraemia and ICT positivity in children (6–7 years) was reduced to 0%. By 2011 and 2012, the prevalence of ICT-positivity in 6–7 year-old children showed an increase, although microfilaraemia was not detected. Despite a steady increase in CFA prevalence in 6–7 year-old children, there was a marked decrease in CFA prevalence rates over the same five year period among those ≥ 8 years of age (trend $\chi^2 = 7.361$, $df = 3$, $P = 0.0067$). This decrease is consistent with attrition over time of established worms. Despite the increasing CFA prevalence in children, our data are most consistent with interruption of LF transmission infective Anopheles, and the decreased CFA prevalence in the older age group. Nonetheless, close monitoring in areas of previously high transmission is necessary to detect early resurgence of transmission and to generate data that may guide and improve the elimination process.

When prevalence was estimated using different tools (Og4C3 ELISA and Wb123 immunoassays) at a single time point (2012), ICT consistently gave a higher prevalence rate compared to the two other tests, although the differences in prevalence were not statistically significant. Higher prevalences using ICT compared to Og4C3 ELISA was also observed in Togo during a school-based TAS conducted three years after stopping MDA [14], although the reasons for this are unclear. *Loa loa* microfilaraemia has been shown to be associated with ICT-positivity at both the community and individual levels [15, 16]; however, the same studies showed no association between ICT-positivity and the prevalence of *M. perstans*, the only other filarial parasite endemic in the study area [17].

Re-emergence of infection after just a few years of surveillance has been reported in Nigeria in some but not in all districts [18]. In India after 10 years following MDA implementation, new infection among children was also reported [19]. Using 6–7 year-old children as the sentinel population makes sense in the Malian context because this group remains in the villages, whereas many adults travel from place to place because of seasonal migration for agriculture and may acquire infection in areas that have not yet started MDA [20].

The approach to post-MDA surveillance is still being perfected. Antibody testing (e.g. Wb123) has been proposed as a potential better tool than antigen testing for the early identification of on-going transmission, as antibody positivity typically occurs months prior to positivity in adult antigen-based circulating antigen testing [21–23]. As there was good concordance between Wb123 prevalence and that of the CFA testing in the children (see Table 1) and with both tests now being point of care (POC) [8, 24], it is possible that the Wb123 rapid diagnostic test may be considered as a major surveillance tool in the near future.

Although screening of vector populations for the presence of infective larvae has been one of the 2 pillars of assessing transmission interruption in onchocerciasis [25, 26], its widespread use in LF has not taken hold to date. However, using both standard (dissection) and molecular techniques on both HLC and PSC collected mosquitoes (*n* = 9,072) only a few positives were found (and only just after the cessation of MDA). This is probably due to the drastic reduction of microfilaraemia prevalence after the seven consecutive MDA treatments and to the relatively low number of mosquitoes collected and the low sensitivity of the dissection [7]. Since RT-PCR, a more sensitive method to detect infective stage L3 larvae in the vector, is available [7], screening of larger numbers of mosquitoes and pool screen-based molecular techniques will need

to be assessed.

The observation that *An*. *pharaoensis* and *An*. *rufipes* were more frequently biting humans and their identification as secondary vectors of *W*. *bancrofti* in West Africa [27], raises the possibility that transmission can be sustained by a number of vectors other than the most prevalent (*An*. *gambiae* complex). The rain pattern in 2012 (frequency and abundance) likely played a role in the increased vector density, as well as in the increase in *An*. *pharaoensis* and *An*. *rufipes* frequencies [28, 29]. However, what is needed is an adequately designed prospective study of *W*. *bancrofti* transmission dynamics and vector control in this region of Mali. In addition, HLC was much more effective at collecting *Anopheles* than PSC; because of potential ethical issues related to HLC [30], better collection methods are needed. With very low human infection and vector infectivity rates, there is no evidence that *W*. *bancrofti* transmission has re-emerged in the study villages in the present study [5, 12].

Nevertheless, new entomological studies are needed to understand transmission dynamics in the context of post MDA surveillance. Mosquito vectors transmit *W*. *bancrofti* in two primary patterns, limitation and facilitation. Limitation is typically exhibited by *Culex* species and allows more efficient L3 development when microfilaraemia loads are low. Conversely, facilitation (usually exhibited by *Anopheles* species) leads to decreased numbers of developing L3 when microfilaraemia loads are low. Because limitation of *An*. *gambiae* (*sensu stricto*) has been observed in Ghana [31], it should also be assessed in other geographic locations (e.g. Mali) given the possibility of adaptation or specific mutation that can modify mosquito's transmission pattern [32]. From our previous studies, in the same area, WHO criteria were met but the mosquitoes were still infective (infectivity rate of 0.02%) when the MDA was stopped [6]. Taking into account the entomological data

and determining a threshold could be beneficial to be able to safely stop MDA in highly LF endemic.

Despite a dramatic and stable decrease in the prevalence of infection in the older age groups and in mosquitoes five years following the cessation of MDA in six villages previously highly endemic for LF, a significant increase in the prevalence of LF antigenemia as assessed by ICT occurred among 6–7 year-old children. Although the ICT prevalence in this age group met WHO criteria for restarting MDA (> 2% ICT-positive) [5], the prevalence using the Og4C3 ELISA and Wb123 antibody ELISA were below the threshold. Furthermore, the observed prevalence increase within this group contrasted with the entomological data that showed an absence of LF transmission and the absence of microfilaraemia in all individuals tested.

Conclusions

Using a set of LF testing methods (ICT, Wb123, Og4C3 ELISA, and vector surveillance), we demonstrated differences among the various techniques considered important for post-MDA assessments. Our data suggest, nevertheless, that an integrated assessment strategy that combines serologic- and vector-based techniques may be useful in the assessment of transmission interruption following cessation of MDA in LF-endemic areas.

Declarations

Abbreviations

ABR: annual biting rate; CFA: circulating filarial antigen; DNA: deoxyribonucleic acid; GPELF: Global Program to Eliminate Lymphatic Filariasis; HLC: human landing catch; ICT: immunochromatographic card tests; IgG4: immunoglobulins g4;

LF: lymphatic filariasis; MDA: mass drug administration; NIAID: National Institute of Allergy and Infectious Diseases; NIH: National Institute for Health; POC: point of care; PSC: pyrethrum spray catch; RNA: ribonucleic acid; RT-PCR: reverse transcriptase polymerase chain reaction; SPSS: Statistical Package for Social Sciences; TAS: transmission assessment surveys; UNDP: United Nations Development Programme; WHO: World Health Organization.

Ethics approval and consent to participate

The protocol and consent form were approved by the Ethical Committee of the Faculty of Medicine and Odontostomatology of Bamako, Mali (No. 01-51/FMOS). A local guide in the presence of a research team member explained the goals, procedures and risks of the study to the volunteers. All participants provided informed assent or consent before the blood collection. For volunteers <18 years of age, a parent or an adult tutor provided consent.

Consent for publication

Not applicable.

Availability of data and material

The datasets generated and analysed during this study are presented in Tables 1–4. Additional information can be available from the authors upon reasonable request.

Competing interests

The authors have no competing interest.

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Authors' contributions

YIC, SFT, YTT, DK designed and conceived the study; YIC, MS, MD, YTT, LKH, KP, SFT, MB, AK, TBN approved final version of the manuscript and helped with the analysis and drafting of the manuscript; SYC, HD, SK, AAD, SSD, LS, MEC, ID, MBS, BD collected and processed the samples and drafted the manuscript; YIC, BD, HD, ID, MBS, SK, SYC, HD, AAD managed the data, did the statistical analysis and helped to draft the manuscript. All authors read and approved the final manuscript.

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Table 1 Sampling according to different activities per year

	Study Human Sample			Mosquito Collection		
Year	Total population	6-7 years old	≥8 years	Number Collected	Technique used	Number of collection rounds
2009	4,431	289	800	4,448	HLC	72
2011a	4,761	301	795	2,962	HLC	72
2012	5,044	285	1,812	7,168/1,907	HLC/PSC	72/36b
2013c	5,225	309	50	nd	nd	nd

a= in 2011, a random sample of 92 subjects from the 6 villages was tested with Wb123 ELISA.

b= In 2012, the 6 villages were visited once a month from July to December (collection in 30 rooms per visit per village);

c = in 2013 the 50 subjects ≥8 years tested were the ones found positive using ICT in 2012

nd = Not Done

Table 2 Characteristics of the study population per village throughout the surveillance period in the 6 study villages of the Sikasso district

Village	6–7 years		8 years and above			Overall
	M/F	Total	M/F	Median age (Range)	Total	
			Survey 1 in 2009			
Dozanso	20/29	49	60/73	34 (12–79)	133	182
Missasso	26/20	46	64/94	40 (15–76)	158	204
Gondaga	22/21	43	55/64	33 (12–75)	119	162
Niatanso	30/24	54	91/106	31 (12–69)	197	251
N'Torla	23/16	39	50/49	37 (12–72)	99	138
Zanadougou	28/30	58	31/63	37.5 (13–77)	94	152
Total	149/140	289	351/449	35 (12–79)	800	**1,089**
			Survey 2 in 2011			
Dozanso	21/17	38	42/71	32 (15–82)	113	151
Missasso	22/31	53	51/99	35 (15–86)	150	203
Gondaga	21/17	38	58/73	29 (15–84)	131	169
Niatanso	25/29	54	73/60	31 (15–82)	133	187
N'Torla	35/26	61	53/80	31 (15–88)	133	194
Zanadougou	26/31	57	49/86	31 (15–89)	135	192
Total	150/151	301	326/469	38 (15–89)	795	**1,096**
			Survey 3 in 2012			
Dozanso	20/16	36	95/137	32 (15–82)	232	268
Missasso	21/27	48	101/171	33 (15–79)	272	320
Gondaga	27/21	48	100/177	28 (15–85)	277	325
Niatanso	26/25	51	134/182	28 (15–83)	316	367
N'Torla	22/15	37	127/208	30 (15–89)	335	372
Zanadougou	34/31	65	137/243	30 (15–80)	380	445
Total	150/135	285	694/1,118	30 (15–89)	1,812	2,097
			Survey 4 in 2013			
Dozanso	24/24	48	8/15	41 (8–75)	23	73
Missasso	26/21	47	1/4	38 (31–68)	5	52
Gondaga	30/25	55	0/5	28 (8–58)	5	60
Niatanso	32/23	55	1/3	25.5 (8–63)	4	60
N'Torla	18/24	42	1/6	46 (24–66)	7	49
Zanadougou	31/31	62	4/2	29 (8–58)	6	68
Total	161/148	309	15/35	38 (8–75)	50	359

M/F= male/female

Table 3 Circulating filarial antigen (CFA) and microfilaraemia prevalence rates within 6–7 year-old children and those of 8 years and above from 2009 to 2013

	Survey 1 (2009)	**Survey 2 (2011)**	**Survey 3 (2012)[a]**	**Survey 4 (2013)**	
Sample size and target	Targeted sample size	1,107	1,107	2,530	372
	Total population	4,431	4,761	5,044	5,225
	Number tested (*n*)	1,089	1,096	2,097	359
ICT	≥ 8 years % Positive(*n*/*N*)	4.9% (39/800)	3.5% (28/795)	2.8% (50/1,812)	
	[95% CI]	[3.53–6.67]	[2.40–5.12]	[2.08–3.65]	–
	6–7 years % Positive (*n*/*N*)	0% (0/289)	2.7% (8/301)	3.9% (11/285)	4.5% (14/309)
	[95% CI]	[0.00–1.64]	[1.24–5.37]	[2.04–7.00]	[2.60–7.66]
Mf	≥ 8 years % Positive (*n*/*N*)[b]	2.6% (1/39)	10.7% (3/28)	0% (0/50)	
	[95% CI]	[0.06–13.48]	[2.81–29.37]	[0.00–8.89]	–
	6–7 years % Positive (*n*/*N*)[b]	0	0% (0/8)	0% (0/11)	0% (0/14)
	[95% CI]		[0.00–40.23]	[0.00–32.15]	[0.00–26.76]
PCR	≥ 8 years % Positive (*n*/*N*)	5.13% (2/39)	np	np	np
	[95% CI]	[0.89–18.63]			
	6–7 years % Positive (*n*/*N*)	0	np	np	np
	[95% CI]				
Wb123	≥ 8 years %Positive (*n*/*N*)	np	np	4.7% (2/43)	nd
	[95% CI]			[0.81–17.06]	
	6–7 years % Positive (*n*/*N*)	np	np	1.8% (5/285)	nd
	[95% CI]			[0.65–4.27]	
Og4C3	≥ 8 years ICT % Positive (*n*/*N*)	np	np	4% (2/50)	np
	[95% CI]			[0.70–14.86]	
	6–7 years % Positive (*n*/*N*)	np	np	1.8% (5/285)	np
	[95 % CI]			[0.65–4.27]	

[a]In 2012, the ELISA test was done on all the children and the 50 ICT positive adults

[b]Only the ICT positive subjects were tested for Mf

Abbreviations: ELISA, Enzyme-Linked Immuno-Sorbent Assay; ICT, Immunochromatographic Card Test; ICT+, ICT positive; Mf, microfilaraemia; n,

number positive; N, number examined; nd, not done; np, not planned; PCR, polymerase chain reaction; Wb123, filarial antibody test

Table 4 Annual variation of mosquito densities and biting rates over the surveillance period from 2009 to 2012

Collection method	Years	Species	No. of mosquitoes collected	No. of mosquitoes dissected Frequency [95% CI]	ABR	Parity Frequency [95% CI]	Infection Frequency [95% CI]
HLC	**2009**	**GA**	4,443	4,375	232	3,671	2
				98.47 [98.05–98.8]		83.9 [82.78–84.98]	0.05 [0.01–0.18]
		FU	5	5	0	4	0
				100 [46.29–100]		80 [29.88–98.94]	
		PH	0	0	0	0	0
		RU	0	0	0	0	0
	2011	**GA**	2,911	2,803	152	2,364	0
				96.29 [95.52–96.93]		84.34 [82.92–85.65]	
		FU	3	3	0	3	0
				100 [31.00–100]		100 [31.00–100]	
		PH	39	38	2	30	0
				97.44 [84.92–99.87]		78.95 [62.22–89.86]	
		RU	9	9	1	9	0
				100 [62.88–100]		100 [62.88–100]	
	2012	**GA**	7,138	5,691	368	5,006	0
				79.82 [78.86–80.74]		88.9 [88.05–89.70]	
		FU	3	3	0	3	0
				100 [31.00–100]		100 [31.00–100]	
		PH	23	23	1	22	0
				100 [77.08–100]		94.1 [69.23–99.69]	
		RU	1	1	0	1	0
				100 [5.46–100]		100 [5.46–100]	
PSC	**2009**		nd	nd	nd	nd	nd
	2011		nd	nd	nd	nd	nd
	2012	***An.* spp.**	1,907	115[a]	12[b]	nd	0

[a]Number of pools of 20 mosquitoes tested with the RT-PCR

[b]The number of half gravid and blood fed mosquitoes divided by the number of sleepers in the rooms visited the night before the collection

Abbreviations: *An*. spp., *Anopheles* species; HLC, HBR, human biting rate; Human landing catch; FU, *Anopheles funestus*; GA, *Anopheles gambiae*; PH, *Anopheles pharaoensis*; PSC, Pyrethrum spray catch; RU, *Anopheles rufipes*; nd, not done