Epidemiology of human filarial infections in Angola revealed by rapid surveys coupled with serological and molecular assays

Thesis submitted in accordance with the requirements of the University of Liverpool School of Tropical Medicine for the degree of Master of Philosophy in Tropical Medicine

By

Rossely Paulo

30th November 2020

Declaration

I hereby certify that this dissertation constitutes my own product, that where the language of others is set forth, quotation marks so indicate, and that appropriate credit is given where I have used the language, ideas, expressions or writings of another.

I declare that the dissertation describes original work that has not previously been presented for the award of any other degree of any institution.

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We confirm that the work reported in this thesis was carried out by the candidate and has been submitted for examinations with our approval as University supervisors.

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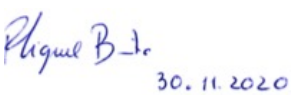
Signed: 

ABSTRACT:

The Republic of Angola is a priority country for onchocerciasis and lymphatic filariasis (LF) elimination, however, the co-distribution of loiasis, caused by the filarial parasite *Loa loa*, is a significant impediment. This is due to the increasing risk of severe adverse effects (SAEs) associated with ivermectin treatment(s) when used in mass drug administration (MDA) campaigns in *Loa loa* endemic areas. In Bengo province, Angola a putative high-risk, but under-surveyed, endemic zone for loiasis exists where alternative intervention(s) may need to be implemented. Within this province, the presence and geographical overlap(s) of the three filarial infections are not well defined. Furthermore, knowledge gaps in the vector biology of *Loa loa* exist. To this end, I undertook a two-phased epidemiological investigation of the three filarial diseases and I provided an up-to-date assessment of the biology of *Chrysops*, the insect vector of loiasis. In the latter I placed special focus upon opportunities to develop better vector control.

In Phase 1, 2007 individuals from 29 communities from Bengo province were surveyed. Community prevalence estimates were determined by Rapid Assessment Procedure for Loiasis (RAPLOA) and Rapid Epidemiological Mapping of Onchocerciasis (REMO) together with two questions on LF clinical manifestations (presence of lymphoedema and hydrocoele). Overall, low levels of endemicity, with different overlapping distributions were found. Loiasis was found in 18 communities with a prevalence of 2.0% (31/1571). This contrasted to previous results which predicted this area to be a high-risk zone. Onchocerciasis prevalence was 5.3% (49/922) in eight communities, and lymphatic filariasis prevalence was 0.4% for lymphoedema (8/2007) and 2.6% for hydroceles (20/761 males) in seven and twelve communities, respectively. The clinical mapping survey method helped to highlight that all three filarial infections are present in this zone of Bengo province and their fine scale spatial patterns.

In Phase 2, a novel combination of clinical, serological and DNA diagnostics was applied, where additional information was collected on participants' duration of residency, access to mass drug administration, knowledge of insect vectors and use of bednets. A total of 1616 individuals (38.1% male, 61.9% female), with an average age of 43 years, were examined. For *Loa loa*, 6.2% (n = 100/1616) individuals were found to have eyeworm, based on the rapid assessment procedure for loiasis (RAPLOA) surveys, and 11.5% (n =178/1543) based on nested PCR analyses of venous blood. *Loa loa* prevalence in long-term residents (>10 years) and older individuals (>60 years) were significantly higher,

and older men with eyeworm were better informed about *Chrysops* vectors. For *O. volvulus*, 4.7% (*n* = 74/1567) individuals were found to be positive by enzyme-linked immunosorbent assay (Ov16 ELISA), with only three individuals reporting to have ever taken ivermectin. For *W. bancrofti*, no infection was found using the antigen-based immunochromatographic test (ICT) and real-time PCR analysis; however, 27 individuals presented with LF related clinical conditions (lymphoedema = 11, hydrocoele = 14, both = 2). Just under half (45.5%) of the participants owned a bednet, with the majority (71.1%) sleeping under it the night before. Our approach of using combination diagnostics reveals the age-prevalence of loiasis alongside low endemicity of onchocerciasis and LF. Future research foci should be on identifying opportunities for more cost-effective ways to eliminate onchocerciasis and to develop innovative surveillance modalities for clinical LF for individual disease management and disability prevention. Taken as a whole, my results evidence that the utility of the earlier RAPLOA derived maps, based on surveys undertaken over a decade ago, are in need of revision and updating given the extent of population movement and environmental change, particularly deforestation. In future, further fine scale micro-mapping is required to more precisely delineate most appropriate interventions required for these complex co-endemic diseases.

Keywords: *Wuchereria bancrofti, Onchocerca volvulus, Loa loa*, prevalence, endemicity, Bengo, Angola.

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Dedication

To my parents, Julião Mateus Paulo and Rosa Cunha, for their dedication and unconditional love that helped me to become the human being that I am. To my brothers and sisters' thanks for the support. A special thanks to Célio N'Ginga and Filipa Vaz, my guiding friends, stimulating presences and critical thinkers. Big thanks to all my dear friends that I've shared my work with.

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To our community willing to participate in this project.

Leaving no one behind - WHO

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LIST OF ABBREVIATIONS

APOC	African Program for Onchocerciasis Control
CDC	Centers for Disease Control
CDDs	Community Directed Distributors
CDTi	Community-Directed Treatment with Ivermectin
DEC	Diethylcarbamazine
DNA	Deoxyribonucleic Acid
ELISA	Enzyme-Linked Immunosorbent Assay
ESPEN	Expanded Special Project for Elimination
FTS	Filariasis Test Strip
GPELF	Global Program to Eliminate Lymphatic Filariasis
ICT	Immunochromatographic Test
IVM	Integrated Vector Management
L. loa	*Loa loa*
LF	Lymphatic Filariasis
Mf	Macrofilariae
MDA	Mass Drug Administration
mf	Microfilariae
NHDP	National Health Development Plan
NTDs	Neglected Tropical Diseases
Ov	*Onchocerca volvulus*
OCP	Onchocerciasis Control Program
OEPA	Onchocerciasis Elimination Program for the Americas
PCR	Polymerase Chain Reaction
RAPLOA	Rapid Assessment Procedure for Loiasis
REMO	Rapid Epidemiological Mapping of Onchocerciasis
SAEs	Side Adverse Effects
STHs	Soil-Transmitted Helminthiases
SDGs	Sustainable Development Goals
UNDP	United Nations Development Program
WHO	World Health Organization
Wb	*Wuchereria bancrofti*

CHAPTER ONE: INTRODUCTION

This chapter presents the general concept of Neglected Tropical Diseases (NTDs) and more specifically on the helminth infections, the most common causes of chronic human infections worldwide – particularly schistosomiasis, lymphatic filariasis (LF), onchocerciasis and loiasis (Table 1.1). The aim is to provide an overview of current knowledge of filarial epidemiology, research and control activities globally and in Angola. Core content on the WHO report is that tackling NTDs significantly advances the Sustainable Development Goals (SDGs) agenda in all its breath and diversity; as such diseases are associated with poverty, poor sanitation, lack of access to safe water, and exposure to vectors.

They are classified as neglected diseases because until recently they received little attention at the global level and in affected countries. This thesis will then concentrate on LF, onchocerciasis and loiasis in Angola, which are considered endemic, based on recent national report.

Table 1.1: NTDs in the world today.

Bacteria	**Parasite**	**Virus**	**Injury**
Buruli Ulcer	Helminth	Dengue	Snakebite
Yaws	Cystiscercosis	Chikungunya	Podoconiosis
Leprosy	Dracunculiasis	Rabies	
Trachoma	Echinococcosis		
Mycetoma*	Foodborne trematodiases		
	Soil-transmitted helminthiases		
	Chagas disease		
	Leishmaniasis		
	Human African Trypanosomiasis		
	Schistosomiasis		
	Scabies		
	LF		
	Onchocerciasis		

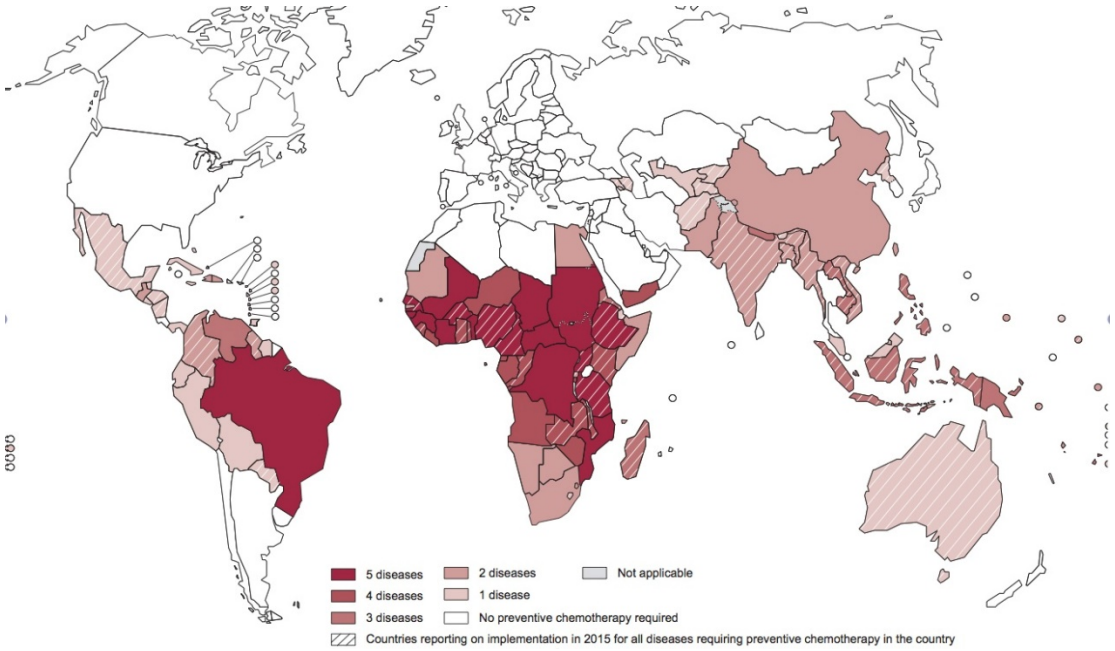
*Mycetoma is also classified as a fungus disease

Source: Adapted from WHO, 2017.

1.1 Background

NTDs are a diverse group of parasitic and communicable diseases that prevail in tropical and sub-tropical regions of the world (Figure 1.1). Populations living in poverty, without adequate sanitation and in close contact with insect vectors of NTDs and often zoonotic sources e.g., companion animals and livestock, are those worst affected. Most of the NTDs can be controlled or even eliminated through mass drug administration (MDA), alongside with, efforts to control the vectors such as mosquitoes and black flies that transmit such diseases.

Figure 1.1: Worldwide countries requiring and implementing preventive chemotherapy for five NTDs, by number of diseases including, LF, onchocerciasis, schistosomiasis, soil-transmitted helminthiases and trachoma diseases.



Source: WHO, 2017.

Elimination of NTDs has recently emerged on the global health agenda and gained prominence with the release of the global strategy and plan to combat them by the WHO. For instances, owing to the availability of sufficient intervention tools, the Global Program to Eliminate Lymphatic Filariasis (GPELF) was launched in 2000, with the aim of eliminating this disease as a public health problem by the year 2025 (WHO, 2013).

Historically, such diseases have been a serious public health concern in sub-Saharan Africa and hindered socioeconomic development in the affected areas. With the introduction of community MDA, preventive chemotherapy campaigns have been significantly scaled-up since the mid-1990s such that diseases have been much reduced while in Angola, they no longer pose an immediate public health problem although significant disease remains (Angola NTDs, 2017).

More broadly, NTDs affect the 1 billion poorest people in the world, and they stand in the way of achieving the SDGs. These diseases are most relevant to the goal to ensure healthy lives and ensure well-being for all at all ages (SDG 3), but they also affect and are affected by many of the other development areas covered by the 2030 Agenda for Sustainable Development (WHO, 2017). For example, NTDs have specific relevance for the SDGs aimed at poverty reduction (SDG 1) and ensuring the availability and sustainable management of water and sanitation (SDG 6) and ensuring that cities are sustainable, safe, resilient and inclusive (SDG 11). Efforts to mitigate the impact of NTDs will have a direct influence on the overall progress made towards achieving the SDGs (Figure 1.2).

The main objectives with NTDs among SDGs are integrating such diseases into global health and development; mainstreaming them in the context of Universal Health Care; and monitoring and financing such their control in the context of SDGs.

Figure 1.2: Sustainable Development Goals.

Source: SDG, 2019.

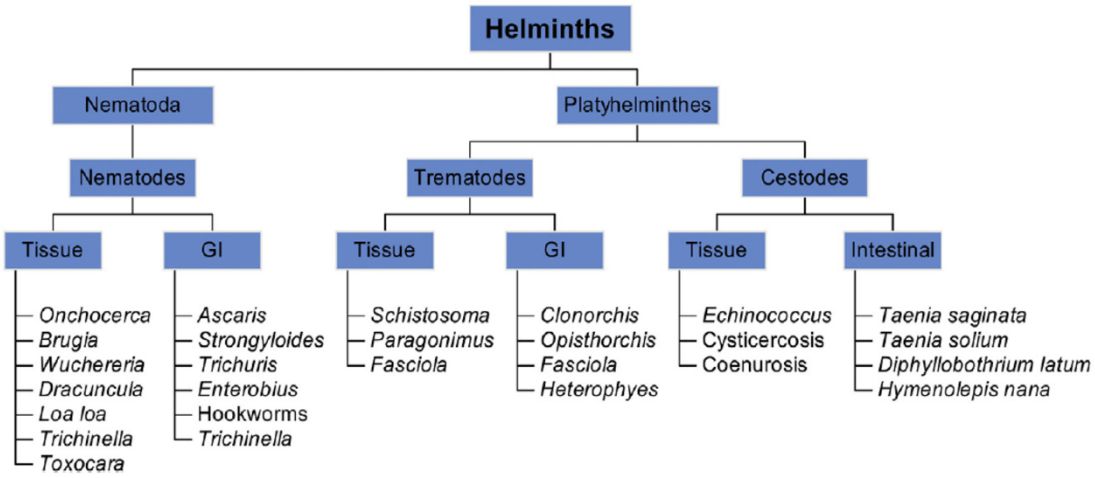
1.2 Introducing the NTDs

Collectively, the NTDs have several things in common as they proliferate in under-developed areas in countries across the income spectrum; settings where large numbers of people have little or no access to adequate health care, clean water, sanitation, housing, education and information. They are neglected in terms of the research and control funding allocated to them both by developing world governments and other donors (WHO, 2017).

Among the NTDs, there are parasitic diseases that can be controlled or eliminated by preventive chemotherapy, such as, LF, onchocerciasis, schistosomiasis, geohelminthiasis and trachoma (Figure 1.3). The soil-transmitted helminthiases (STHs) include hookworms (*Ancylostoma* & *Necator*), whipworms (*Trichuris*) and round worms (*Ascaris*) that inhabit the human gut, and their eggs are passed out in the feces. For schistosomiasis, adult *Schistosoma* worms live in the blood vessels of the human host and the eggs laid by the female worm cause the major symptoms. There are two forms of schistosomiasis, urogenital and intestinal, where host organs such as the bladder or bowel undergo progressive damage respectively. The schistosomes utilize certain freshwater

snails as obligate intermediate hosts, and these snails become infected when human excreta (feces and urine) are deposited in fresh water. Blindness due to trachoma is caused by the after-effects of conjunctivitis caused by *Chlamydia* infections carried by flies (Table 1.2).

Figure 1.3: Diagram of helminth nomenclature.



Source: King, 2019.

The challenges ahead for preventive chemotherapy have been recognized and might interfere with future progress after 2020, also now being in sight of WHO 2021-2030 NTD Roadmap targets. As more than 1 billion tablets of albendazole and mebendazole are administrated annually through preventive chemotherapy programs, open questions remain whether these nematodes evolve towards drug resistance and therefore challenge the long-term success of chemotherapy approaches alone (King, 2019). Furthermore, the systematic issues of non-compliance in individuals that frequently miss or refuse treatment may be able to sustain ongoing transmission (Farrell *et al*, 2018)

Indeed, current and future deficiencies in adequate sanitation increases transmission of STHs. It has been predicted that STH will remain a public health concern beyond 2020 in many countries where STH is currently endemic (WHO, 2017).

1.3 The anti-filarial drugs in MDA

In order to extend the health system coverage, to reduce poverty and social ostracism as well as advocating reasonable integrated public health perspectives, MDA has been the recommended strategy for control where at least 75% of the population infected by certain NTDs is targeted for treatment (WHO, 2010). The rationale for MDA in filarial infections is to lower the circulating populations of microfilariae (mf) therefore lowering the transmission without the need for additional vector management (Molyneux *et al*, 2018). The WHO has launched global elimination programs, involving MDA, where the GPELF is based on an annual single dose of diethylcarbamazine (DEC) or ivermectin combined with albendazole, while the APOC is based on MDA of ivermectin only.

Anti-filarial drugs used in MDA for LF are majorly microfilaricidal and these include albendazole, ivermectin and DEC. The latter drug is not recommended to use in patients from Africa due to loiasis or onchocerciasis co-infection and associated SAEs (Muller, 2002; Cano *et al*, 2018). Although these regimens are significantly safe and efficacious against mf (the larval progeny stage), these are not considered to exert a powerful macrofilaricidal (adult stage killing) effect on the long-living adult worms (Cano *et al*, 2018). In order to interrupt transmission, MDA must be continued, at high levels of treatment coverage and adherence, for at least as long as the duration of the reproductive lifespan of the adult worms, therefore ranging from 4-12 years for *Wuchereria bancrofti* (Stolk *et al*, 2015).

For loiasis treatment, DEC kills both adults and mf for a period of 14-21 days, however, SAEs including oedema, nausea, meningoencephalitis and even coma might occur (Muller, 2002). Ivermectin decreases mf levels by 90% and reduces prevalence from 30%-10% but these might return within a period of 2 years (Muller, 2002). Albendazole causes mf numbers to fall slowly and may have an adulticidal action (Muller, 2002).

The WHO strategy against onchocerciasis consists of MDA with community-directed treatment with ivermectin (CDTi) that has shifted its goal from morbidity control to disease elimination by 2025 (WHO, 2012b). Currently, this is the only drug in widespread use that has completely replaced DEC, which is no longer advocated for this condition (Muller, 2002). Ivermectin is a very efficient microfilaricide as it sterilizes temporarily

the female worm mf production for several months, therefore lowering morbidity and transmission (Cano *et al*, 2018). Thus, in order to interrupt transmission, MDA must be continued for another 9-11 years for *O. volvulus*, with 95% of the worms ending reproduction by the age of 13-15 years (Cano *et al*, 2018). Nevertheless, ivermectin resistance has been reported in parasitic nematodes including *O. volvulus* due to common markers, mainly the *β-tubulin* gene in human *O. volvulus* (Lustigman *et al*, 2007). Furthermore, Campille *et al* (2020) has reported for the first time, a limited prophylactic effect on *O. volvulus* by reporting a raising count of nodules in 17.7% of the subjects administered with ivermectin for 3-monthly as to those treated annually. Since presently there are no powerful drugs against adult worms, major efforts have been put on a promising drug named as auranofin, able to kill adult *Onchocerca* and adult *Brugia* spp. *in vitro* (Bulman *et al,* 2015).

For mansonelliasis, DEC has no effect while albendazole and mebendazole given for 10 days have given significant results (Muller, 2002). Ivermectin appears to reduce mf for a period but probably has no long-term effect (Muller, 2002).

The endosymbiotic *Wolbachia* is a vital bacterium in the most common filarial parasites of humans as it promotes growth, development, fertility and survival (Taylor *et al*, 2005). As a result, clinical trials have reported that extinguishing *Wolbachia* from *O. volvulus* and *W. bancrofti* by administering oral doxycycline, perpetually sterilizes female worms and shrinks the adult worm long-life, promoting powerful macrofilaricidal activity. Doxycycline has been reported to be a highly safe drug as it reduces antifilarial activity of anti-*Wolbachia* therapy by avoiding inflammatory responses linked to prompt killing of micro- or mf within patients attending clinical settings (Taylor *et al*, 2010). Therefore, these functions are highly superior to those drugs previously mentioned used for antifilarial activity (Taylor *et al*, 2010; Walker *et al* 2015).

Overall, there are three main perspectives in which anti-*Wolbachia* therapy could be applied in the population based on ‘test and treat’ which include 1) Offering doxycycline as an alternative in *Loa loa* co-endemic area with onchocerciasis and/or LF instead of ivermectin; 2) It can be also used to ‘clean’ residual infections in suppressed but not yet interrupted foci areas, and, 3) Key backup method in areas where minimal efficacy of ivermectin have been recorded (Taylor *et al*, 2010; Taylor *et al*, 2009; Churcher *et al*, 2009).

Table 1.2: NTDs and control strategies.

NTD	*Status*	*Control Strategy*
Diseases controllable by MDA		***Mass Drug Administration (MDA)***
Soil Transmitted Helminths	Over 1 billion people infected globally	Annual treatment with albendazole or mebendazole
Schistosomiasis	200 people million infected – mostly in Africa from water contact	Treatment with praziquantel, improved water supplies
Lymphatic filariasis	120 million people infected in Africa and Indian continents, but elimination is possible	Elimination strategy by six Mass Drug Administration rounds with albendazole + ivermectin (in Africa) or albendazole + DEC (elsewhere)
Trachoma	192 million people at risk infection in worldwide	Annual treatment with Zithromax, as part of SAFE strategy: surgery for those with trichiasis, antibiotic treatment to clear conjunctival infection cleanliness and environmental improvement to reduce transmission
Onchocerciasis	26 million people infected in sub-Saharan African countries	Control of symptoms by annual treatment with ivermectin
Yaws	89 million people are at risk in 13 countries where treponematoses are endemic	Single-dose azithromycin
Mycetoma	There is currently a lack of accurate data on mycetoma's incidence, prevalence and distribution. 75% of cases were reported from Mexico, Sudan and India	Treatment options of the causative organism. Bacterial mycetoma requires long-term treatment with a combination of antibiotics. For the fungal type, treatment is based on anti-fungal agents, usually followed by surgical excision of the lesions
Food-bore trematodiasis	Asia and Latin America are the most affected	Depending on the condition, triclabendazole and praziquantel are recommended respectively. Cooking food and behavioral changes in food hygiene
Guinea worm	Close to eradication	Individual case finding case containment, clean water provision and filtration, vector control. Regular surveillance of endemic villages. No vaccine or medication is available to prevent or treat the disease
Scabies	Occurs worldwide	Permethrin cream, Sulphur (4%) ointment. 15% benzyl benzoate. Oral ivermectin.
Diseases requiring individual treatment		***Case control***
Leprosy	Close to elimination	Case finding followed by multidrug therapy for 6-12 months
Buruli ulcer	Endemic in 30 countries -Americas, Africa and SE Asia	Early diagnosis, treatment with antibiotics or surgery
Chagas diseases	Limited distribution in South America – a disease of poor housing	Control of the 'kissing bugs' which carry the diseases
Human African trypanosomiasis	Narrow in Africa dictated by tsetse fly distribution	Case finding and treatment, vector control where appropriate
Cutaneous leishmaniasis	12 million people presently infected worldwide	Early diagnosis and prompt treatment; control sandfly populations through residual insecticide spraying of houses and through the use of insecticide-impregnated bed nets
Visceral leishmaniasis	500,000 cases per year	Case finding and treatment with meglumine antimoniate or sodium stibogluconate
Dengue	250 million at risk and 50 million cases per year in over 100 countries	No effective medications exist to treat dengue infection. Fluids and possibly transfusion and vector control. A limited use attenuated dengue vaccine has been licensed since 2015. Two new live attenuated dengue vaccines are currently in phase III efficacy trials, OSV4 indigenous tetravalent dengue subunit vaccine in under-development
Chikungunya	It occurs in Africa, Asia and the Indian subcontinent. In recent decades, there have been outbreaks of the disease in countries that have never recorded cases before	No effective antiviral medications exist to treat chikungunya infection. Fluids and vector control. Several vaccine candidates are currently being developed
Snakebite	An estimated 5.4 million people are bitten each year with up to 2.7 million envenoming. Around 81 000 – 138 000 people die yearly	Snake venoms
		Animal zoonosis
Neuro-cysticercosis	Up to 20% infections in rural Africa and South America	Tape worm control and strict pig meat inspection. Vaccination and chemoprophylaxis in pigs have been widely used as a control strategy
Echinococcus	Unknown numbers with cysts in liver	Tape worm control in dogs and careful surgery plus albendazole to remove unbroken cysts
		Animal reservoir
Rabies	Transmitted by dog bites	vaccination

Source: Adapted from WHO, 2017; Smits, 2009.

1.4 Lymphatic Filariasis

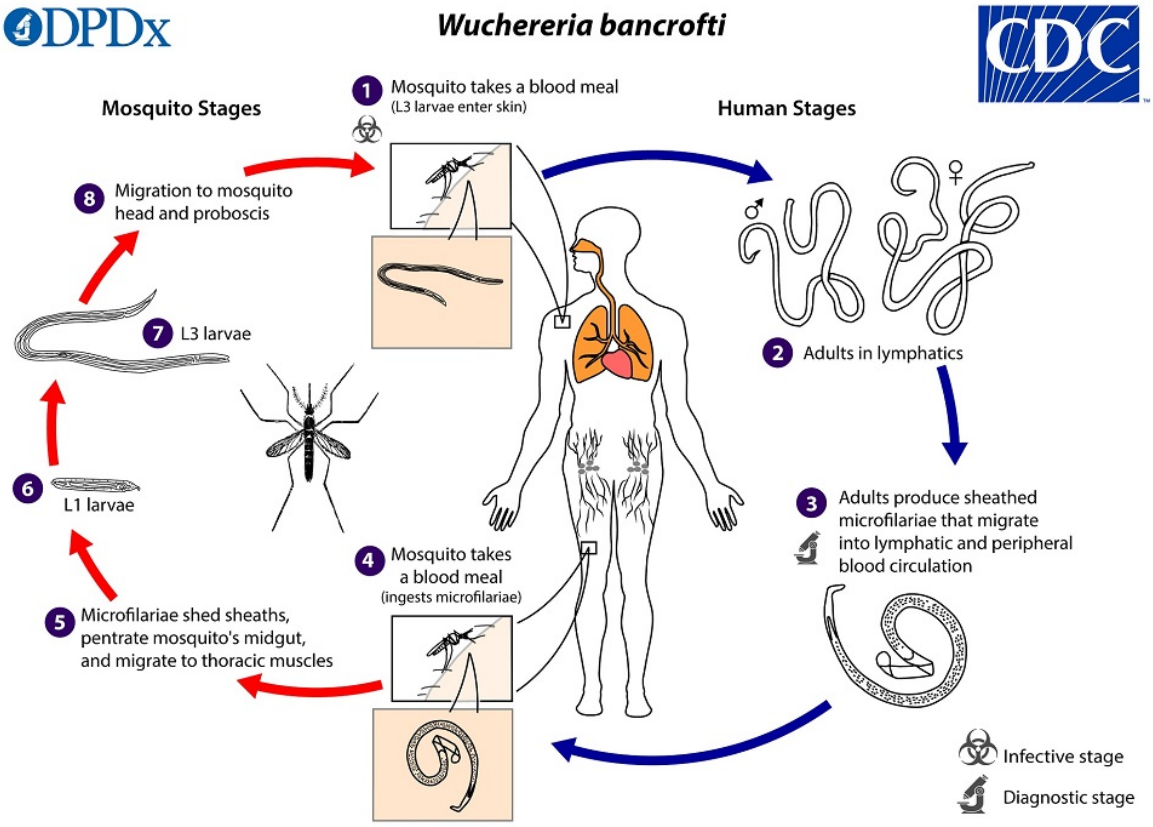
1.4.1 Parasites

LF is caused by an infection with the parasites *W. bancrofti*, *Brugia malayi* or *Brugia timori*, which are classified as nematodes of the family Filariodidea (Figure 1.9 Panels A-C). *W. bancrofti*, the most widespread of the three species, is responsible for more than 90.0% of cases (WHO, 2017). The sheathed mf circulates in the bloodstream and, in most parts of the world show a marked nocturnal periodicity as they are found in the peripheral circulation from 10pm-2am, while during the diurnal period they hide within the capillaries of the lungs (Mathison *et al*, 2019).

The transmission cycle of *W. bancrofti* occurs in a way that when the mf is ingested with a blood meal by the mosquito, they lose their sheath within 15-30 min in the stomach of the insect (Figure 1.4). Fractions of the mf manage to diffuse into the stomach wall prior the formation of a peritrophic membrane and migrate to the thoracic muscles within 24h. Within two days the mf has evolved into sausage-like larvae, measuring 150 μm x 10 μm. Within five days the larvae have evolved further and moved to the alimentary canal, where it has matured into the second-stage larvae measuring 250 μm x 25 μm. On the second week the larvae evolved more and elongate greatly (1.2-1.8 mm), reaching the infective third-stage larvae (L3), moving to the head, where they infiltrate the labium and proceed through the tips of the labella while the mosquito is feeding.

Succeeding the infiltration into the skin of a human, larvae migrate to the lymph vessels, nodes and tissue, where they moult twice again and evolve and the females reproduce mf within 1 year. The adult female worms reproduce mf for a period of 5 years, although longer periods of 10-17 years have been observed in the literature (Muller, 2002). Furthermore, adults of *Wuchereria* and *Brugia* spp. contain a bacterium, *Wolbachia*, in the cords sideways and it has latterly been suggested that these bacteria are accountable for inflammatory actions, which is a protective effect for the filariae from the host immune response, hence, being vital for the continuous survival of the filarial (Muller, 2002). According to Slater *et al*, (2012), humans are the only reservoir host of the LF parasite in Africa.

Figure 1.4: Life cycle of *Wuchereria bancrofti*.



Source: CDC, 2018.

1.4.2 Vectors

These nematode parasites are transmitted by various species of mosquito vectors from the genera *Anopheles, Aedes, Culex, Mansonia, Coquillettidia* and *Ochlerotatu*s (Cano *et al*, 2018). The major *Anopheles* vectors of LF in Africa are *Anopheles gambiae s.l*, and *Anopheles funestus* group. These species complexes are made up of distinct species, which are morphologically indistinguishable and may occur in sympatric situations (Souza *et al*, 2012). Five chromosomal forms namely, 'Forest', 'Bissau', 'Bamako', 'Mopti' and 'Savannah' have been described (McGreevy *et al*, 1982). The Mopti form of *Anopheles gambiae s.s*, for example, is believed to be associated with *W. bancrofti* compared with other species (Souza *et al*, 2012).

Therefore, understanding the roles of different vectors in LF transmission and the implications for accelerated interruption of transmission in Africa where the LF vectors are also targeted through malaria control efforts is important (Souza *et al*, 2012). LF shares the same vectors with malaria in most African countries and the practices for controlling the vectors of malarial parasites, such as the use of insecticide treated bednets, indoor-residual spraying for personal and community protection, can at the same time be effective against both malaria and LF (Souza *et al*, 2012).

1.4.3 Disease

This condition causes a wide range of clinical signs and symptoms, including lymphoedema, hydrocele, lymph scrotum, chyluria, tropical pulmonary eosinophilia, adenopathy, hematuria, and various manifestations of worms in ectopic sites (Addiss *et al*, 2003).

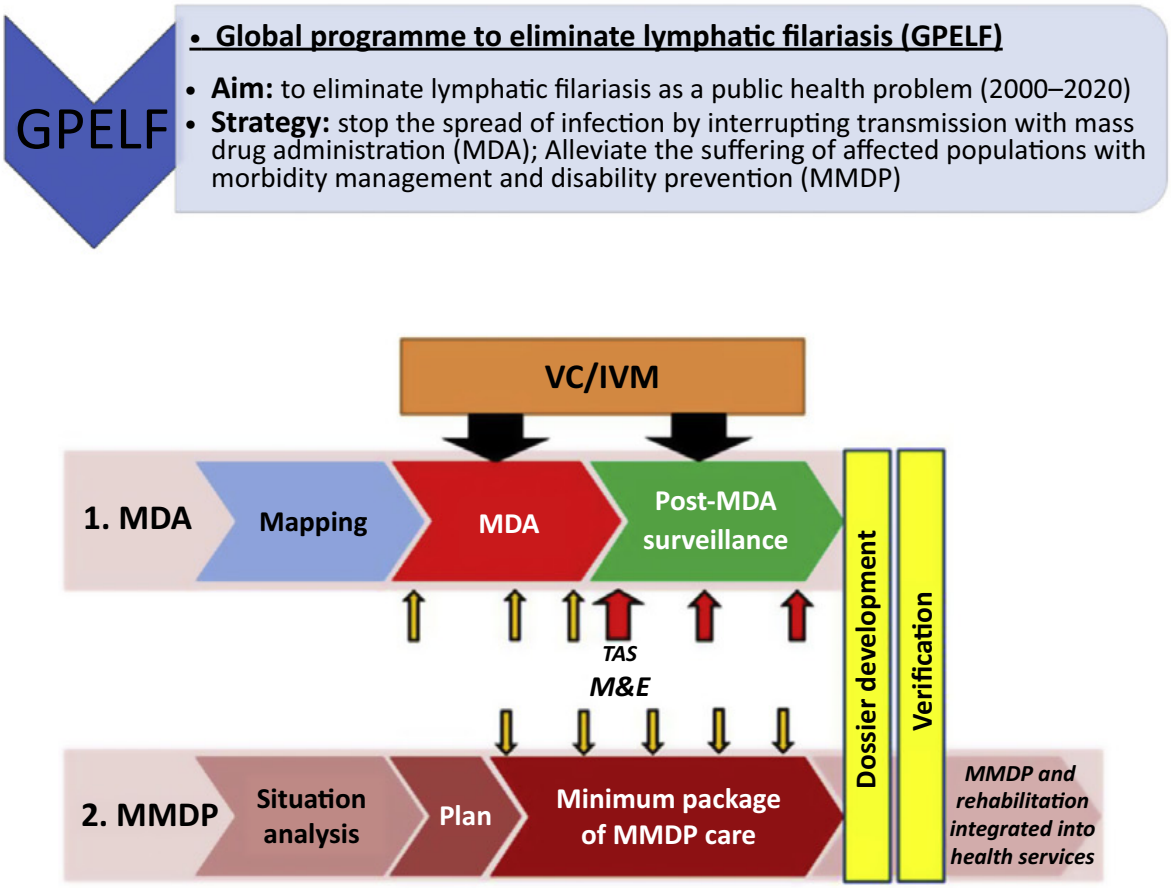
Individuals living in the endemic area can be categorized into two major groups, infected and uninfected individuals based on their clinical and parasitological status. The infected group includes asymptomatic mf carriers, acute disease, chronic disease (both elephantiasis and hydrocele) and cryptic infection. Asymptomatic patients are mf positive without any symptoms. Cryptic individuals are free from mf and acute chronic symptoms but positive for circulating filarial antigen, which is the marker of live adult worms. Acute filarial diseases are identified as those having localized signs and symptoms of pain.

Uninfected individuals are free from mf, circulating filarial antigen and filarial symptoms (Bal *et al*, 2015). The earliest onset of lymphoedema in filariasis-endemic areas is usually observed around the time of puberty, and the prevalence increases with age (Addiss *et al*, 2003). In many areas where bancroftian filariasis is endemic, lymphoedema of the leg is more common in women than in men. Scarification of the skin, a traditional practice in many filariasis-endemic areas, is considered a risk factor for rapid progression of filarial elephantiasis because of the increased risk of acute dermatolymphangioadenitis (Addiss *et al*, 2003). Another form of non-filarial elephantiasis, that is often not identified correctly and misdiagnosis as LF due to its similar etiology, is podoconiosis that also occurs in tropical settings in genetically prone individuals who are unprotected to irritant volcanic soils (Chandler *et al*, 2020; Deribe *et al*, 2018). Furthermore, other causes of secondary lymphedema to have in account involve rheumatic heart disease, endemic Kaposi sarcoma, river blindness and liver failure (Deribe *et al,* 2015).

Hydrocele is the most frequent disease manifestation of *W. bancrofti* infection in men, yet its pathogenesis is poorly understood. Hydroceles develop most commonly during adulthood and are a consequence of the accumulation of excess fluid between the serosal and visceral layers of the tunica vaginalis, which lies between the testis and scrotal sac. Hydroceles may be a consequence of unreduced inguinal hernias or orchitis and epididymitis secondary to infectious agents such as *Mycobacterium tuberculosis*. In *W. bancrofti* infection, it is likely that hydroceles result from inflammation of the tunica vaginalis or impaired lymphatic drainage through the spermatic cord (Tobian *et al*, 2003).

A major goal of the GPELF (Figure 1.5) aims to interrupt the infection through MDA and alleviate suffering among people with chronic disease through morbidity management. The strategy that WHO recommends achieving these aims is large-scale annual treatment MDA of all eligible people in all areas where infection is present to stop transmission and managing morbidity through a minimum package of care to alleviate and prevent the disabling manifestations of the disease. A more recent estimate of the impact of MDA from 2000 to 2012 suggests that the burden has been almost halved to around 67 million people infected and as many as 36 million living with hydrocele and lymphoedema. The roadmap sets a target for global elimination of LF as a public health problem by 2020.

Figure 1.5: GPELF strategy.



Source: Ichimori *et al,* 2014.

Note: VC= vector control, IVM=integrated vector management; TAS= transmission assessment survey, M&E monitoring and evaluation

Another major goal of the GPELF is to provide basic care for individuals who suffer from the major forms of filariasis-related morbidity, both acute such as inflammatory episodes and chronic such as lymphoedema and hydrocele (Addiss *et al*, 2003). In LF, stigmatization is a common and serious problem for those affected, as individuals might experience suicidal thoughts, dissolution of marriage plans due to diminished economic productivity and attractiveness; often receiving insults and being excluded at social events (Kebede *et al*, 2018).

According to the GPELF, the primary intervention includes the use of footwear, regular foot hygiene and the application on floor coverings to reduce the contact between feet and the irritant soil (Negussie *et al,* 2018). Secondary and tertiary interventions focus on the management of the lymphoedema-related morbidity and include wound care, exercise, elevation of the legs, treatment of acute attacks and providing psychological and socio-economic support to those affected. However, before morbidity management and disability prevention activities for both diseases can be implemented, better patient estimates and an understanding of the distributions of LF at community level are vital (Kebede *et al*, 2018).

1.4.4 Diagnostics

The general available diagnostic techniques for LF have the purpose to detect mf, circulating filarial antigen, anti-filarial antibodies and molecular based tests. The most conventional techniques used for detection of mf include the thick blood smear method also known as TBS20; the Knott's concentration method, the counting chamber method and the membrane filtration technique (McMahon *et al*, 1979; Melchers *et al*, 2020).

The main limitations of this group of techniques include low sensitivity and longer time required examining the specimen. The most common techniques for detection of circulating filarial antigens include the immunochromatographic test (ICT) and the Og4C3 ELISA (Njenga *et al*, 2001). The unit price is a major limitation for extensive field application and the requirement of a well-equipped laboratory infrastructure and staff, respectively for each technique. The most common technique for detection of anti-filarial antibodies includes IgG4 ELISA and the main handicaps are possible cross-reactivity and a well-equipped laboratory and equally well-trained staff (Lammie *et al*,

2004). Molecular based tests include polymerase chain reaction (PCR), which detects parasite deoxyribonucleic acid (DNA) extracted from mosquitoes or from human blood samples (Simonsen *et al*, 2008).

1.4.5 Control and Elimination

Vector control is among the five strategies recommended by the WHO for prevention, control, elimination and eradication of NTDs in its road map for implementation. Prior to 2012, the WHO strategy for LF was based primarily on chemotherapy (Souza *et al*, 2012).

Vector control is an important component in preventing and controlling vector-borne diseases, specifically for transmission control. For instances, the mosquito *Culex quinquefasciatus*, which breeds in stagnant and organically polluted water, is globally the most important vector for *W. bancrofti* and is an important target for vector control (Smits, 2009). Community-based actions to combat *Anopheles* comprise activities to educate and empower communities to identify and remove mosquito-breeding habitats in households and the immediate vicinity, as well as other settings where human-vector contact occurs, such as schools, hospitals and workplaces. Mosquito breeding can also be prevented through the provision of reliable piped water and regular solid-waste management and by installing screens in houses. Further vector-control methods include ensuring personal protection by using insect repellents and insecticide-treated bed nets and by providing indoor residual spraying. However, increasing resistance to insecticides may reduce their effectiveness over time (WHO, 2017).

1.5 Onchocerciasis

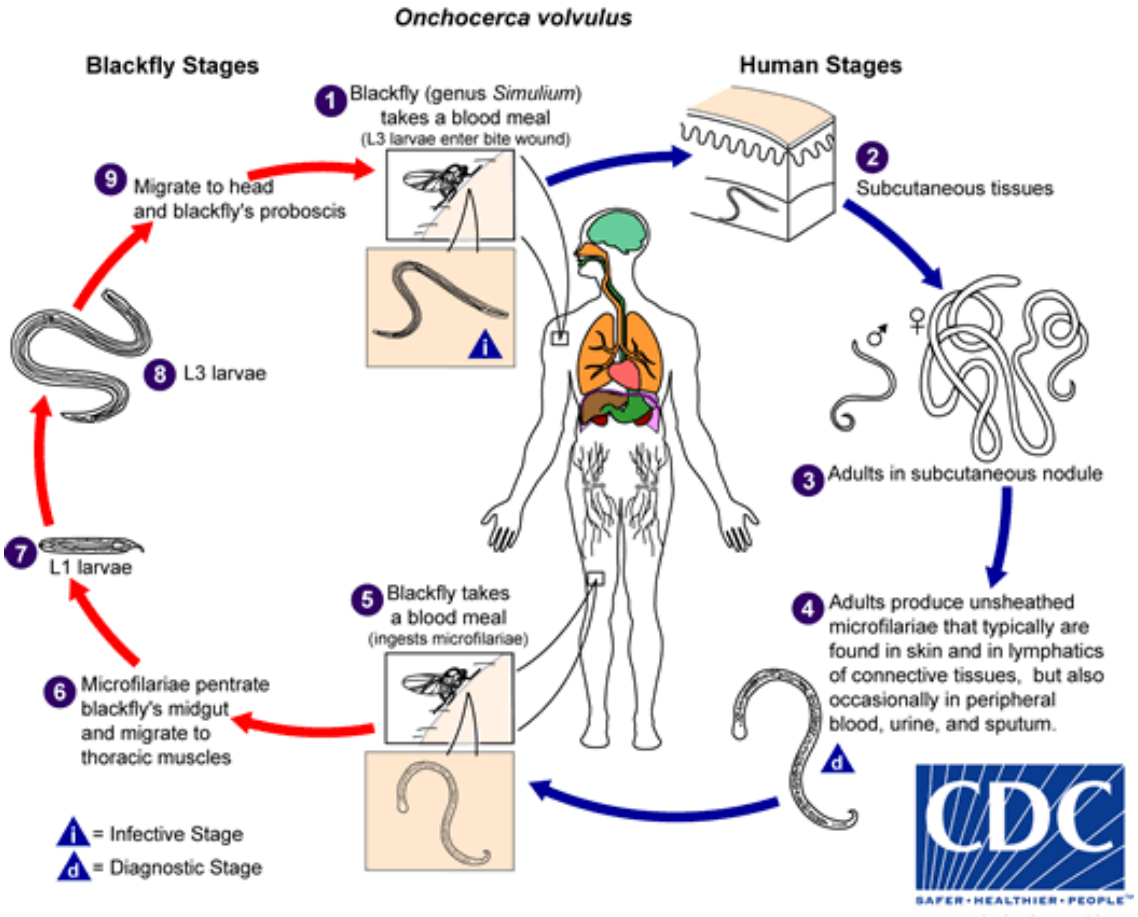
1.5.1 Parasites

The life cycle of *O. volvulus* comprises a human stage and a vector stage, with the blackfly vector having its own distinct lifecycle, also known as holometabolous (Figure 1.6). The transmission cycle of *O. volvulus* occurs in a way that when the mf are ingested by blackflies of the genus *Simulium*, they are attracted to the biting site by saliva injected into the puncture wound. As a result, a portion of the mf ingested by the blackfly clear out the midgut prior the formation of the peritrophic membrane and diffuses into the thoracic muscle, distinctly the flight muscles cells, while others are confined in the midgut and die.

The first moult in the muscles of the fly generates a sausage stage, succeeding by the second moult that generates the infective third-stage larvae (L3) which then progress to the head and proboscis, concluding their maturation within 12 days, determined by the ambient temperature as no growth takes place in conditions lower than 18ºC (Muller, 2002). The infective third-stage larvae disseminate through the puncture wound when an infected *Simulium* blackfly bites another individual. They move to the subcutaneous tissues, moult twice and outreach sexual maturity within 15 months. The *O. volvulus* mf measure 280-330 μm x 6-9 μm. They are unsheathed and have a tapered tail, often flexed and non-nuclease (Figure 1.9 - Panel D).

Macrofilariae can be detected in the skin within 18 months succeeding the infection but not usually in the blood, unless after the treatment DEC treatment (Muller, 2002). The mf reproduces and live on about for 9-11 years, and exceptionally live for more than 13 years mainly in the subcutaneous and deep tissue nodules regions; while mf can live in the skin for up to two years (Muller, 2002; Mitra *et al*, 2017). Periodicity of mf of *O. volvulus* in the skin is non-specific (Mathison *et al*, 2019; Kuesel, 2016).

Figure 1.6: Life cycle of *Onchocerca volvulus.*



Source: CDC, 2013.

1.5.2 Vectors

The blackfly *Simulium damnosum s. l.* transmits the causative agent *O. volvulus*, which causes blindness and skin pathologies in humans. Nine sibling species of *S. damnosum* complex have been taxonomically identified and documented in East Africa. The species include *S. sirbanum, S. damnosum sensu stricto, S. dieguerense, S. sanctipauli, S. soubrense, S. squamosum, S. yahense, S. leonense* and *S. konkorense* (Ibeh *et al*., 2006). The first three species are known as savanna flies which transmit the savanna strain of *O. volvulus* while the rest belong to the forest group and transmit the forest strain of the parasite which causes more skin disease than blinding disease (Ibeh *et al*., 2006).

Blackflies changes in the distribution patterns of *S. damnosum* occur annually in association with dry and wet seasonal climatic changes. Other factors influencing species distribution include the physical and chemical properties of rivers and human activities that change the habitats of blackflies, e.g., deforestation and hydropower dams (Zarroug *et al*, 2016).

1.5.3 Disease

The most common symptoms of the disease range from itching to blindness, which are caused by an inflammatory reaction due to immunological response to the death of the microfilariae (WHO, 1995). Non-tender nodules, also known as onchocercomata are often reported which can be felt and seen over bony prominences. Furthermore, depending on the geographical region, if reported in Africa, those nodules are often found in the lower parts of the body such as the pelvic region, knees and lateral chest, contrary to the Americas where they are often found in the upper part of the body including the head (Muller, 2002). Other skin conditions related to onchocerciasis are pruritus, itchy rash and 'leopard skin' in a later stage. Interestingly, skin changes often mimic those seen in vitamin A deficiency (Muller, 2002).

Besides the skin, the eye is affected as the mf in the anterior chamber induce chronic inflammation leading to sclerosing keratitis and blindness if untreated, giving the disease its popular name 'river blindness'. Overtime the inflammatory response to dying *mf* can cause severe itching, disfiguring skin disease and visual loss or blindness (CDC, 2013)

1.5.4 Diagnostic

Traditionally, microscopic analysis through parasite mf counting of skin snip biopsies used to be the gold-standard technique, but, because of the high insensitivity and invasiveness reported in the literature, it has been replaced by the Ov16 serological test (Thiele *et al*, 2016).

1.5.5 Control and Elimination

Onchocerciasis has been the pivot of the international community; as it is strongly linked to morbidities such as blindness, skin disease and an association with childhood epilepsy (Colebunders *et al*, 2018). The concern about this infection has led to the implementation of control interventions essentially vector control (*Simulium* control) and CDTi (WHO, 2017a). The main long-term control programs that target the filarial parasite *O. volvulus* are the Onchocerciasis Control Program (OCP) in West Africa, the Onchocerciasis Elimination Program for the Americas (OEPA) and the African Program for Onchocerciasis Control (APOC) in the remaining part of Africa.

The OCP was established in 1974 and ceased in 2002 in West Africa countries, and has been described as successful, as onchocerciasis is no longer considered a public health concern in the savanna regions of ten OCP countries (Cupp *et al*, 2019; WHO, 2015). OCP's primary goal was based only in vector control intervention, targeting at the immature stages of the *Simulium* vectors, but later was aggregated to the interruption of parasite transmission through annual or biannual MDA treatment with ivermectin in that region also known as Special Intervention Zone (Remme *et al*, 2017; Stolk *et al,* 2015; WHO, 2015).

OEPA was acknowledged in 1990 with the purpose of eliminating the disease in every region from thirteen isolated endemic foci in six Latin American endemic countries, and as a strategy, a mass bi-annual ivermectin treatment takes place, in contrast to Africa (Webster *et al*, 2014). By 2013, transmission has been verified to be eliminated by WHO, in four of the six OEPA countries which still requiring monitoring for the following years ahead (Webster *et al*, 2014).

In 1995 APOC was implemented in view of eliminating onchocerciasis through annual CDTi in all lasting 20 countries in Africa that were not covered by the OCP, which had more than 80% of the global burden of the onchocerciasis, therefore representing 14.9 million infected individuals at the time (Remme, 1995; O'Hanlon *et al*, 2016). APOC main goal was to achieve morbidity control by targeting only communities with high infection rates (>20% nodule prevalence in adult males) through community directed annual treatments and Rapid Epidemiological Mapping of Onchocerciasis (REMO) (Cupp *et al*, 2019; Zouré *et al*, 2014). Unfortunately, APOC was not successful in making the transition from a morbidity control program to a transmission elimination program, and as a result it has formally ended in December 2015, where its considerably achievements are now being built by the Expanded Special Project for Elimination (ESPEN) of NTDs (WHO, 2015). In addition to working towards onchocerciasis elimination, this expanded program is also focused on accelerating the reduction and elimination of other NTDs from the African Region by 2020, namely LF, schistosomiasis, STHs and trachoma (WHO, 2017).

The current status of the global program to eliminate the transmission of onchocerciasis reports that although some countries including Angola have implemented MDA with less than 100% geographical coverage, the number of people identified as residing in areas at risk for infection is expected to enhance as another couple of countries had not reported treatment data at the time of the most recent report (WHO, 2018; ESPEN, 2017).

As there are no critical animal reservoirs of *O. volvulus* to preserve the transmission dynamics autonomous from the human population, long-lasting elimination of transmission of onchocerciasis can be obtainable, as reported in four countries in the Americas and in particular parts of Africa (Katabarwa *et al,* 2014; Diawara *et al*, 2009). However, the shifting towards elimination has identified particular issues that has been slowing this process down which include lacking coverage, access to treatment; treatment adherence; sub-optimal responses to ivermectin; and cross-border issues (Colebunders *et al*, 2018a). The lack of coverage and access to treatment has been challenging in many African countries where prevalence is greater than 60% and CDTi is performed annually, therefore requiring more coverage and longer treatment durations (Kim *et al,* 2015; Kamga *et al*, 2016). Issues in regards the treatment might include access and poor treatment compliance such as inadequate supply; restrict access to remote areas,

insufficient training and poor resources (Njim *et al*, 2017). Furthermore, treatment adherence issues also include cross-border issues, seasonal migration of workers, lack of incentives for community directed distributors (CDDs) and population reservation regarding side effects, and, ivermectin treatment in loiasis co-endemic areas that can result in SAEs (Senyonjo *et al*, 2016).

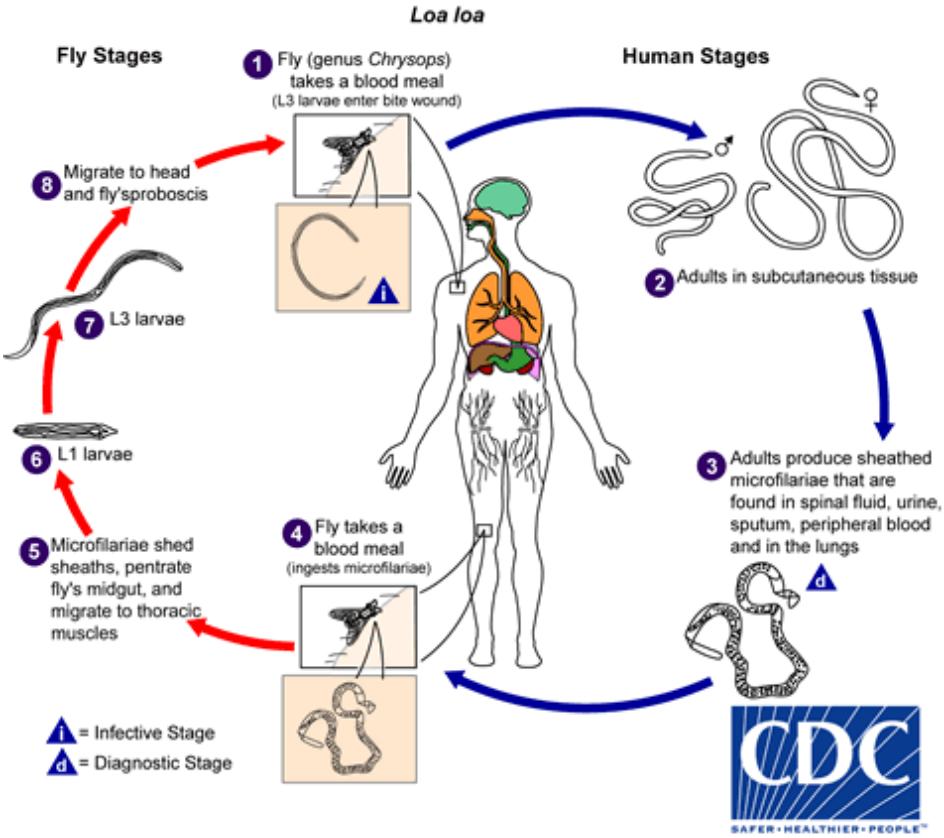
1.6 Loiasis

1.6.1 Parasites

The adults are thin transparent worms, the females measuring 70 mm x 0,5 mm, the males 30-35 mm x 0,3-0,4 mm, while the mf are sheathed and measure 250 (230-300) μm x 6-8 μm (Figure 1.9 – Panel E). The tail is short and relatively thick, with large nuclei continuing to the tip. The sheath does not stain with Giemsa or Wrights stain, it does with haematoxylin, so that the mf is sometimes mistakenly identified as being unsheathed (Muller, 2002).

Mf detected in the peripheral blood suggests a diurnal activity, therefore, being greatly abundant from 8am to 5pm. As the mf is ingested within female *Chrysops'* blood meal, they misplace their sheaths in the internal side the stomach of the fly and invade the gut wall following 6 hours (Figure 1.7). They mature in the cells of the fat body and moult twice. The third-stage larvae (L3), sizing 2 mm x 25 μm, diffuse primarily to the thorax and then to the head afterwards where they become infective within 12 days. The larvae migrate down to the labium during a new human blood meal, entering into the wound. They move through the human subcutaneous connective tissues and muscle fasciae, maturing twice and growing into adults within 12 months. The adults live for 4-17 years (Muller, 2002). Humans are the primary reservoir for *Loa loa* (Slater *et al*, 2012).Furthermore, it is shown that age affects the prevalence of *mf* in such a way that the percentage of mf carriers increases and reaches a plateau at about 45 years. The low proportion of mf in younger age group is supported by the fact that their exposure time has not been sufficient to facilitate biting by infected *Chrysops* (Djikeussi *et al*, 2014).

Figure 1.7: Life cycle of *Loa loa.*



Source: CDC, 2019.

1.6.2 Vectors

Chrysops silacea and *Chrysops dimidiata* are the main vectors of *L. loa* parasite which are confined to the tropical rainforest of Central and West Africa, but also *C. centurionis; C. distinctpennis, C. langi, C. longicornis, C. zahrai* and possible *C. streptobalius* could act as vectors, mainly in the forest-fringe region (Akue, 2016). Furthermore, *Chrysops* also known as the mango, mangrove or softly-softly fly, rubber plantations and palm oil groves, that commonly bites lower the knee and is a pool feeder (Akue, 2016). They belong to the Tabanidae family living in the forest canopy and are particularly attracted by smoke of wood fires and darker colors or the color blue/light blue (Akue, 2016).

1.6.3 Disease

The main features that characterize *L. loa* infection can evolve from local involvement to become a systematic disease. The most common symptoms are Calabar edema or Calabar swellings, which commonly appear on the arm. The ocular passage of an adult worm is another common sign. Loiasis can develop to a more sensitive life-threatening disease such as endomyocardial fibrosis or lymphoma. Further symptoms include pulmonary loiasis and encephalitis (Akue, 2016).

1.6.4 Diagnostics

The gold standard indicators and diagnostics for loiasis involve parasitological identification in diurnal blood smears to detect microfilaremia performing with light microscopy (Boatin *et al*, 2002). Furthermore, clinical indicators cover the skin analysis for Calabar swellings, ocular analysis to verify any adult worm. Lastly and more recent has been the use of Rapid Assessment Procedure for Loiasis (RAPLOA) survey for infection prevalence evaluation at community and individual level (Zouré *et al*, 2011).

A good characterization of the occult infected individuals is principal for the understanding of mechanism, which control the infection. Besides the microscopic technique and the ocular passage of adult worms, new methods such as detection of *L. loa* specific IgG4 by ELISA are now available. By using some of these methods, it

appears clearly that occult infected individuals are more prevalent than microfilaremic ones (Djikeussi *et al*, 2014).

The most recent progression in regard to loiasis diagnostics comprehends the LoaScope which involves the blood smear assessment for mf using a smart phone with image analysis software that allows the quantification of the number of mf/ml of blood placed into a amplifying tool (Kamgno *et al*, 2018). This modern technology affiliated to the new 'Test and (not) Treat' strategy for onchocerciasis elimination as a way to rule out the high-risk population from therapy (Kamgno *et al*, 2017). Furthermore, a novel immunological indicator that has only been used for research purposes for *L. loa* infection includes the Loa Antibody Rapid Test that adapts the recombinant antigen LI.SXP-1 to a lateral-flow assay (LFA) platform (Drame, 2014).

1.6.5 Control and Elimination

Although restricted to central African and some West African countries, this filarial disease is now emerging as a public health problem due to increasing human movement throughout the world (Akue *et al*, 2016). The transition to filarial elimination has also created a more urgent need to finalize the strategy for implementing ivermectin treatment, or alternative treatment strategies, in areas that are co-endemic for *L. loa* infection (WHO, 2017). In such regions, the most significant impediment for programs is the co-distribution of loiasis, due to the risk of SAEs including encephalopathy and death, which have been associated with ivermectin when given to individuals with high *L. loa* mf loads in the blood (> 30,000 mf/ml) (Boussinesq, 2006; Gardon *et al*., 1997; Kelly-Hope *et al*., 2014; Addiss *et al*, 2003). Furthermore, it has been estimated that, based on the actual strategies in course, at most 31 000 co-infected individuals will demand for onchocerciasis therapeutics by 2025 amid being at risk of SAEs (Melchers *et al*, 2020)

Furthermore, loiasis is still not included within the WHO's list of NTDs, as the lack of funding for research maintains, the lack of treatment and control programs action delays the disease awareness within the communities (Metzger *et al*, 2014; Chesnais *et al*, 2017; Whittaker *et al,* 2018). A specific feature of *L. loa* is the fact that the prevalence of mf carriers in endemic zones rarely exceeds 30%, while in most cases amicrofilaraemic individuals account for 70% of infected people (Boussinesq, 2006). This spectrum

suggests the existence of a potential mechanism for clearing mf from the peripheral blood. The importance according to *L. loa* today is mostly related to its negative impact on mass chemotherapy for eradication of other filarial parasites such as *O. volvulus* in co-endemic areas. Furthermore, albendazole should be administered twice yearly for LF in areas where *L. loa* infection is co-endemic for a minimum period of six years (Boussinesq, 2006; Pion *et al*, 2017).

Apart from the risk of SAEs associated with implementing ivermectin treatment in *L. loa* co-endemic areas, excessive *L. loa* microfilaraemia has been linked with the elevated mortality rate, probably as a consequence of a direct and indirect outcome of mf or to mechanisms promoting co-infections or comorbidities, proposing that loiasis is not as benign as it was considered previously, and therefore should receive more awareness due to its outcome in regards the onchocerciasis and lymphatic control strategies (Chesnais *et al*, 2017).

Since at least 60% of the global population relies on medicinal plant for their primary health-care needs, this alternative therapy should not be neglected, as a significant number of plants have been reported to treat *L. loa* infections such as *Alstonia congensis, Costus lucanusianus J, Senna occidentalis, Portulaca oleracea, Nicotiana tabacum*, among others (Wink, 2012). Furthermore, WHO-traditional Medicine Centers indicated that about 80% of 122-targeted components have been used for the same reason of treating *L. loa* infections (Van *et al*, 2004, WHO, 2011).

1.7 Mansonelliasis

1.7.1 Parasites

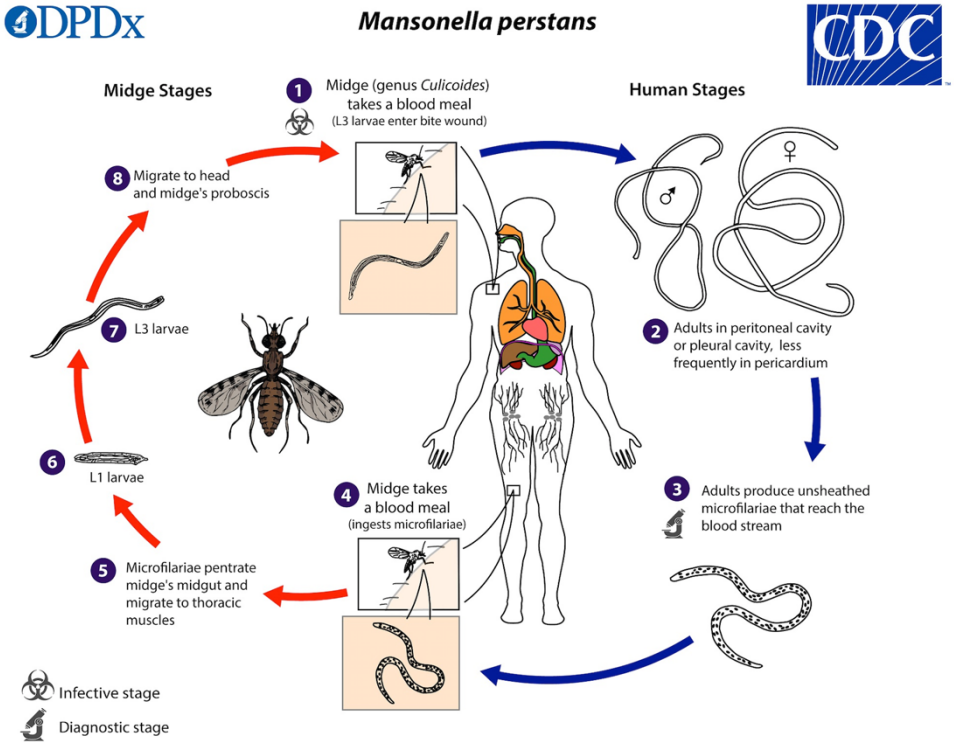
There are three species of *Mansonella*, a vector-borne filarial nematode genus that are associated with human infections, which are *Mansonella perstans, M. ozzardi* and *M. streptocerca* (Figure 1.9 Panels F-H).

The early medical literature referred to this nematode as *Acanthocheilonema perstans* or *Dipetalonema perstans* (Agbolade *et al*, 2005). These species vary in their geographic occurrence and localization within the host. For instances, *M. perstans* appears to be

associated only with humans, while *M. ozzardi* and *M. streptocerca* seem to be associated with both humans and primates (CDC, 2019a). The adult females measure 70-80 mm x 0.12 mm, males 35-45 mm x 0.06 mm; both sexes have a smooth cuticle and a ventrally curved tail, which has trilobed appearance (Baird *et al*, 1987). The tail of the male has four pairs of preanal and one pair of postanal papillae and there are two, very unequal, rodlike spicules (Baird *et al*, 1987). The mf in the blood is unsheathed and measure 200 µm x 4.5 µm; they have curves when fixed and stained. The tail ends bluntly and contains nuclei to the tip (Figure 1.9 F-H).

The life cycles for all three species are similar, and there is no marked periodicity, but mf is more abundant in the peripheral circulation at night (Garcia, 2007; Mommers *et al*, 1994; Service, 2004). The mf is ingested in a blood meal by small female ceratopogonid midges of the genus *Culicoides*, which are pool feeders (Figure 1.8). The larvae enter the thoracic muscles, moult twice and reach the infective third stage by 9 days, when they migrate to the head (Muller, 2002). They exit from the labium of the midge while it is feeding. The parasites take months to become mature, moulting twice more, in the human body (Muller, 2002).

Figure 1.8: Life cycle of *Mansonella* spp.



Source: CDC, 2019a.

1.7.2 Vectors

In Africa the vector species are female *C. austeni* and *C. milnei* (midges) which have a short flight range of about 100 m. Eggs are often laid in mud or wet soil in batches or in water in banana stumps (Miller, 2002). Male flies do not suck blood but feed mainly on plant juices (Gordon *et al*, 1978). Recent studies have proposed the capacity of another vectors of *M. perstans* such as *Simulidae, Tabanidae*, or *Culicidae* (Bassene *et al*, 2015).

1.7.3 Disease

This condition is often asymptomatic and has been related to allergic symptoms including itching, pruritus, joint paints, fever, enlarged lymph glands and uncommon abdominal symptoms, followed by elevated eosinophilia (Muller, 2002). In the host, *M. perstans* are usually found in body cavities, whereas *M. streptocerca* is often located in dermal and subcutaneous tissue, and *M. ozzardi* in subcutaneous tissues (Garcia, 2007; Heyman, 2004). A complicating feature of *Mansonella* infections is that their geographic distribution overlaps that of other filarial diseases that might have similar clinical manifestations, therefore, it is difficult to confirm that symptoms identified in cross-sectional studies in endemic areas were due to *Mansonella* or to co-infection with other parasites (CDC, 2019a). For instances, *M. ozzardi* have been linked with symptoms that resemble those of bancroftian filariasis (CDC, 2019a). Furthermore, the symbiotic relationship between filarial nematodes and *Rickettsia* bacteria can make these filariasis significant parasitic diseases (Rajan, 2003)

1.7.4 Diagnostics

The gold standard diagnostic for mansonelliasis is through microscopy by identifying the mf of *M. perstans* and *M. ozzardi* in stained blood smears whereas *M. streptocerca* is usually diagnosed through skin snips (Figure 1.9 F-H; CDC, 2008). Usually, more than ten mf per milliliter may be present, together with *L. loa* and *W. bancrofti* parasites (Muller, 2002; Debrah *et al*, 2017).

In the clinical context, molecular detection has been often used to differentiate mf of *M. streptocerca* from *O. volvulus* in skin snips, whereas in the research context, real-time

PCR technique is usually performed to identify and quantify *M. perstans* and *M. ozzardi* mf, and loop-mediated isothermal amplification assays (LAMP) have been developed for field use (CDC, 2019a).

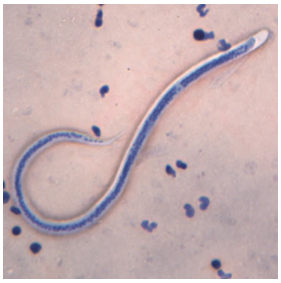
Furthermore, a new possible species of *Mansonella* was identified through molecular analysis, known as *Mansonella* sp 'DEUX', which was only reported in febrile children in Gabon. It is thought to be a new species as it differs from *M. perstans* ITS1 with only 94% similarity between them, while *M. perstans* has been reported homogenous throughout Africa (Jimenez *et al*, 2011; Marcos *et al,* 2012).

1.7.5 Control

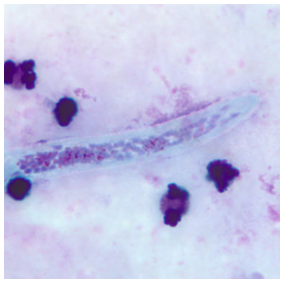
Currently, while no specific treatment is available for mansonelliasis, ivermectin appears to reduce mf for a period but probably has no long-term effect, while albendazole and mebendazole given for 10 days have been given equivocal results, and DEC has no effect at all (Muller, 2002; Wanji *et al*, 2016; Garcia, 2007; Heyman, 2004).

Figure 1.9 Microfilariae parasites under the microscope in blood smears with exception of *O. volvulus*. **Panel A –** The mf of *W. bancrofti* are sheathed and measure 240-300 μm. **Panel A1 –** Close up of the anterior end of the *W. bancrofti*. **Panel A2** – Close up of the posterior end of *W. bancrofti*. **Panel B -** Mf of *B. malayi* are sheathed and measure 175-230 μm. **Panel C –** Mf of *B. timori* are sheathed and measure on average 310 μm. **Panel D –** Mf of *O. volvulus* from a skin nodule stained from hematoxylin and eosin, measure 300-315 μm in length (H&E). **Panel E –** Mf of *L. loa* are sheathed and measure 230-250 μm. **Panel E1 –** Adult of *L. loa* removed from the eye of a patient. **Panel F –** Mf of *M. perstans* are unsheathed and measure 190-200 μm. Panel G – Mf of M. ozzardi are unsheathed and measure 160-205 μm. Panel H – Mf of *M. streptocerca* are unsheathed and measure 180-240 μm by 3-5 μm. All measuring bars = 30 μm (x100 magnification).

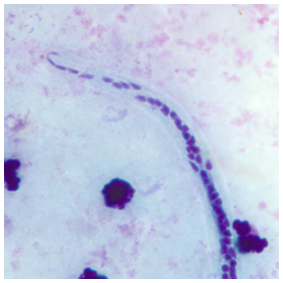
A - *W. bancrofti*



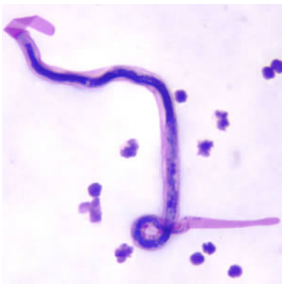
A1- *W. bancrofti*



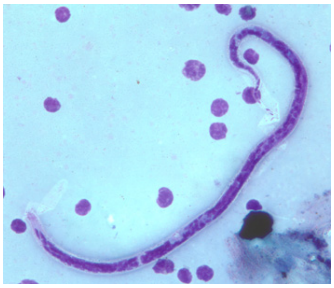
A2 - *W. bancrofti*



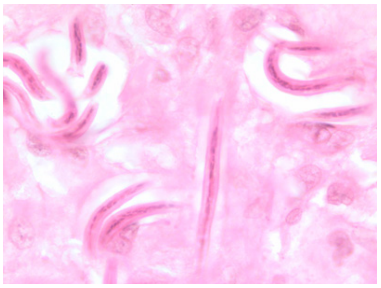
B - *B. Malayi*



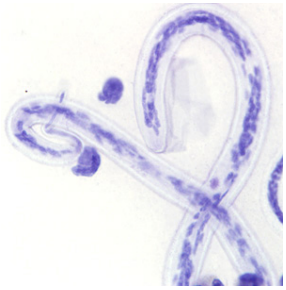
C - *B timori*



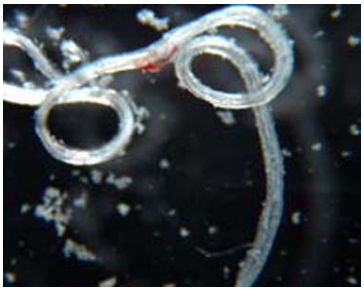
D - *O. volvulus*



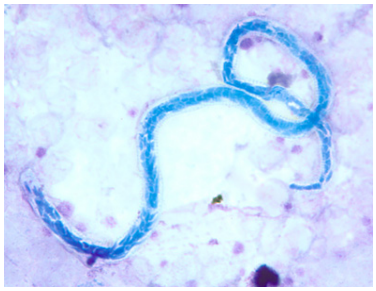
E – *L. loa*



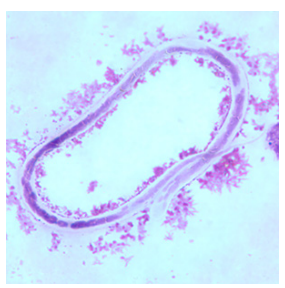
E1 – *L. loa*



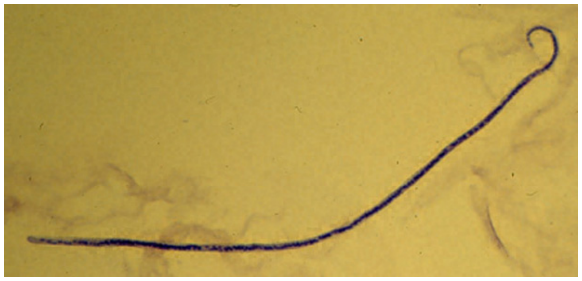
F - *M. perstans*



G. *M. ozzardi*



H. *M. streptocerca*



Source: Adapted from CDC, 2019.

1.8 Current status of Angola

1.8.1 Angola

Angola is an expansive country with an area of 1247 million km^2 and a population density of 20.6 inhabitants per square kilometer. The gradual increase in population is due not only to the natural growth rate of the population (estimated at 2.7% per year), but also to the return of Angolans, once refugees in neighboring countries, as well as an increase in life expectancy. The overcrowded population in the capital is due in part due to the migration (20%) in search of security and better life opportunities (Angola NTDs, 2017).

1.8.2 Geography

Located on the west coast of Africa the country is limited on the West by the Atlantic Ocean, the North by the Democratic Republic of Congo, and the East by the Republic of Zambia and the South by the Republic of Namibia (Figure 1.10). From the West to the East, the topography is characterized by a transition zone formed by plateaus that reach an altitude of more than 1500 m, and a zone of variable climate influenced by numerous factors like the latitude and altitude. The savannah vegetation covers a great part of the country; the forest is denser to the north while steppe and desert are present to the south of the country. The climate includes two seasons: the rainy season, hotter period, which occurs between September and May, and the Cacimbo or drought season, which is less hot and occurs between May and September. On average, temperatures in the country range from 17.0 °C to the lowest, and 27.0 °C, the maximum (Angola, NTDs, 2017).

Figure 1.10: Administrative division of Angola.

Source:www.d-maps.com, 2007.

1.8.3 Demography

Like most developing countries, Angola has a relatively youthful population. The demographic indicators are a challenge to the country's sustainable development. According to the 2012-2025 National Health Development Plan (NHDP) and the United Nations Development Program (UNDP) statistical indicators included in the 2014 Human Development Report, the population is estimated at about 24.3 million in habitants, with a high annual growth rate of about 3.0%. About 62.3% of the population lives in urban and peri-urban areas while 37.7% lives in rural areas. There is an internal migratory movement of about 20.0% from the rural to the peri-urban areas (Censo, 2014).

1.8.4 Economy

Oil represents 55.0% of Gross Domestic Product and 95.0% of exports. The non-oil sector has the potential to employ the majority of the economically active population. The industrial sector is in the process of reconstruction and rehabilitation. The rural sector with agriculture, forestry and livestock activities is the second largest manufacturing sector in the country, in which GDP is currently around 8.0% despite ongoing demining actions. The official currency is the Kwanza (Angola NTDs, 2017).

About 46.0% of the population gets water from improved sources (piped water, public fountains, wells or wells, protected 'cacimbas' and springs, rainwater); the most common source of water in the urban area is the mobile water tanker. The country is ranked 160th in the poverty index, in a list of 173 countries where more than 61.0% of the population live below the poverty line, of which 26.0% live in extreme poverty (Angola NTDs, 2017). Since the beginning of the economic boom, Angola has been facing an increase of 0.4% in complex migration trends, including an intensification of mixed migration movements (Angola NTDs, 2017).

1.8.5 Health status

Three decades of civil war ended in 2002 leaving its footprints marked by dilapidated health infrastructure, with nearly half of its total population lacking any access to health-care services (Njau, 2013; Gebrezgabiher *et al*, 2019). The main causes of disease and death are infectious and parasitic diseases, diseases strongly associated with poverty, sanitation and malnutrition, such as malaria, HIV/AIDS, tuberculosis, and NTDs (Angola NTDs, 2017). Added to this is loiasis, which is commonly found in the north and northeast of the country (WHO, 2013).

One of the main major health challenges in Angola are 1) the high communicable disease mortality and morbidity, frequent epidemics and a sharp rise (as yet unmeasured) in the prevalence of noncommunicable diseases, 2) Increased vulnerability of the country to various potentially health-threatening situations due to high mobility of persons and goods within and outside the country, and extensive open borders shared with neighboring countries, and 3) No systematic mapping of regions, localities, and vulnerability and/ or at-risk populations to facilitate timely response through selected interventions (WHO, 2016).

While many countries in Africa have made steady progress in scaling up their national programs, several countries, including Angola, are behind the targets if the WHO Roadmap is to be accomplished (Bockarie *et al*, 2013; Molyneux *et al*., 2018; WHO, 2012). Human migration is another challenge as people search for employment, which has been a common and increasing phenomenon in developing countries such as Angola. Both permanent or long-term and temporary migrations are common in many communities (Ramaiah KD, 2013). Information is scarce on the role of migrants in creation of new foci and/or reintroduction of transmission into the 'cleansed' areas, which are potential threats to LF elimination efforts (Ramaiah, 2013). Four categories of migration have implications for LF elimination efforts. These are (i) migration from endemic areas to nonendemic areas, (ii) migration from endemic rural areas to endemic urban areas, (iii) migration from endemic areas to the areas that achieved control/elimination of LF, and (iv) transborder migration (Ramaiah, 2013).

As for the main constraints there is the need to increase efforts at the national level to eliminate NTDs, the small number of partners involved in the control of these diseases and the lack of specialized human resources. Challenges for the effective control of NTDs include finalizing coordinated mapping of such diseases, updating data on the prevalence of geohelminthiases and schistosomiasis; the effective implementation of large-scale MDA improving the performance of the current projects and the extension of de-worming campaigns with praziquantel throughout the territory (WHO, 2013).

1.8.6 Neglected Tropical Diseases in Angola

The coverage index NTD mass treatment developed by WHO has reported that about 3.1 million people have received treatment in Angola in 2016, and 11.4 million people in need did not receive treatment simultaneously (WHO, 2016b). Donor support from The End Fund in regards NTD treatments has covered for LF only 1% of the population, onchocerciasis 2%, schistosomiasis 50%, STHs 26% and the status for trachoma is currently unknown (WHO, 2016b; USAID, 2018). Over the past couple of years, epidemics of diarrhea (bloody stool and viral), human and animal rabies, dengue fever and chikungunya have been notified, investigated and controlled in Angola. Outbreaks of dengue and chikungunya have been reported in Angola since 2013, and due to the presence of other febrile illnesses, mostly malaria, and deficient clinical diagnosis, laboratory analysis and case reporting, all of which also hampers systematic vigilance, leading to underreporting of this condition (WHO, 2017). Recently, it has been reported that about 1,000 individuals have been infected annually with NTDs in Angola according to the local MoH reports, but no donor support for morbidity management & disability prevention has been provided so far (USAID, 2018).

Table 1.3 NTDs current status in Angola.

Status	Disease	Description
Reported	Buruli Ulcer	It was first reported in 1998, in Bengo province by Bar. 27 Angolan refugees were reported in DRC in 2003 (Kibadi *et al*, 2003). Foci of infection have been identified along Kwango river between Angola and DRC frontier in 2008 (Kibadi *et al*, 2008).
	Chikungunya	An outbreak was reported between 1970-1971 in Luanda simultaneously with a yellow fever outbreak (Filipe *et al*, 1973; Pinto *et al*, 1973). An imported case from Angola was reported in Portugal (Parreira *et al*, 2014). This virus has been diagnosed in a traveler from Angola to Japan during yellow fever outbreak (Takaya *et al*, 2017).
	Cysticercosis	Few cases of bovine cysticercosis and taeniases reported between 1996-2005 (Dermauw *et al*, 2018).
	Dengue	An outbreak of dengue was reported in 2013 confirming 1,008 clinical cases and 10 fatalities (Schwartz *et al*, 2013).
	Helminths/other	Human strongyloidiasis, giardiasis, cryptosporidiosis and blastocystosis have been reported in Bengo province and Cubal region (Dacal *et al*, 2018; Mirante *et al*, 2016; de Alegria *et al*, 2017)
	Human African Trypanosomiasis	There has been shrinkage of the frequency of human African trypanosomiasis by 49% since 2011 (WHO, 2016).
	Leishmaniasis	A single case report of *Leishmania infantum* infection in Angola was published by Jimenéz *et al,* in 1994. Prevalence surveys in 1.9% of dogs in Luanda province (Vilhena *et al*, 2014). Suspected cases of human cutaneous leishmaniasis in Huambo province region in 2017 (Cortes *et al*, 2019).
	Leprosy	It ceased to be categorized as a national public health concern by 2005, when the prevalence estimate lowered to less than one case per 10 000 inhabitants. A prevalence estimates superior the national average has been registered in some provinces. Available data from Angola presented that 378 new cases were reported. The prevalence reported in 2013 was 1141 cases, which is in line with the elimination target (0.6 cases/10 000 inhabitants) (WHO, 2016).
	Lymphatic Filariasis	Endemic in the northern part of the country. About 76.5% of the population are at risk of Bancroftian filariasis as of 2000 (Berger, 2014)
	Onchocerciasis	The highest prevalence was found 7 provinces (Strangway *et al*, 1950). About 100,000 were infected and 2,000 blinds in 1985 (Berger, 2014). Interestingly, the most updated data indicates that from 2005-2016, at least 1690 people were infected with river blindness in Angola.
	Schistosomiasis	Majority of the cases were reported in all regions (Grácio, 1978). About 32,216 cases of schistosomiasis were reported in 1980 (Berger, 2014). Sporadic cases have been reported among travelers to Angola (Hua *et al*, 2013). An outbreak was reported between 2008-2009 including 254 casas and 15 fatalities in Zaire province (Promed, 2008)
	Snakebite	The prevalence of cases of snakebites accidents in Angola is unknown, however, it can be estimated that it is high, taking into account the data for neighboring countries. The inexistence of epidemiological data and the fact that it is not a mandatory reporting disease, hinder the country's real casualties and, consequently, the management of the supply of antiophidic serums for the treatment of the respective poisonings (Oliveira, 2017).
	Soil Transmitted Helminthiasis	Reported cases in Bengo (Soares *et al*, 2013)
	Rabies	Most human rabies is acquired from dogs (Berger, 2014). Notable outbreaks were reported in Luanda, Uíge and Bié (Promed 2009, 2011).
Not reported	Podoconiosis Dracunculiasis Foodborne Trematodiases Yaws Echinococcosis Mycetoma Scabies Trachoma Cystiscercosis Chagas disease	Suspected cases have been reported (Deribe *et al*, 2018).

1.8.6.1. Lymphatic filariasis in Angola

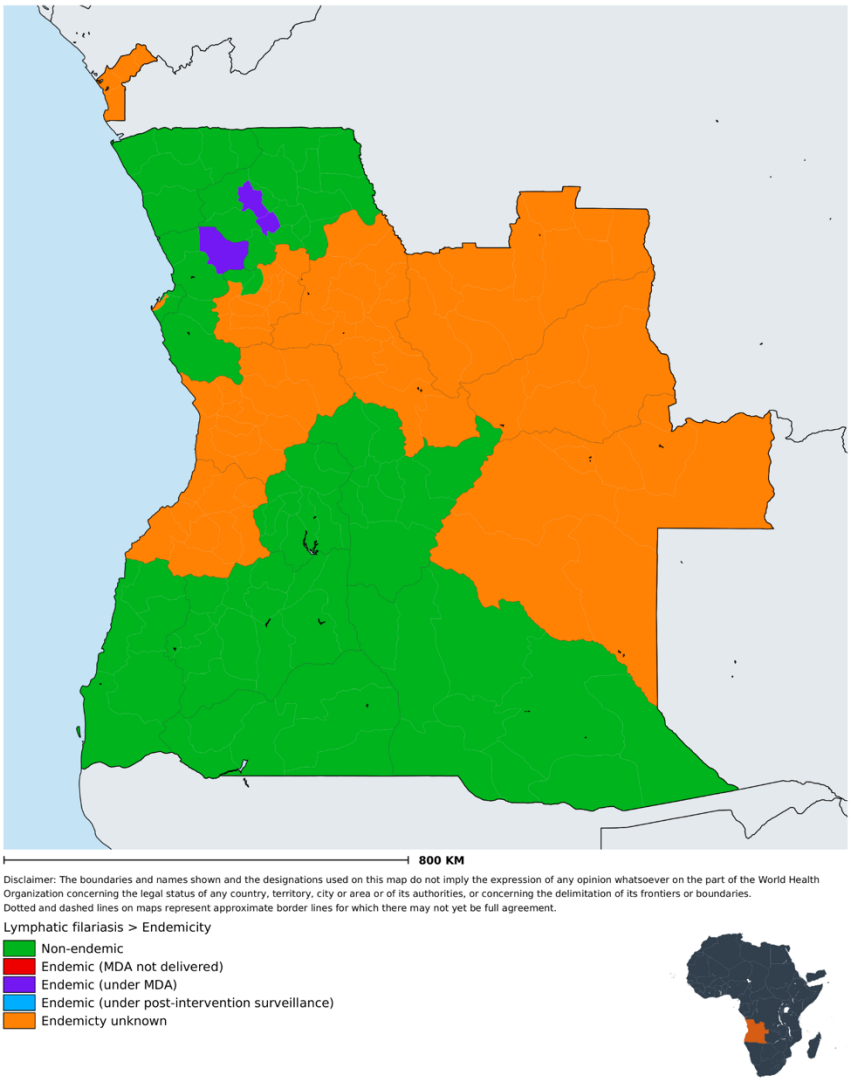
Angola is a country where LF is endemic. The national mapping of the occurrence of the disease began in June 2015. The mapping is being carried out according to the WHO mapping script to detect occurrence of transmission of *W. bancrofti* infection in each municipality of the country, the basic unit of implementation of health interventions (Angola NTDs, 2017). The mapping survey has been carried out using as a diagnostic tool based on blood smear; ICT and Filarial Test Strip (FTS) (ESPEN, 2017).

According to the current results, LF is endemic in the country, with the North, Central and Eastern provinces being the most affected (Uíge, Bié, and Kuando Kubango). It is estimated that the treatment coverage has slightly increased from 0% in 2015 to 1% in 2016, where about 5.35 million are at risk of contracting this disease (WHO, 2016). The map shown below in Figure 1.11 indicates the status of LF elimination in Angola (ESPEN, 2017).

For LF, the problem of the cross-reactivity in *L. loa* endemic areas with the FTS card highlights that an alternative method is required, and in the absence of any new or alternative diagnostic tool, the presence of the main clinical symptoms of lymphoedema and hydrocoele may help to identify if LF is a public health problem.

With the current global interest in the control of NTDs, the Angolan Government and partners have ensured the implementation of NTDs mapping step by step throughout the country. Accurate mapping of the distribution of LF is a crucial step for its elimination programs due to loiasis co-endemicity that can lead to serious adverse events in people are treated with ivermectin (Bakajika *et al*, 2014; Brito *et al*, 2017).

Figure 1.11: Status of lymphatic filariasis elimination in Angola.



Source: ESPEN. 2019.

LF is transmitted by certain anopheline species and the precise incrimination of those species in Angola associated with transmission is limited. Of the culideos described in Angola in the 60s, the following *W. bancrofti* vectors were considered: *Anopheles gambiae, Culex pipiens fatigans, Anopheles funestus, Mansonia* and *Aedes aegypti* (Casaca, 1966). However, in many regions in Africa the LF vectors, such as *Anopheles funestus* and *Anopheles gambiae,* are the same as those, which are found across all regions of Angola at varying distributions and species compositions.

In line with the global trends to improve efforts in malaria control Angola has put in measures to mitigate malaria transmission including vector control using insecticide-treated mosquito nets, which were distributed during community mass distributions and had covered less than 25.0% at household level in 2017 (Angola NTDs, 2017).

1.8.6.2 Onchocerciasis in Angola

According to WHO, the CDTI might include several communities where it is being carried out, and as a result, these communities can span a municipality, province or even an entire country. Treatment coverage denotes the number of people treated multiplied by 100 and divided by the total population (exposed population), and in Angola context it slightly increased from 0% in 2015 to 2% in 2016, meaning that about 5.55 million are still at risk of infection while 124,000 people have received treatment (WHO, 2016a). Angola has seven high-risk onchocerciasis areas being targeted with the annual CDTI strategy, one of which overlaps with part of the loiasis high-risk zone (WHO, 2016; Zouré *et al*., 2014). The CDTI areas were identified through the REMO (Noma *et al.,* 2002), which is based on community prevalence of skin nodules ≥20.0% in adults, as part of APOC's strategy to target and control blinding onchocerciasis (Zouré *et al.,* 2014). However, the recent change in strategy to expand and eliminate onchocerciasis, by treating low transmission areas (nodule prevalence < 20%) poses several challenges, which are being considered by ESPEN (Molyneux *et al.,* 2014; WHO, 2015).

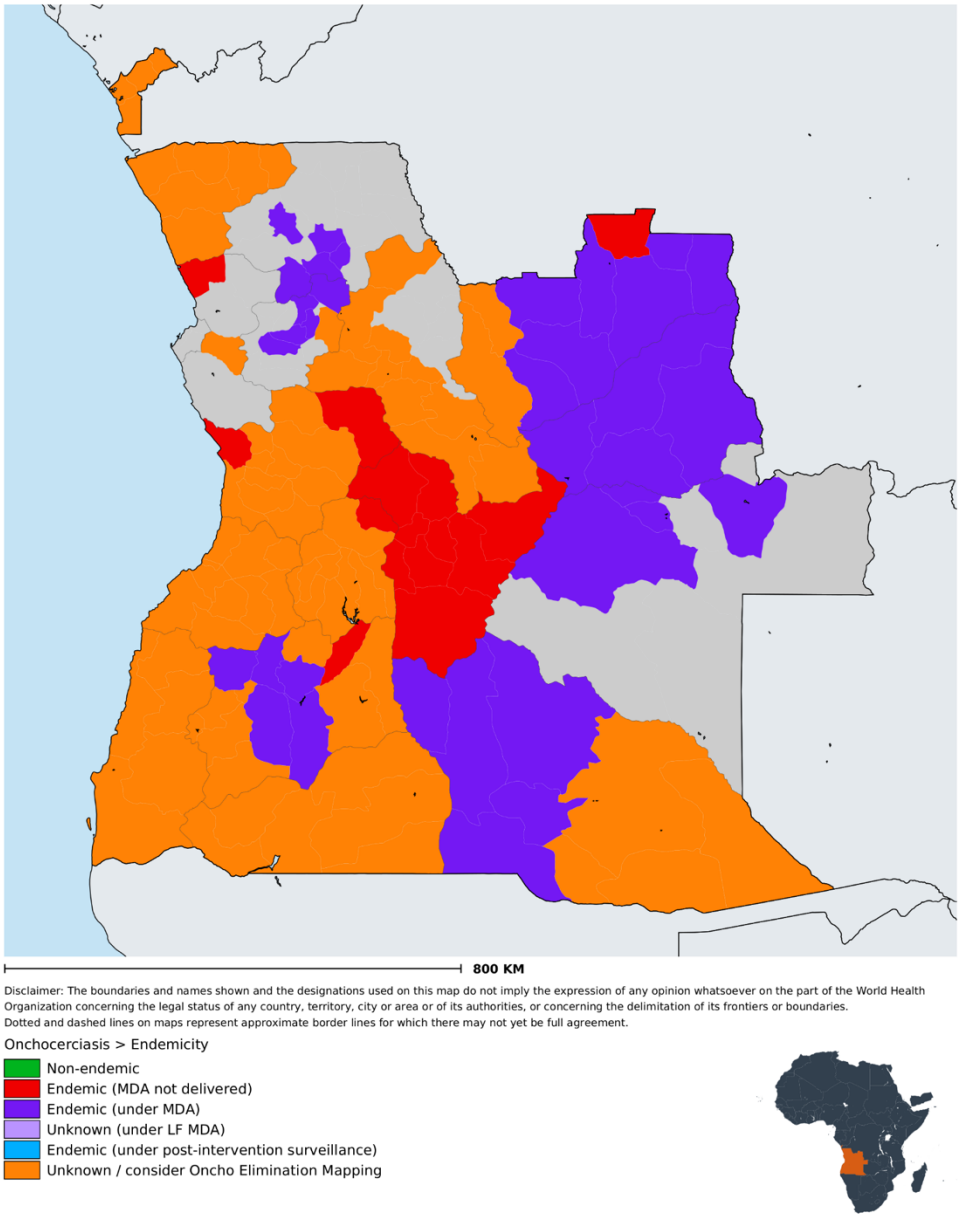
Onchocerciasis is still endemic in 44 municipalities in 9 provinces. The mappings produced between 2004-2011 show that 3,240 communities are affected and approximately 2.5 million individuals are at risk, thus requiring mass treatment which suggests no re-assurance with regard to elimination of the disease (Figure 1.12). Mapping

of onchocerciasis began in the country in a first phase in 2002 using the REMO methodology supported by the APOC. A total of 535 villages were selected, but only 114 villages were assessed between January 25 and February 28, 2002. The second phase of REMO, held in July 2011, covered the remaining 421 villages selected (Angola NTDs, 2017).

Based on these results, 8 community-directed treatments with ivermectin projects were established in 8 provinces of the country, which implemented an MDA ivermectin between 2004 and 2015 (Angola NTDs, 2017). Considering the recent change in the onchocerciasis control paradigm for elimination, between July and December 2015, 177 villages were selected for further mapping and finally only 76 were mapped by the cutaneous biopsy technique to investigate *mf* of *O. volvulus*. The integration of the results of the two mapping exercises indicates that onchocerciasis is endemic in 12 provinces of the country.

For the Angolan onchocerciasis and LF national programs to implement MDA using ivermectin as a constituent drug, it is critical to understand the extent to which the three filarial infections overlap geographically. This will ensure that safe treatment strategies are implemented and monitored for impact and potential SAEs. The large-scale RAPLOA and REMO surveys provide essential baseline information; however, they were completed at different times, and on different and relatively large geographical scales. Micro-mapping and overlap-mapping are new approaches developed to delineate risk, define co-endemicity and target interventions which may be more useful in this loiasis high risk zone, which comprises both hyper- and hypo-onchocerciasis, and an unknown LF prevalence (Kelly-Hope *et al*., 2014). Given that the programs in Angola are behind targets for the achievement of elimination and have minimal funding support, the method to initially assess co-endemicity needs to be simple, rapid and relatively cost effective. Further for onchocerciasis, the use of skin snips and/or the new OV16 rapid diagnostic test are currently not feasible or affordable at a large scale in this low-resource setting (Kelly-Hope *et al*., 2014)

Figure 1.12: Status of onchocerciasis elimination in Angola.



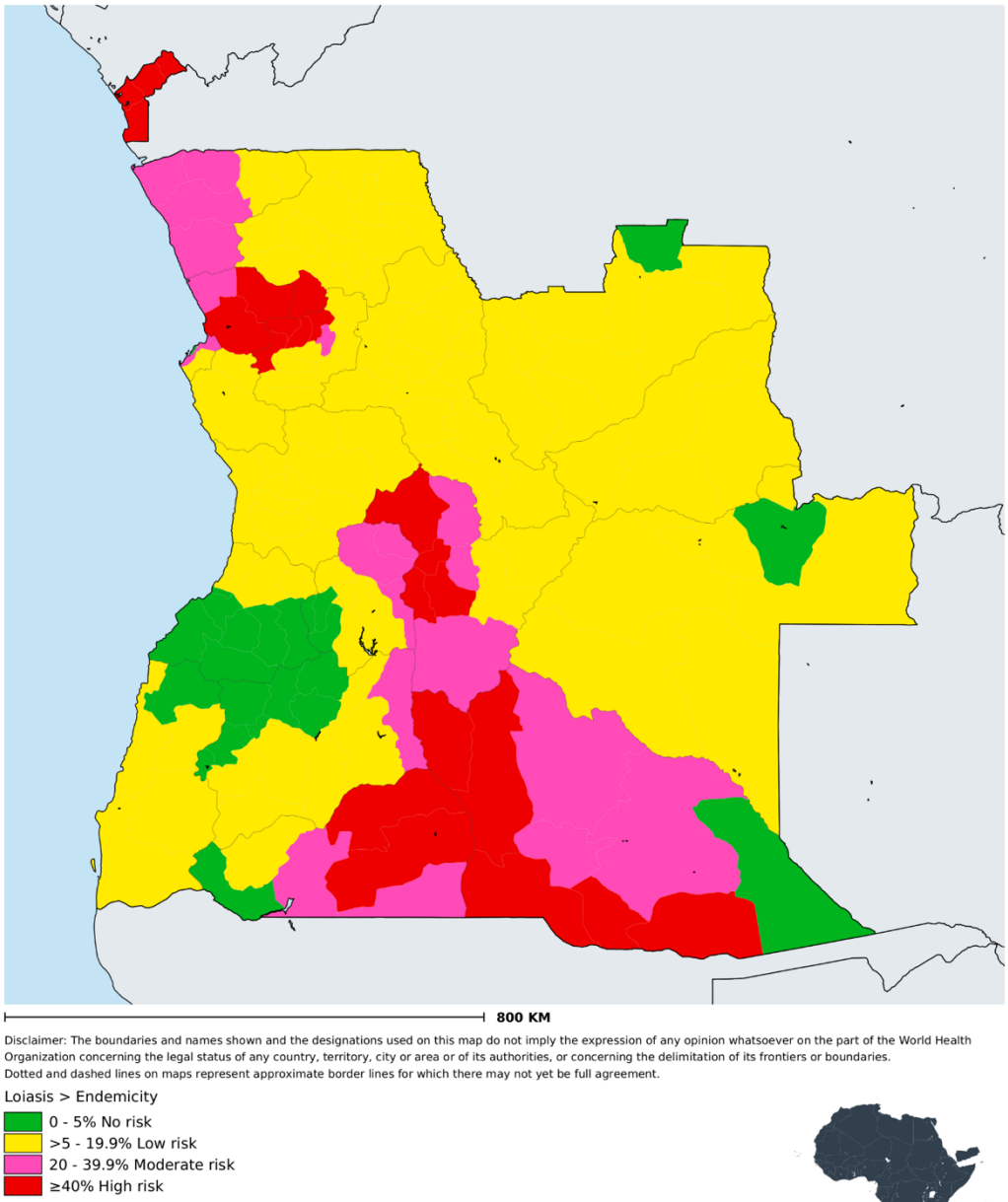
Source: ESPEN, 2019.

1.8.6.3 Loiasis in Angola

Knowledge of the distribution and level of endemicity and density of mf of the *L. loa* is essential to decide the strategy of MDA of ivermectin in areas co-endemic with onchocerciasis or LF in the face of the risks of SAEs in individuals with high levels of mf. In this perspective, following the mapping of onchocerciasis between 2008 and 2011, RAPLOA surveys were carried out in the areas indicated for the massive distribution of ivermectin. RAPLOA evaluates the history of the presence of worm in the eye in a sample of individuals at the place of inquiry. Figure 1.13 shows the delineation of the prevalence areas of *L. loa* determined by RAPLOA. This survey was developed as a more economically feasible alternative to the standard microscopy-based mf detection in blood smears. This information was used to decide the viability of the treatment, which indicates that the areas of greatest endemicity are located in the provinces of Bengo, Bié and Cuando Cubango (Angola NTDs, 2017).

More recently, in 2015, during the survey of onchocerciasis mapping in 76 villages using the cutaneous biopsy technique, the research and determination of *L. loa* mf in the subjects were included in the survey. The mf levels detected do not represent a risk for the occurrence of SAEs after ivermectin treatment, with the highest mf density per microliter being detected in the municipality of Quitexe (6,820 mf/ul). Despite the low densities detected, this does not preclude the need to establish a pharmacovigilance system to detect any adverse events that may exceptionally occur following the massive administration of ivermectin (Angola NTDs, 2017).

Figure 1.13: Estimated loiasis endemicity.



Source: ESPEN, 2019.

Angola has a *L. loa* high-risk area in Bengo Province in the northwest region of the country with high prevalence estimates (> 40.0%) and associated risk of SAEs (Zouré *et al*, 2011). These risks were defined by RAPLOA surveys conducted in 2003, 2004 and 2008, and support historical studies on filariasis, which also found widespread onchocerciasis caused by *O. volvulus* and transmitted by the *Simulium* vector, but little evidence of the LF parasite *W. bancrofti* (Casaca, 1966). The current prevalence of LF in this area is not known but is expected to be low based on the historical data and recent modeled map estimates developed by Cano *et al* (2018). Baseline mapping is required to determine the way forward. However, it will be important to take into account the ongoing activities of the onchocerciasis program that currently operates in some areas of Bengo Province.

The situation for implementing an alternative strategy for LF elimination in *L. loa* co-endemic areas is more straightforward, but not without challenges. While the WHO and GPELF recommends the alternative strategy of twice a year albendazole together with the use of long-lasting/insecticide treated bed nets (LLIN/ITNs) (Kelly-Hope *et al*, 2014; WHO, 2012); only one or two countries out of the 10 *L. loa* co-endemic countries have started to implement this alternative strategy. The reasons for such delays are multi-faceted, but mainly related to political instability, poor infrastructure and difficult access to communities (Molyneux *et al*., 2014). A new practical approach for scaling up the alternative strategy may help countries to develop action plans, however they will need considerable funding and support at a national level (Kelly-Hope *et al*, 2017). In some LF-loiasis co-endemic areas more refined mapping and definition of risk factors are important where there is uncertainty about the risk of SAEs and if CDTI or alternative intervention strategies should be used. The other main challenge relates to measuring endemicity, which has largely been defined in Africa through mapping community prevalence of filarial antigen detected with the rapid diagnostic immuno-chromatographic test card (BinaxNOW Filariasis), and now more recently the Filariasis Test Strip (Weil *et al*, 2013; Weil, 2007).

Large-scale parasitological surveys undertaken across Angola over the past decade have produced broad risk maps and provided some insights into the geographical factors and potential challenges associated with co-endemicity. However, to fully understand the potential risks, it is important to examine data on a finer scale, as prevalence can vary

greatly within a short distance and according to ecological factors such as riverine breeding sites and forest areas (Kelly-Hope *et al*, 2014).

1.8.6.4 Mansonelliasis in Angola

Mansonella perstans is categorized as a human filarial nematode that is mostly endemic in particular areas through sub-Saharan Africa and South America with undefined clinical symptoms (Akue *et al*, 2011). The main demographic aspects of this condition are the fact that in highly endemic even within the children population and tends to escalate with age (Bassene *et al*, 2015). Globally, more than 100 million people might be infected and roughly 600 million people reside in the 33 countries including Angola. The blood sucking Culicoides flies are the main vectors and are extremely abundant in tropical ecosystems (Bassene *et al*, 2015).

The problem of the existence of filariasis in Angola, its incidence and distribution, is still far from being solved, due to the lack of concrete data regarding the various species that are considered possible to find in the African continent. Except for the specific case of onchocerciasis, the remaining filariasis need to be the subject of a careful survey, and it is important at this time to review all the elements that we can gather and have at our disposal, which are bibliographic, whether statistical, whether observations or other existing data (Casaca, 1966).

The first record of *M. perstans* in Angola was made in the 1940s, in the northern region, and confirmed by microscopy. Since then, although no rigorous identification of the infesting species has been made, most of the 417 cases reported up to the 1960s, must correspond to *M. perstans*, due to their morphology and dimensions (Simonsen *et al*, 2011). From all the observations, it can be concluded that this filariasis is widely distributed in the northern part of the country, namely in the provinces of Cabinda, Zaire, Lunda and Kwanza-Norte; transmitted by Culicoides and Stegomia (Casaca, 1966).

1.9 Study rationale

Filarial diseases are endemic infections in Angola, representing a serious public health problem, and the mapping and study of their prevalence is of importance for better management. In 2010, the population at risk was estimated by the APOC, with onchocerciasis and loiasis co-endemic in the north and northeast of Angola (WHO, 2013b). However, what is the actual situation of filarial diseases in the Municipality of Dande, Bengo province? Furthermore, loiasis has recently emerged as a disease of public health importance when neurological SAEs were reported in individuals with high *L. loa* mf after ivermectin treatment. This had a negative impact on the control of onchocerciasis and LF in areas of co-endemicity with loiasis therefore more detailed knowledge at the local scale is needed.

In this thesis, the study of the prevalence of filariasis was chosen because it is still a public health problem worldwide. Understanding LF-loiasis-onchocerciasis co-distribution at a micro-level, delineating in relation to forested areas and how people move in and around these areas, may provide insights into where SAEs are more likely to occur, and if the Test-and-not-Treat (TNT) strategy should be used to help minimize risk (Brant *et al*, 2018). Furthermore, following the baseline parasitological survey from this study, the resulting data will be analyzed to better understand the epidemiology of filarial diseases in the study area. The information from this baseline analysis will help to implement targeted interventions, including monitoring the effects of the interventions.

1.10 Main Aims

The main objective of this study is to support the National NTDs Program through an original research agenda that addresses critical gaps in the epidemiology and control of NTDs in Angola in the region of Bengo province. This will develop and assess an integrated filarial mapping survey to help determine the presence and co-distribution of onchocerciasis and LF in an area previously identified as one at high-risk *L. loa* SAEs. The study builds on the RAPLOA-LF-REMO survey method by adding LF clinical symptoms, combined with field diagnostics including RDTs, and laboratory techniques including real-time and nested-PCR, to monitor emerging filarial infections, both at the individual and epidemiological levels. Furthermore, a finer

geographical scale map within a high-risk zone than previously attempted will be implemented. This will help in construction of a rapid integrated filarial 'RAPLOA-LF-REMO' clinical survey using a new micro-mapping approach.

1.11 Specific Objetives

1. To conduct the most recent status of the literature for *L. loa* parasite in regard to its vector transmission characteristics in *Chrysops*.
2. To examine the use of the integrated RAPLOA-LF-REMO survey, based on key clinical manifestations of filarial diseases in the northern region of Angola.
3. To use a map as a tool for filarial epidemiological analysis based on GIS mapping, molecular and serological methods in the northern region of Angola for the first time.
4. To consolidate the overall work, successes and challenges in filarial control.

CHAPTER TWO: A CURRENT REVIEW OF *LOIASIS* AND ITS VECTORS

In light of the knowledge gaps identified in Chapter 1 concerning the vector management of *Loa loa* and the fact that it is not yet formally listed as an NTD by the WHO due to its wrongly classified benign nature, this chapter seeks to provide an up-to-date assessment based on the literature for this parasite in regard to its vector transmission characteristics that may be targeted for control. Nevertheless, the aim of this chapter is to provide within this chapter a new insight into the potential for integrated vector management (IVM) for loiasis and its significant epidemiological impact on the elimination programs of the filarial conditions. Furthermore, the novelty about this chapter is that it is the first comprehensive literature review of vectors of *L. loa* for the past 50 years.

The control of *Chrysops* has not been considered as a viable cost-effective intervention, and here, the current knowledge of *Chrysops* vectors is reviewed to assess the potential for its control, as well as, identified areas for future research. As results, 89 primary published papers were identified concerning the two main *L. loa* vectors, *Chrysops silacea* and *Chrysops dimidiata*. These papers were gathered into a database and categorized by field and laboratory procedures, species distributions, insect ecology, habitats and methods of vector control. The majority of the articles were from the 1950-1960s. *C. silacea* has been described as the most significant and dominant vector species. The latter vector has been described to be the most attracted to wood fire due to dissemination of odorous particles rather than carbon dioxide (CO_2) involved in the smoke in the canopy. Main vector targeted measures proposed to impact on *L. loa* transmission included defensive such as personal repellents, household screening, clearing vegetations, while the aggressive control measures included adulticides, larvicides and indoor residual spraying (IRS). Long ago, control depended mainly on the regime of insecticides and clearing of vegetation neighboring the residences, but contemporarily, repellents, trappings and destruction of the canopy are the best-proposed options around residencies.

This chapter has been published by Kelly-Hope *et al*, in part in as entitled as '*Loa loa* vectors *Chrysops* spp.: perspectives on research, distribution, bionomics, and implications for elimination of lymphatic filariasis and onchocerciasis', 2017, *Parasite and Vectors,* 10:172. In this article, Louise Kelly-Hope, Brent Thomas and myself contributed for the collation and chronological listing of articles (Appendix 9). David Molyneux support through the CouNTDown operational projects to the LSTM. This

study is dedicated to those staff of the LSTM and others who worked in West Africa during the 1950s on *L. loa.*

2.1 Introduction to *Loa loa* and its vectors *Chrysops* species.

L. loa (Figure 2.1) is transmitted through biological transmission by tabanid flies that belong to the Arthropoda phylum whose mainly characteristics are the division of the body into clusters of segments notably the head, thorax and abdomen (Kouam *et al*, 2017). Furthermore, they belong to the Insecta class based on three pairs of legs in adult stage, a single pair of antennae and broad tagmatisation of the body into the head, thorax and abdomen (Kouam *et al*, 2017). These species are part of the Diptera order or true flies, which are characterized by a thorax bearing a single pair of functional wings (Baldacchino *et al*, 2014). Their sub-order is Brachynera whose short antennae are usually composed of different sized segments. The Brachycera face is bulbous and there is no arise of the antennae (Kouam *et al*, 2017). Predominantly, most of the economically tabanids are in the Chrysopsinae, distinctly the genus *Chrysops*, and the Tabaninae (Mullens, 2002).

There are two main anthrophilic vector species of tabanid flies of the genus *Chrysops* that are *C. silacea* and *C. dimidiata,* however, *Chrysops distinctiennis*, *Chrysops zahri, Chrysops centurionis* and *Chyrsops longicornis* have been reported to be non-primary vectors of the human strain of *L. loa* (Akue, 2016; Duke, 1955; Mullens, 2002; Whittaker *et al,* 2018). Instead, the later vectors have been reported to support the maintenance of the simian form of the infection due to the differing biting behavior and periodicities of microfilarial circulation in the peripheral blood (Whittaker *et al*, 2018; Duke *et al*, 1958).

Overall, *C. silacea* and *C. dimidiata* are similar with a characteristic color, longitudinal black stripes on abdomen, mottled wings and large head and eyes (Figure 2.1 – A & B). In some parts of Africa, *C. silacea* is known as the 'Red Fly' due to its bright orange abdomen with short black stripes, which was considered distinct from *C. dimidiata* with its paler color and broader longer stripes (Kouam *et al*, 2017). Furthermore, adult females of the Tabanidae are known to live on a mixed diet, feeding on sugar and blood, whereas adult males feed exclusively on carbohydrates, averaging about 25 mg per meal in natural conditions while the average varies about 40 mg in laboratory conditions (Crewe W, 1956; Whittaker *et al*, 2018). In contrast to mosquitoes, the minor food intake observed

in *Chrysops* might be explained by their preference of feeding from a pool of blood formed by laceration, in alternative to a capillary, therefore, delaying the agglomeration of microfilariae within the pool (Gordon *et al*, 1953; Whittaker *et al*, 2018).

Figure 2.1: A. Female *C. silacea*, B. Female *C. dimidiate*. Figures measuring bars = 50 µm.

A B

Source: Wheeler L, 2018.

Moreover, *C. silacea* was found to be predominant in the cleared forest, particularly in the villages and in their immediate vicinity, whereas *C. dimidiata* prefers natural vegetation, particularly in the rainforest (Iboh *et al*, 2012). Smoke of wood fire is extremely attractive to *C. silacea*, due to dissemination of odorous particles rather than CO_2 involved in the smoke in the canopy (Kouam *et al*, 2017). As a result, this mechanism increases the opportunity of contact between humans and the flies (Duke, 1955). Further research suggests temperature as an important factor influencing transmission of *L. loa* vectors as *C. silacea* is mostly found in warmer locations ranging around 20-28 ºC, optimal for larvae development, adult density and biting infection, compared to *C. dimidiata* (Badia-Rius, *et al* 2019).

Vector control for *Chrysops* is particularly difficult due to the scale and remote location of breeding sites. *Chrysop silacea* and *C. dimidiata* bite mostly in the morning and evening leaving an irritating area on the skin of the person on which they have fed (Akue, 2016). Interestingly, it has been reported that the biting frequency of both species is quite reduced, only biting once every 5 days, depending on temperature and humidity (Kershaw *et al*, 1957; Whittaker *et al*, 2018)

2.1.1 Perspective on research

To date the control of the *Chrysops* vector of *L. loa* has not been considered as a potential alternative or additional strategy to address the problem co-endemic loiasis presents to the LF and onchocerciasis elimination programs. It is possible it could play an important role if correct vector strategies are deployed. However, a better understanding of the major vectors transmitting *L. loa* is essential and timely given the WHO defined Roadmap targets for the elimination of LF and onchocerciasis, and the challenges identified (WHO, 2012).

The first comprehensive review of the *L. loa* vectors for several decades by Kelly-Hope *et al* (2017) highlights the key vector transmission characteristics that may be targeted for vector control providing insights into the potential for IVM, with multiple diseases being targeted simultaneously, with shared human and financial resources and multiple impacts. IVM programs for filarial infections, especially in low transmission areas of onchocerciasis, require innovative approaches and alternative strategies if the elimination targets established by the WHO are to be achieved.

2.2 Methodology

A systematic search and collation of data in the peer-reviewed published literature on the two main *Chrysops* spp. of the vectors of *L. loa* was conducted using PubMed, JSTOR, SCOPUS and Google online sources. Information on the articles was collated into a database in Excel, summarized as the following: publication profile, study features, field and laboratory procedures, species distribution, ecology and habitats; factors influencing spatial-temporal transmission, and methods of vector control.Information on the study locations included in the published documents were geo-referenced and imported into the geographical information system software ArcGIS 10.1 (ESRI, Redlands, CA) to produce a new sector distribution map based on the knowledge synthesized in this review.

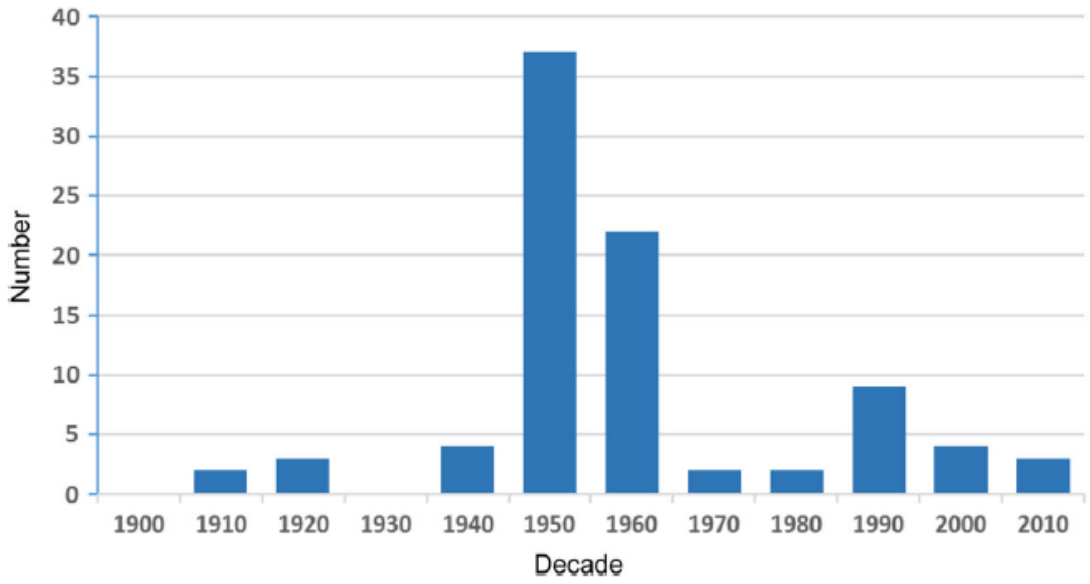
Based on the information reviewed, key points related to field and laboratory procedures, species distribution, ecology and habitats, spatial-temporal transmission and methods of vector control were highlighted in a series of excerpts, and areas for potential future research were summarized.

2.3 Results

2.3.1 Publication profile

A total of 89 primary published documents were identified on the two main *L. loa* vectors *C. silacea* and *C dimidiata*. These were collated into a database summarizing the publication, field and laboratory procedures, species distributions, ecology, habitats and methods of vector control. The majority of articles were from the 1950–1960s (Figure 2.2). Field studies conducted in Cameroon, Democratic Republic of Congo, Equatorial Guinea, Nigeria and Sudan highlighted that *C. silacea* is the most important and widespread vector. This species breeds in muddy streams or swampy areas of forests or plantations, such as banana, rubber and plantain plantations, descends from forest lower canopies to feed on humans during the day, is more readily adapted to human dwellings and attracted to wood fires. Main vector targeted measures proposed to impact on *L. loa* transmission included personal repellents, household screening, IRS, community-based environmental management, adulticiding and larviciding. Therefore, it has been recognized that in order to control loiasis, a better understanding of the *Chrysops* species vectors driving transmission was required.

Figure 2.2: Number of articles per decade 1900-2010.



Source: Kelly-Hope *et al*, 2017.

2.3.2 Study features: location, type and period

The majority of research was conducted in Cameroon, Nigeria, Congo, DRC, Equatorial Guinea, Gabon and Sudan. The most common type of study was field-based (n=30) or a combination of field/laboratory-based (n=28) with only a few exclusively laboratory-based studies (n=6). Overall, information on the study period was irregular with the year the study started most regularly documented.

2.3.3 Field and laboratory procedures

2.3.3.1 Collection methods

All field-based studies involved outdoor collections either of adult (hand nets) or immature-stage/larval (wooded frame sieve) stages and were mainly related to measuring transmission patterns including species abundance and infection rates.

2.3.3.2 Species identification

Information on species identification were not commonly documented, however, from the articles published, both *C. silacea* and *C. dimidiata* have only been identified and distinguished from each other by morphological features., specifically the bold colors and patterns on different body parts such as eyes, and body size.

2.3.3.3 Infection detection

L. loa was documented to be found in the fat bodies of abdomen and to a lesser extent the fat-bodies of the thorax and head of *Chrysops* species. *L. loa* larvae were classified into different stages including sausage (L1), larval stage 2 (L2) and larval stage 3 or infective stage (L3), with the development of microfilariae to the infective stage estimated to take between 10 and 12 days based on laboratory experiments. Dissecting vectors, also known as parity, was the only method used for detecting infection, and therefore, the dissection of *Chrysops* species under a microscope involved separating the head, thorax and abdomen manually, and identifying the presence (parous) or absence (nulliparous) of *L. loa* larva.

2.3.4 Species distribution, ecology and habitats

2.3.4.1 Distribution and ecology

The broad distributions of the main vectors, *C. silacea* and *C. dimidiata* are shown in the following map (Table 2.1), which were based on available georeferenced data of study locations and historical maps. Overall, *C. silacea* and *C. dimidiata* have been found throughout the greater part of the tropical equatorial rainforest. They are considered to become less dominant on the fringes where other species such as *C. langi, C. centurionis, C. zahrai* and *C. longicornis*, may replace them as vectors.

Table 2.1: Summary of primary and secondary *Chrysops* species main characteristics.

Species	Ecological distribution	Peak biting time	Putative host	Main biting location
C. silacea	Forest	Day	Human	Ground
C. dimidiata	Forest	Day	Human	Ground
C. langi	Forest	Crepuscular/Nocturnal	Monkey	Canopy
C. centurionis	Forest	Crepuscular/Nocturnal	Monkey	Canopy
C. zahrai	Forest-fringe	Crepuscular	Monkey/Human	Canopy/Ground
C. longicornis	Forest/Savanna/ Wooded areas	Crepuscular	Monkey	Canopy
C. distinctpennis	Savanna	Crepuscular	Monkey/Human	Canopy/Ground

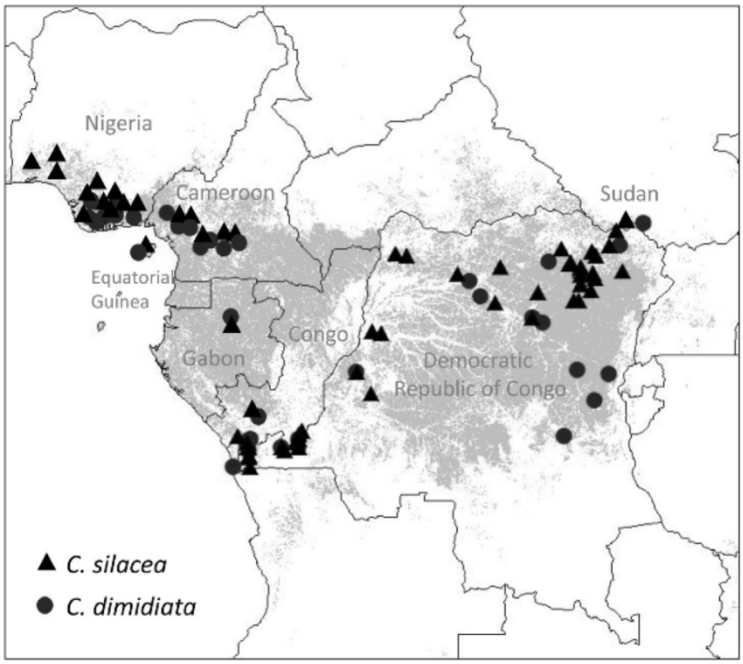
Source: Kelly-Hope *et al*, 2017.

Overall, *C. silacea and C. dimidiata* were considered to have similar habitats, and in addition to rainforests, have been found in rubber plantations, palm oil groves and fringes of mangrove swamps (Figure 2.3). Although these species frequently occur together, however, in some areas one species was found to dominate the other across different ecological settings with *C. silacea* more likely to adapt to human influenced environments.

2.3.4.2 Immature stage habitats

The *Chrysops* larvae and pupae were found to have well defined microhabitats, which were characterized by densely shaded streams and swamps, shallow slow flowing or standing water, with fine soft mud covered by layers of decaying leaves.

Figure 2.3: Map showing reported species distribution.



Source: Kelly-Hope *et al,* 2017.

2.3.4.3. Adult habitats

C. silacea and *C. dimidiata* were considered to be forest canopy dwellers descending to bite the human population in the forested or plantation areas. *C. silacea* in particular has been reported to avoid the deepest shade and the brightest sunlight and found to be most abundant in the patchy lightshade of intermediate areas. This vector has been found to bite at all levels of the forested areas, and throughout plantations, and will leave shelter to cross small clearings to enter houses or attack local workers.

2.3.4.4 Adult host seeking

C. silacea and *C. dimidiata* were considered to be practically noiseless, persistent daylight feeders and attack the ankles and the lower limbs most commonly. They are considered to hunt mainly by sight and noted to be attracted to color and movement; however, specific studies on host seeking behavior also found an olfactory stimulus related to forest leaves burning in wood fires; this attraction to fires is perhaps due to the CO_2 derived from them. *C. silacea* was reported to be more attracted to darker colors or the color blue/light blue.

Both *Chrysops* vectors peak biting times were closely associated with the diurnal periodicity of microfilariae of *L. loa* in humans. Specifically, the biting activity of *C. silacea* appeared to increase with a rise in temperature to 66-85 ºF and decrease with a rise in relative humidity of 56-100%.

2.3.4.5 Host preference and patterns

While *C. silacea* and *C. dimidata* were associated with the transmission of human *L. loa*, it was noted that they may attempt to feed on monkeys and other animals during the day; however, with monkeys there was minimal opportunity to take microfilaria from the nocturnally periodic *L. loa* found in monkeys. Gordon *et al* (1953) raised the importance of understanding the relationship between *Chrysops* infective density and human infection rates for control and curative measures, and aimed to define the different levels of risk, and explain why there may be disparities within and between populations and subgroups such as adults, children, African and Europeans.

2.3.4.6 Factors influencing spatio-temporal transmission

2.3.4.6.1 Abundance pattern measures

Several factors were identified as influencing the biting cycles and infection rates, which were primarily related to spatial and temporal environmental and anthropogenic factors. Adult *Chrysops* density was based on biting rates, number of flies caught per man per hour or tabanid per man per day.

2.3.4.6.2 Spatial environmental factors

Spatial environmental factors were related to the changes in forest density and light intensity both vertically and horizontally. Several studies examined the relationship between forested and cleared areas and found decreasing biting rates with deforestation related to anthropogenic plantation and human development. However, the rate of reduction varied between sites depending upon the amount and distance from forested vegetation, as well as by species with *C. dimidiata* noted to be more confined to forested areas. *C. silacea* was more dominant in villages whereas *C. dimidiata* was rarely found in the open environment, favoring primary and secondary forested areas.

2.3.4.6.3 Temporal environmental factors

Temporal environmental factors were related to climate and seasonality. For instances, the rainy season suggests better conditions for the breeding of the Chrysops vectors due to the presence of high moisture, humidity and very low temperature (Iboh *et al*, 2012). On the other hand, extreme conditions are avoided by the *Chrysops* spp, from bright sunlight to deep shade in forested areas (Noireau *et al*, 1990).

2.3.4.6.4 Wood fires

Wood fires were identified as an additional anthropogenic factor influencing transmission as it was observed that the smoke of wood fires appeared to attract *C. silacea* and detailed studies found a six-fold increase in biting densities of *C. silacea*, but not *C. dimidiata*.

2.3.5 Methods of vector control

In relation to the control of the *Chrysops* vector, overall few practical measures have been suggested; however, several historical articles referred to studies and potential methods of control can be divided into two main categories and sub-categories including the following:

(i) ‘Defensive Methods Control’ such as repellents, screening, clearing forest and bush.

(ii) 'Aggressive Methods of Control', which includes measures directed against adult and immature stages of *Chrysops*, such as adulticides and larvicides respectively. For immature stages, spraying foliage where eggs are laid has been suggested, and also the possibility of clearing brush and trees to remove shade or the canalizing of streams to remove stagnant vegetation may help to reduce fly density. For adults, it has been suggested that IRS may help to reduce density as they potentially rest on walls and ceilings waiting to obtain their blood meals or spraying the undergrowth in the vicinity of the oviposition sites may be of value.

2.3.6 Areas of potential future research

Based on the extensive research summarized in this study, the following areas for future research should be considered:

1- Alternative trapping methods for collecting adult *Chrysops* spp. that do not involve human landing catches such as Nzi traps or F-traps.
2- Review and assess the potential range of attractants, including wood-fires and trap color that may increase adult catch numbers.
3- Determine the optimal time and labor efficient methods for identifying breeding sites and collecting larvae for analysis within high-risk communities.
4- Determine the relationship between *Chrysops* infection rates and human loiasis risk, and if xenomonitoring could play a role in determining the level of risk within a community.
5- Determine the capacity of local entomologists, community members and field workers to identify main *Chrysops* spp. high-risk breeding and biting sites within communities and workplaces to help target control measures.
6- Determine if the ecological and climatic aspects of vector habitants and behavior, including the extent of deforestation and the potential role in reducing risk, can be predicted over larger geographical areas using remote sensing satellite imagery and modeled environmental data.
7- Determine the geographical extent of overlapping vector-borne disease infections to better determine how ivermectin could be effectively implemented.
8- Considering the identification problems with the use of traditional taxonomic tools for *Chrysops*, as molecular tools may play a pivotal role for the accurate

identification through mitochondrial cytochrome c oxidase subunit 1 (COI) gene sequences which became a standard method of identification of species of most invertebrates (Mugasa *et al*, 2018; Hebert *et al*, 2003). Therefore, this technique is not influenced by sex or a reproductive stage of the targeted taxa, and it is capable to reveal genetic diversity, which can solve species complexes (Banerjee *et al*, 2015).

2.4 Discussion

This is the first comprehensive review on the two main *L. loa* vectors *C. silacea* and *C. dimidiata* in more than 50 years. This is important as these vectors transmit loiasis, which although not formally listed as an NTD by the WHO due to its wrongly classified benign nature, has a significant epidemiological impact on the elimination programs for LF and onchocerciasis (WHO, 2012; Mogoung-Wafo *et al*, 2019; Kamgno *et al*, 2017). Studies on the epidemiology of loiasis, and the *Chrysops* vectors that drive transmission should have more prominence as these studies highlight the potential clinical impact of loiasis on individuals (Chesnais, 2017). Efforts to scale up elimination activities for other co-endemic filarial diseases such as LF and onchocerciasis have been prioritized, and all possible methods of control need to be considered. This review recommends that the control of *L. loa* vectors be considered as an additional strategy to reduce its transmission where the elimination of LF and onchocerciasis is compromised by the risk of *L. loa* induced encephalopathies; this may be particularly pertinent in hypo-endemic onchocerciasis areas where there are currently no safe chemotherapy options recommended (Kelly-Hope *et al*, 2017).

The review highlighted that the majority of studies were conducted in the 1950s and 1960s, when there was a surge of interest in the control of loiasis as an important disease. This was most likely related to the high prevalence found in local populations, rubber plantation workers and palm grove estates. The work done in Cameroon, and the significant body of related work published in several series of research papers, has provided an important and comprehensive foundation from which to build further work in this field, specifically in relation to the distribution, ecology and epidemiology in high-risk areas, and methods of targeted vector control, which could be integrated with other vector-borne diseases (Zouré *et al*, 2011). However, this will require a further significant

surge in interest, funding and purpose for capacity strengthening, as currently there is a general shortage of medical entomologists in Africa, and only a small pool of scientists currently working on *L. loa*.

Moving forward with any form of *Chrysops* control is likely to be multifaceted given that *C. silacea* and *C. dimidiata* are day-biting vectors that breed in densely shaded muddy streams and swamps, and rest in forest canopies high above ground-level. While these characteristics pose significant challenges, several studies indicated that vector control activities could impact on *L. loa* transmission (Kelly-Hope *et al*, 2017). Therefore, *Chrysops* control or repelling the biting of humans should be considered as an additional approach to be used in conjunction with other strategies. While this may not be a solution to reducing the risk of SAEs in the short-term given the duration of the transmission cycle, it would provide long-term benefits by reducing the number and intensity of infections, and thereby reducing the frequency of individuals with high microfilariae loads. Interestingly, a recent cross-sectional study in regards the prevalence and intensity of *L. loa* in Cameroon by Mogoung-Wafo *et al* (2019), has suggested a noncumulative nature of loiasis disease over a period of 23 years, as its transmission remained stable over time. Therefore, the latter study suggests that post-ivermectin SAEs is unlikely to occur in untreated communities with this profile. The use of modern tools and technology to identify local 'hotspots' and initiate vector control/repellency studies could be successful if targeted at the right place, at the right time, with the right intervention (Kelly-Hope *et al*, 2017).

For the immature stages of *Chrysops*, the use of community-based environmental management and larviciding with new formulations may be considered. Environmental management including drainage, filling, or clearing of vegetation around onchocerciasis breeding sites may be possible on a small scale, but is not practical in vast forested areas. The application of insecticide-based larvicides such as temephos or biological control agents such as *Bacillus thuringiensis* that specifically kill dipteran larvae through regular spraying offers an alternative method. These interventions have low toxicity and have been used widely in Africa for the control of onchocerciasis (*Simulium spp*.), control of *Dracunculus* (guinea worm) intermediate hosts (copepods) and malaria (*Anopheles* spp*.)* control (Monnerat *et al*, 2014).

For the *Chrysops* adult stages, the use of personal protection, household screening, IRS, and community-based insecticide spraying, or trapping may all help to reduce vector-human contact and transmission. Standard insect repellents have been shown to provide protection to people if applied regularly, especially in the morning peak biting times, however, new methods involving transfluthrin-impregnated hessian strips being trialed against outdoor exposure of malaria (*Anopheles*), urban filariasis (*Culex*) and Zika (*Aedes*) vectors may also be promising for loiasis (*Chrysops*) (Govella *et al*, 2015).

Furthermore, the information collected in this review has been used for a recent study for modeling habits based a maximum entropy species distribution modeling known as MaxEnt method (Badia-Rius *et al*, 2019). As a result, insights into the spatial and ecological parameters of the *L. loa* vectors driving transmission have provided a large-scale environmental analysis through the *Chrysops* spp. database where it can actually be used to delineated loiasis risk, which will be major to implement filariasis control and elimination programs in the equatorial rainforest of Central and West Africa (Badia-Rius et al, 2019).

These examples also provide insights into the potential for IVM with multiple diseases potentially being targeted simultaneously with shared human and financial resource and multiple impacts. However, it will be important to first conduct a situational analysis of each disease, including an assessment of the epidemiology and entomology, the extent of geographical overlap, vector control needs and available resources (WHO, 2016; WHO, 2012a). A systematic review and field assessments of tabanid trapping and control methods in other regions of the world may also help to determine what could realistically be trialed and used in Africa (Mizell *et al*, 2002).

Historically, a variety of traps, which the concept is on flight interception foundations and attraction to great targets, have been commonly used to trap tabanids flies (Blahó *et al*, 2012). For instances, different trapping methods such as the Nzi trap have been used to monitor *Chrysops* species abundance, and attractants such as CO_2 and octanol have been shown to potentially improve capture rates, which may be better than the use of wood fires (Mihok, 2002). Furthermore, Vavoua trap has been also useful for *Chrysops* spp. (Desquesnes *et al*, 2005). Interestingly, a novel trapping technique based on horizontally polarizing photovoltaic surface has shown a dual function where reflected light signal engages with polarotactic tabanids; and provides the electricity indispensable to rotate the

wire; therefore, reporting an efficiency of 92% in full sunshine (Blahó *et al*, 2012). The development of a trapping-attractant method for the loiasis vectors in Africa could also help with large-scale monitoring. *Chrysops* xenomonitoring has never previously been proposed as tool to determine community risk but may be a more cost-effective option than labor-intensive human seroprevalence surveys or RAPLOA (Kelly-Hope *et al*, 2017).

2.5 Conclusion

The review provides the most recent summary on the current knowledge on the two main *Chrysops* vectors, highlighting main field and laboratory procedures, species distributions, ecology, habitats and potential methods of vector control. Importantly, these factors may help determine the feasibility of how vector control may be implemented to reduce *L. loa* transmission and microfilariae loads in high prevalence communities, and if as a consequence, could also reduce the risk of SAEs associated with the drug ivermectin for LF and onchocerciasis elimination. This is particularly important in areas where a high prevalence of *L. loa* is co-endemic with hypo-endemic onchocerciasis 'hotspots' and the need for alternative strategies and novel approaches is critical if elimination targets are to be achieved. Focusing on those already infected ignores the role that the vector plays in driving the epidemiology and the consequent risk of SAEs.

2.6 Future Work

Future work could involve the need to evaluate the genetical differences between *C. silacea* and *C. dimidiata*. There is a gap in the knowledge of species and also in the genetic variability that may occur within and among species. Whether sibling species or subspecies occur in the *Chrysops* species is not known.

Since mathematical transmission models have been greatly used within the NTDs as guidance and as an indicator of quantification of the effect of intercession towards control and elimination of NTDs, therefore, bettering the health of many individuals. Since no mathematical model currently exists for loiasis, there could be a window of opportunity here to explore this area in a way to deeply comprehend the basis of population of biological development of this condition through research and advocacy for mobilization of resources for its control and management (Chesnais *et al*, 2017; Whittaker *et al*, 2018).

CHAPTER THREE: MICRO-EPIDEMIOLOGY OF FILARIAL CO-INFECTION IN MUNICIPALITY OF DANDE, BENGO PROVINCE

The Republic of Angola has been categorized as a priority country for onchocerciasis and lymphatic filariasis elimination, but the unknown co-distribution of the filarial *Loa loa* has become an impediment due to side adverse effects (SAEs) associated with ivermectin used in mass drug administration (MDA) campaigns. The Bengo province has been identified with a significant high-risk of loiasis where different intervention might be needed to be considered, although the presence and geographic convergence of the three filarial conditions are not well defined. This study conducted a rapid integrated filarial mapping based on RAPLOA-LF-REMO survey. In total, 2007 individuals from 29 communities in 5 municipalities were surveyed. Overall low levels of endemicity, with different overlapping distributions were found. Loiasis was found in 18 communities with a prevalence of 2.0% (31/1571), which contrasted to previous results defining the area as a high-risk zone. Onchocerciasis prevalence was 5.3% (49/922) in 8 communities, and LF prevalence was 0.4% for lymphoedema (8/2007) and 2.6% for hydroceles (20/761 males) in 7 and 12 communities respectively. The clinical mapping survey method helped to highlight that all three filarial infections are present in this zone of Bengo province. However, the significant difference in loiasis prevalence found between the past and this current survey suggests that further studies including serological and parasitological confirmation are required.

This chapter includes data from the first survey that has been published by Dr Miguel Brito *et al*, in part in *Parasite Epidemiology and Control* journal and entitled as 'Rapid integrated clinical survey to determine prevalence and co-distribution patterns of lymphatic filariasis and onchocerciasis in a *Loa loa* co-endemic area: The Angolan experience', 2017, 71:82, where my main contribution was within the field work as technical supervisor, and later on as data analyst (Appendix 10). Dr. Pedro Van-Dúnem has contributed as the main NTD coordinator through National Directorate of Public Health, Angola MoH. Dr Thomas, Dr. Robert and Dr. Benjamin have contributed the overall implementation of the project through their experience and financially through the College of Public Health, University of Florida. Dr, Louise and Michelle have contributed this project from the implementation to elaboration of the maps through ArGIS 10 software.

3.1 Current status on epidemiology of Neglected Tropical Diseases in Angola

According to WHO recommendations, Angola has started the mapping of NTDs, where LF is endemic in 22 municipalities, in the provinces of Huambo, Bié, Huila, Zaire, Cuando Cubango, Luanda and Bengo. Cases of elephantiasis were reported in the provinces of Huíla, Cuando Cubango and Luanda. The strategy based on chemoprophylaxis with albendazole and DEC in areas of co-infection and ivermectin in nonendemic areas has been successfully achieved (Angola NTDs, 2017).

With the current global interest in the control of NTDs, the Angolan government and partners have ensured the implementation of NTDs mapping step by step throughout the country (Angola NTDs, 2017).

For the Angolan onchocerciasis and LF national programs to implement MDA using ivermectin as a constituent drug, it is critical to understand the extent to which the three filarial infections overlap geographically. This will ensure that safe treatment strategies are implemented and monitored for impact and potential SAEs. The large-scale RAPLOA and REMO surveys provide essential baseline information; however, they were completed at different times, and on different and relatively large geographical scales. Micro-mapping and overlap-mapping are new approaches developed to delineate risk, define co-endemicity and target interventions which may be more useful in this loiasis high risk zone, which comprises both hyper- and hypo-onchocerciasis, and an unknown LF prevalence (Kelly-Hope *et al*., 2011, 2014, 2015).

3.2 Epidemiology of lymphatic filariasis in Angola

Following an exhaustive historical bibliographic review, there was no concrete reference to the existence of filariasis by *Wuchereria bancrofti* in Angola up until the 1960s, although the disease was first reported in Angola in 1920 by Gillet, it is still little-known today. Few studies from the country were noted where Pires *et al* (1959) studied on blood microfilariae in Lunda province (east of the country), therefore diagnosed the existence of species *Acanthoceilonema perstans* (Dipetalonema). Azevedo (1964) refers to the possibility of the existence of filariasis in Angola, without, however, presenting any case properly as diagnosed by the presence of filaria by microscopy. Casaca (1966) was not able to confirm the existence of *W. bancrofti* in Angola as well, as some cases of

elephantiasis were registered, but in none of them was the etiology likely due to *W. bancrofti*. Further reports from northern Angola suggested cases of elephantiasis and hydrocele, especially in the Cabinda, Zaire and Uíge, but nothing could be said about its etiology as well (Azevedo, 1964). There are references from Sasa (1976) being the first to admit the possibility of the existence of filariasis of the species *W. bancrofti* in northern Angola, namely Cabinda. Finally, Pinto (1986), in an integrated study of filariasis, malaria and African trypanosomiasis, studied individuals in the village of Sinde, Municipality of Buco-Zau, and found a prevalence of parasitemia *W. bancrofti* and *L. loa*, having *A. gambiae* and *A. funestus* mosquitoes as being the identified vectors for both LF and malaria.

In Angola, *W. bancrofti* parasite is transmitted by mosquitoes *Anopheles, Aedes, Culex* and *Mansonia* spp. (Pinto *et al*, 1973), which coincidentally are also vectors of transmission of the country's greatest endemic disease, malaria. It is popularly known as elephantiasis, alluding to one of the chronic and stigmatizing forms that affects a number of those with the disease. It affects individuals living in rural areas and an increasing number of urban dwellers in poor sanitation (WHO, 2017).

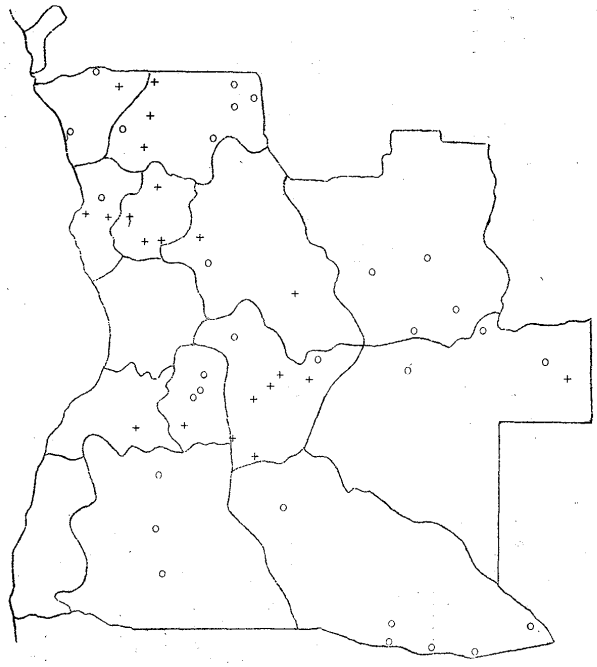
Although bancroftian filariasis may cause a considerable degree of morbidity related to the acute and chronic forms of the pathology, mortality is almost null (Maciel *et al*, 1994). These facts associated with the little information available in regards this disease and the low demand in health services, since most patients only reach for those services in late stages of the disease, often disfiguring, makes this endemic condition a negligence in Angola (Bungo, 2002).The current prevalence of lymphatic filariasis in Bengo region is not known but is expected to be low based on the historical and current data (Brito *et al*, 2017.)

3.3 Epidemiology of onchocerciasis in Angola

From the bibliographic review carried out, few published studies from Angola were noted. And even in these, there is greater emphasis on onchocerciasis, which had its first publication in 1950 by Strangway *et al*. In 1961 the Institute for Medical Research presented the Conference on Onchocerciasis in Africa, which summarized the knowledge available at that time on onchocerciasis in Angola. Table 3.1 shows the geographical distribution at the time of 30 *Similium* species identified so far in the Angola territory in the 60s. Of all these species, only *S. damnosum* and *S. albivirgulatum* were clearly anthropophilic. However, the only species that behaves as a vector is the *S. damnosum*, whose distribution is schematized on the Figure 3.1.

Onchocerciasis has been reported in Angola since the 50s and its annual CDTi strategy has been implemented since 2005 (Angola NTDs, 2017).

Figure 3.1: Distribution map of *S. damnosum* in Angola. *S. damnosum* present (-+), *S. damnosum* absent (-o).



Source: Casaca, 1961

Table 3.1: Distribution of *Simulium* species in northern region of Angola.

Province	Zaire	Uíge					Luanda		
Municipality	S. salvador	Cuango			Damba	Uíge	Zombo	Dande		
Villages	S. Salvador	Cuango	Icoca	Quimbele	Damba	Carmona	Maquela do Zombo	Mabubas	Quicabo	ùcua
Species										
S. adersi									**X**	**X**
S. albivirgulatum	**X**									
S. alcockialcoki										**X**
S. alcoki, var Djallonens	**X**								**X**	**X**
S. bequarti										
S. bovis										**X**
S. cervicornutum	**X**	**X**								
S. colas-belcourtl										
S. dam nosum	**X**				**X**	**X**	**X**			**X**
S. fragai										**X**
S. hirsutum										
S. hirsutum, var Sexien										
S. impukane										
S. ituriense	**X**									
S. janzi										
S. loutelense										
S. mcmahoni										**X**
S. medusaeforme										
S. medusaeforme, var Angolensis										
S. medusaeforme, var hargreavesi										**X**
S. nigritarsis										
S. nill										
S. rodhaini									**X**	**X**
S. ruficorne										
S. schoutedeni			**X**							
S. teniaculum										
S. unicornulum			**X**							
S. unicornulum, var Rofundum										
S. vorax										**X**
S. wellmannni										

Source: Casaca, 1966.

3.4 Epidemiology of *Loa loa* in Angola

Loiasis is widespread mainly in the northern of Angola. Until the 1960s this endemic disease has ever been exclusively a subject of a methodical survey to verify its existence, but doctors who have worked in that region have already mentioned it for a long time. While doing a work in the Mission of identifying tropical diseases and later in the Institute of Medical Research, from 1953 to 1966, it was possible to observe some cases of loiasis, either in the very places where the disease was contracted, or in patients coming from several points of Angola. As a result, almost all cases were clinically diagnosed by the passage of the adult worm by the conjunctiva or by its repetition under the dermis after administration of DEC or by the appearance of Calabar swellings.

Of the known *Chrysops* species, *C. dimidiata* and *C. longicornis*, have already been described as existing in northern Angola which brings up in favor of the possibility of existence of the loiasis only in the northern of Angola (Casaca, 1966). Angola has a *L. loa* high-risk area in Bengo province in the northwest region of the country with high prevalence estimates (>40.0%) and associated risk of SAEs (Zouré *et al*, 2011).

3.5 Methodology

3.5.1 Study location

Bengo province borders Luanda (South), Zaire (North), Uíge (northeast, and Kwanza Norte (East) provinces, and has an area of 31,371 Km^2 and a population of 351 579 with the majority of people living in rural areas basic housing, and limited access to electricity, clean water or sanitation (Costa *et al*, 2012; INE, 2014; Figure 3.2). Bengo province has a tropical climate, with an average temperature of 25 ºC and a rainy season from October to April with peaks in November/December and March/April. It has large areas of rain forest as part of Kissama National Park and Kibinda Forest Reserve, as well as forest-savanna mosaic, savannah and woodland vegetation. Several large rivers run through the province including de Dande River, which flows rapidly from the higher eastern region to the western lowlands where it flows through the capital, Caxito, into the Atlantic Ocean (INE, 2014).

Figure 3.2: Health and demographic surveillance area in the Dande Municipality, Bengo province, Angola

Source: Adapted from Costa *et al*, 2012

3.5.2 Study sites

The study was primarily focused in the CISA area (Centro de Investigação em Saúde de Angola/Health Research Centre of Angola) of Dande Municipality, which comprises the three communes of Caxito, Mabubas and Úcua. The CISA includes the Dande Health Demographic Surveillance System, which operates an ongoing population monitoring system across 69 geo-referenced communities comprising approximately 16,000 households, and a population of approximately 60,000 inhabitants in an area of 4,700 Km^2 (Costa *et al*., 2012).

The population density in Dande Municipaliity is 217 929, with approximately 13 inhabitants per Km^2, and has both urban and rural characteristics, with 16 communities in the main town of Caxito (Censo, 2014). The landscape is primarily savanna with a forest gallery around the riverbanks and higher areas. The Dande, Lifune and Úcua rivers run through the area and have permanent water flow, with many lakes surrounding lower parts of the Dande. Figure 3.3 shows photos of the landscape, including rivers, vegetation and typical houses. The presence of the river and the thick vegetation provide grounds for breeding of mosquitoes and flies.

Figure 3.3: The landscape, including rivers, vegetation and typical houses from Dande Municipality. A, D-Typical vegetation, B, C-Typical river, E, F- Typical houses.

3.5.3 Inclusion criteria

Any willing participant who had lived in the study area for at least three years and was above the age of five years was eligible.

3.5.4 Exclusion criteria

People who had not lived in the study area for the last three years and are younger than 14 years old were excluded from the study.

3.5.5 Mapping strategy and field logistics

As spatial analysis has the capacity to map out the distribution of diseases, it enables integration of information and thus constitutes a powerful tool for planning, monitoring and controlling endemic diseases.

To determine the prevalence and co-distribution of the filarial diseases at a fine geographical scale across in the study area, a rapid integrated micro-mapping activity assessing the evidence of key clinical conditions was conducted in January-February 2014. One community was selected from within a 15 km grid which was created in the geographical information system (GIS) software (ArcInfo 10, ESRI, Redland CA) to demarcate a 5-15km distance between each, to ensure that prevalence was measured at regular spatial intervals across the entire study area and to incorporate a range of environmental characteristics.

In the adjacent five municipalities, only one or two communities were selected to provide insights into the endemicity in the surrounding CISA areas. Communities were selected if they reported more than 100 adult (>15 years) inhabitants according to the guidelines for rapid mapping of bancroftian filariasis in Africa. In each community, all adults presented at the time were invited to participate, and the first 100 individuals accepting the invitation were included in the survey. CISA research team based in Caxito implemented the mapping survey. In total, five research assistants were trained in the detection of LF, onchocerciasis and loiasis clinical conditions using a short survey and physical examination, in accordance with the rapid mapping assessment methods described below.

3.5.6 Filarial clinical indicators

3.5.6.1 Lymphatic filariasis clinical indicators

To determine the presence of lymphatic filariasis, all participants aged above 15 years old underwent physical examination that included evaluation for lymphedema of the extremities (tissue swelling or thickening), hernias, and palpitation of the testicles and scrotum (scrotal swelling), verified by a local medical officer.

3.5.6.2 Onchocerciasis clinical indicators

The clinical presentations of onchocerciasis are predominantly dermal, lymphatic, and ocular in character and are a result of host inflammatory reactions in the tissue to the dead microfilaria (Remme *et al,* 2017).

To determine the prevalence of onchocerciasis, the basis of the REMO method was used (Noma *et al*., 2002). The standard REMO method was developed for APOC to delineate zones of endemicity, identify CDTi-priority areas and estimate the number of people to be treated. It samples a proportion of villages to determine the prevalence in the local area by feeling for sub-cutaneous worm nodules in 50 adult males, who are aged >20 years and have lived in the community for at least 10 years. If ≥20% of adults have nodules, the local area is considered to be a CDTi-priority area, and where nodule prevalence is <20% then clinic-based treatment is applied. In this current study the REMO method was used. However, because the area was hypo-endemic both males and females were included with exception of pregnant women and those that were breastfeeding.

3.5.6.3 *Loa loa* clinical indicators

To determine the prevalence of *L. loa*, the rapid assessment procedure for loiaisis (RAPLOA) diagnostic survey method was used based on Takougang *et al* (2002) and Zouré *et al* (2011). RAPLOA is based on an individual's history of eye worm, with information obtained from a simple short non-invasive survey (Annex 2).

Loiasis was confirmed in an individual when the answers to the three questions were all positive 'yes'. This is considered to be the restricted definition of eye worm or loiasis and has been found to correlate with high *L. loa* microfilariae rates and risk of SAEs (Addiss *et al*., 2003). An unrestricted definition of loiasis is considered to be a positive response

to the first question only. In each community, individuals aged >15 years and whom had resided in the area for at least 5 years were selected for assessment.

3.5.7 Data Analysis and mapping

After running the RAPLOA-LF-REMO survey, the geographic coordinates (latitude, altitude) of the community were collected using a geographical positioning system (GPS) unit in a central point of the village (Appendix 4 and 5).

All survey data were entered into Microsoft Excel 2010 by the CISA team, and IBM SPSS statistical version 21 Software was used for analysis. Prevalence distributions by sex (male, female), and age class (15-19, 20-29, 30-29, 40-49, 50-59, >60) were summarized and statistical differences examined using the chi-square test (p-value significant <0.05). The GPS locations of the communities and households within the CISA area were available from the Health Demographic Surveillance System (HDSS) in Dande. The filarial prevalence in each CISA community was mapped using ArcGIS 10 (ESRI, Redland CA), and communities found to have more than five cases and multiple filarial diseases were further mapped by household to better understand the co-distribution at a micro-level.

3.5.8 Ethics, consent and patient referrals

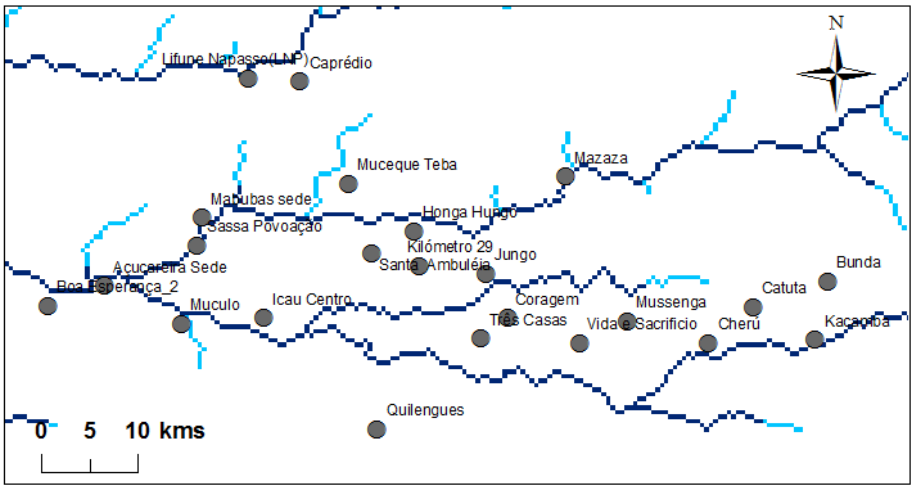
The Ethical Committees from the Ministry of Health of Angola and the Liverpool School of Tropical Medicine Research approved the survey (Appendix 1). Written informed consent was obtained from each respondent and was orally explained if respondents were illiterate (Appendix 1 and 2). For those who refused to participate, no further questions were asked, and no information was recorded. For those individuals who consented, their name, sex, age and years of residence in the community were recorded before proceeding with the questions about filarial clinical conditions. For individuals found to be positive for clinical conditions, information on risk factors and prevention were provided, and they were referred to the local health services, which had previously been informed of the survey's activities. As part of the HDSS in Dande, an established communication system is already in place between the CISA team and all community clinics, therefore good support and follow-up for the patients could be provided.

3.6 Results

3.6.1 Fieldwork

This study was conducted in January-February 2014. In total, 29 community study sites were sampled across the six municipalities, being the majority from CISA area in Dande Municipality (Figure 3.4; Table 3.2). All study sites were within endemic areas of LF, onchocerciasis and loiasis as defined by the modeled maps with a range of prevalence distributions as shown in maps in Figure 3.5 A-D. For loiasis, all sites were in pre-defined high-risk 'hyper-endemic' areas (>40%), for onchocerciasis the majority of sites were in pre-defined low risk 'hypo-endemic' areas (<20%) with approximately one third in CDTi priority areas (>20%), and for LF, all sites were in very low (< 5%) or low (5-20%) 'hypo-endemic' areas as defined by the modeled *mf* map.

Figure 3.4 Study sites in Dande Municipality



About half of the sites (n=13; 48.1%) had lower than the expected number of adults meeting the inclusion criteria, which is likely due to population movements associated with long-term civil conflict, seasonal (agricultural) employment, charcoal activity and rural-to-urban immigration trends, which is now common in this area after the war. This is limited the analysis as it was not possible to include necessary sample size of 100 individuals or assess the prevalence in those individuals, who are most often men, as they were not present, apparently working outside the village.

Figure 3.5 Filarial prevalence distributions in CISA communities. A- Loiasis, B- Onchocerciasis, C- LF hydrocele, D- LF lymphoedema

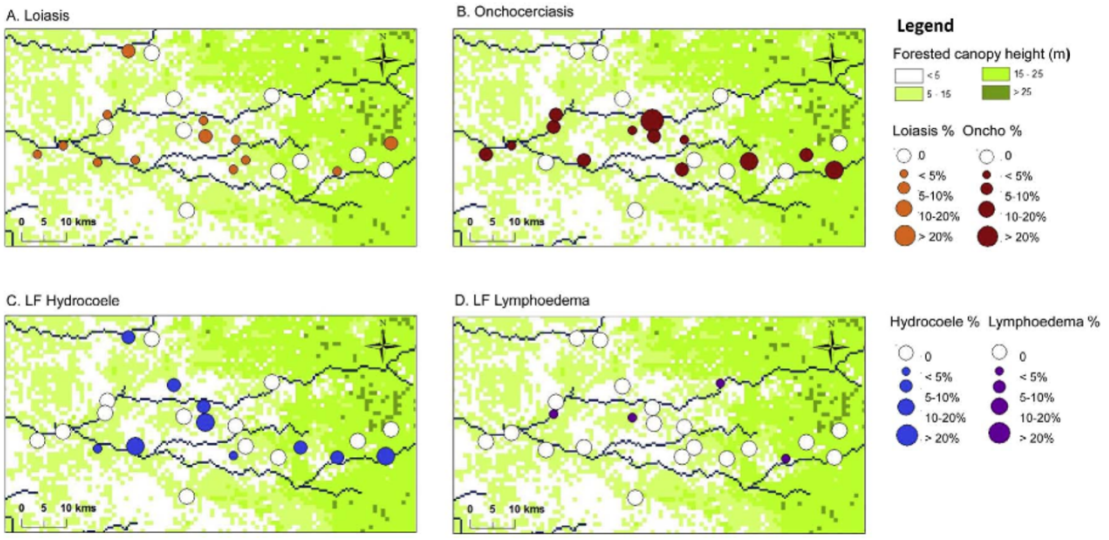


Table 3.2: Summary of study sites, population and individual samples.

Municipality	Population*	N of sampled villages	N examined	N males	N females	Average age (years)
Dande	217929	23	1.380	495	885	41
Ambriz	21806	2	191	51	140	39
Pango-Aluquem	6571	1	99	54	45	31
Dembos- quimbaxe	28202	1	117	56	61	26
Bula Atumba	16047	1	116	60	56	36
Nambuangongo	61024	1	104	45	59	34
TOTAL	**351579**	**29**	**2.007**	**761**	**1246**	**40**

* Total population recorded during the National Census 2014

In total, 2007 individuals were surveyed, with numbers ranging from 9 to 149 in each community study site. There were 761 males (37.9%) and 1246 females (62.1%), with average age of 40 years, ranging from 15 to 94 years based on 1558 individuals reporting their age. Figure 3.6 presents the sampling and analysis framework for each disease with details on the numbers, prevalence rates by age, sex and community study site described below and outlined in Table 3.3-3.7.

3.6.2 RAPLOA survey

For loiasis, 1571 individuals were included in the community analysis based on the RAPLOA inclusion criteria. In total, 346 individuals (22.0%) stated that had experienced a worm moving in their eye; of those 55 individuals (16% of 346; 3.5% of 1571) confirmed the worm with the picture shown by the interviewer, with 31 individuals (56% of 55; 2% of 1571) confirming the most recent episode being between 1 and 7 days (Table 3.2, Figure 3.5). There were no significant differences between males and females in the unrestricted and restricted eye worm definitions (Table 3.4). Of the 29 communities surveyed, a total of 18 communities were found to have loiasis positive individuals based on the restricted definition.

Overall, prevalence ranged from 0%-10%, and the distribution in the CISA areas only is shown in Figure 3.5 A-D, which highlights that positive communities are located throughout the area with no obvious geographical pattern of clustering. When examining prevalence by age and sex (based on 1558 individuals who provided information), overall, no significant differences between males and females or by age class were found (Table 3.3). Community-level age and sex analysis was not possible due to the low numbers, with most communities reporting one or two loiasis cases, and only Tabi (Ambriz Municipality) and Muxaluando (Nambuangongo Municipality) reporting three to five positive cases including both sexes and a range of age class.

Table 3.3: Comparison of loiasis prevalence based on restricted and unrestricted RAPLOA definitions.

	N examined	*N* included in RAPLOA *	*N* yes to question 1	% yes to question 1	N Positives yes to 2 questions	% Positives yes to 2 questions	N Positives yes to 3 questions	% Positives yes to 3 questions
Total	2007	1571	346	22.0	55	3.5	31	2.0
Male	761	592	117	19.7	17	2.9	15	2.0
Female	1246	979	229	23.4	38	3.9	25	2.0

* Persons older than 15 years old and that have been resident in the village for at least 5 years

Table 3.4: Summary of loiasis distribution measured by RAPLOA methods.

Municipality	Village	*N* examined	*N* included RAPLOA *	Positives RAPLOA	% Positive RAPLOA
Dande	**Total Dande**	**1380**	**1010**	**18**	**1.8**
	Açucareira Sede	99	68	1	1.5
	Sassa Povoação	101	76	0	0.0
	Total	**200**	**144**	**1**	**0.7**
	Boa Esperança 2	100	89	2	2.2
	Bunba	41	26	2	7.7
	Honga Hungo	46	33	1	3.0
	Icau Centro	24	22	1	4.5
	Jungo	99	65	2	3.1
	Kilometro 29	27	20	2	10.0
	Mabubas	125	95	1	1.1
	Mazaza	25	18	0	0.0
	Muceque Teba	34	18	0	0.0
	Muculo	52	44	1	2.3
	Quilengues	32	30	0	0.0
	Santa Ambuleia	65	41	0	0.0
	Total	**670**	**501**	**12**	**2.4**
	Caprédio	9	4	0	0.0
	Lifune Napasso Kicabo	53	37	2	5.4
	Total	**62**	**41**	**2**	**4.8**
	Catuta	36	31	0	0.0
	Cherú	48	37	1	2.7
	Coragem	100	67	1	1.5
	Kacamba	25	11	0	0.0
	Mussenga	98	71	0	0.0
	Três Casas	92	74	1	1.4
	Vida e Sacrificio	49	33	0	0.0
	Total	**448**	**324**	**3**	**0.9**
Ambriz	**Total Ambriz**	**191**	**185**	**4**	**2.2**
	Capulo	42	42	1	2.4
	Tabi	149	143	3	2.1
Pango-Aluquem					
	Cazuangono	99	87	2	2.3
Dembos-Quimbaxe					
	Coqueiros	117	87	0	0
Bula Atumba					
	Ibundo	116	106	2	1.9
Nambuangongo					
	Muxaluando	104	96	5	5.2
TOTAL Bengo	**-**	**2007**	**1571**	**31**	**2.0%**

* Persons older than 15 years old and that have been resident in the village for at least 5 years

3.6.3 REMO survey

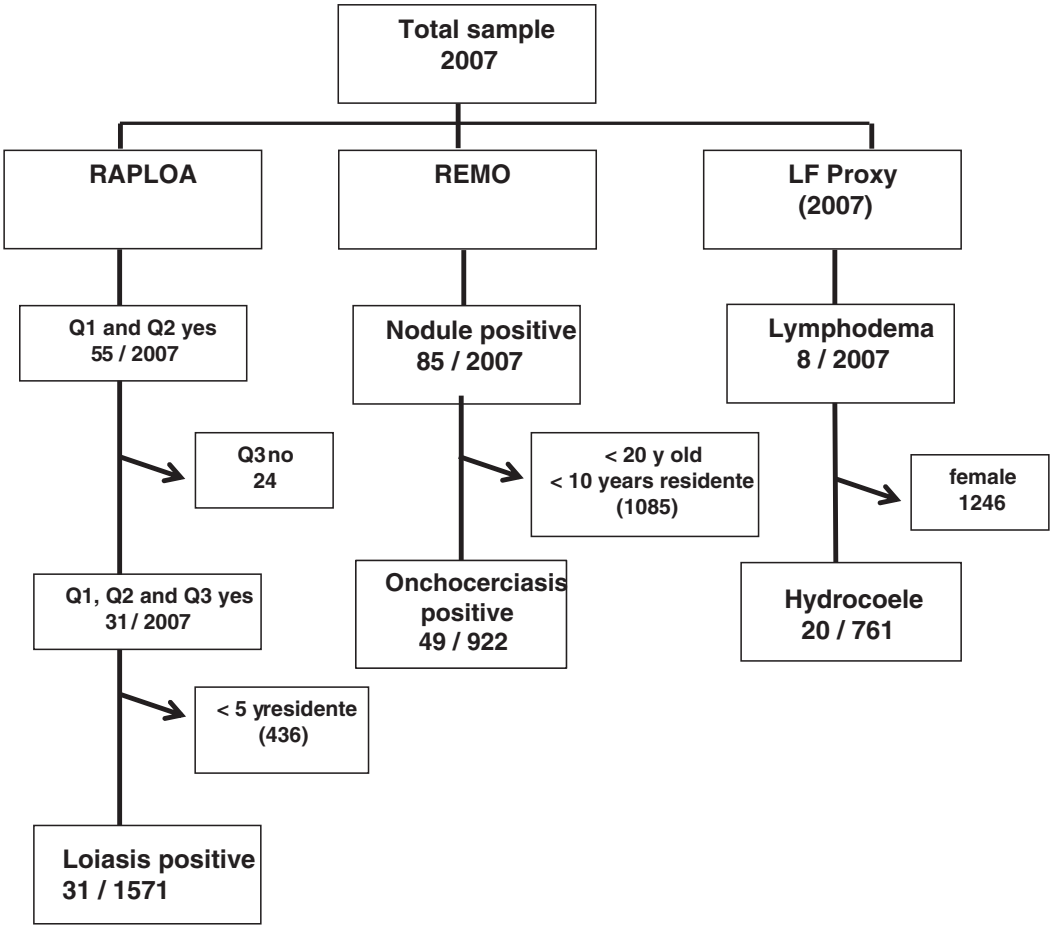
For onchocerciasis, there were 988 individuals from 8 communities who were included in the analysis based on the REMO inclusion criteria, with 49 individuals (5.3%) found to have palpable nodules (Table 3.5, Figure 3.5). Of the 29 communities surveyed, initially 18 communities found positive individuals, however only 8 communities met the required minimum numbers of 50 male adults. For the purpose of this study in a hypo-endemic area, all data were included in the analysis and mapped. Overall, the prevalence ranged from 4.8% to 7.6% in the 8 REMO communities, and from 2.9% and 42.9% in the other communities with fewer individuals surveyed. The distribution of all communities in the CISA area is shown in Figure 3.5 B, which highlights that the positive communities are located throughout the study area with no specific geographical pattern. When examining prevalence by age, overall, there was an increasing prevalence by age, which was found to be significant overall, and in the female sub-group (Table 3.6). Community-level age and sex analysis was not possible due to the low numbers of only 3/7 positive cases found in each community.

Table 3.5: Summary of onchocerciasis nodules distribution measured by REMO method.

Municipality Commune	Village	*N* examined	*N* included REMO *	Positive Nodules	% Positive Nodules
Dande	**Total Dande**	**1380**	**486**	**26**	**5.3**
Caxito	**Total**	**200**	**97**	**6**	**6.2**
	Açucareira Sede	99	34	1	2.9
	Sassa Povoação	101	63	5	7.9
	Total	**670**	**261**	**15**	**5.7**
	Boa Esperança 2	100	66	5	7.6
	Bunba	41	18	0	0.0
	Honga Hungo	46	7	3	42.9
	Icau Centro	24	16	1	6.3
	Jungo	99	23	1	4.3
	Kilometro 29	27	10	1	10.0
	Mabubas	125	55	3	5.5
	Mazaza	25	7	0	0.0
	Muceque Teba	34	8	0	0.0
	Muculo	52	18	0	0.0
	Quilengues	32	7	0	0.0
	Santa Ambuleia	65	26	1	3.8
	Total	**62**	**24**	**0**	**0.0**
	Caprédio	9	1	0	0.0
	Lifune Napasso Kicabo	53	23	0	0.0
	Total	**448**	**104**	**5**	**4.8**
	Catuta	36	21	2	9.5
	Cherú	48	25	0	0.0
	Coragem	100	19	0	0.0
	Kacamba	25	7	1	14.3
	Mussenga	98	8	1	12.5
	Três Casas	92	17	1	5.9
	Vida e Sacrificio	49	7	0	0.0
Ambriz	**Total**	**191**	**163**	**7**	**4.3**
	Capulo	42	33	0	0.0
	Tabi	149	130	7	5.4
Pango-Aluquem	Cazuangono	99	63	3	4.8
Dembos- quimbaxe	Coqueiros	117	50	3	6.0
Bula Atumba	Ibundo	116	86	5	5.8
Nambuangongo	Muxaluando	104	74	5	6.8
TOTAL Bengo	**-**	**2007**	**922**	**49**	**5.3%**

* Persons older than 20 years old and that have been resident in the village for at least 10 years

Figure 3.6: Sampling and analysis framework.



3.6.4 Lymphatic Filariasis clinical signs

About 2007 individuals were eligible for inclusion in this study. In total, 8 individuals (0.4%) were found to have leg lymphedema and 20 men were found to have hydrocele (2.6%) (Tables 3.6 & 3.7; Figure 3.6). Of the 29 communities surveyed, a total of 7 communities reported lymphedema cases, 12 communities reported hydrocele cases and 3 communities reported both clinical conditions, however, different individuals were affected. For lymphedema, no significant differences by age and sex were found; however, for hydrocele, there was an increasing prevalence by age class with significant differences found (P-value <0.001). The highest hydrocele prevalence rates were reported among men aged over 50 years, which ranged from 3.4% to 8.5%. The distribution of LF clinical cases for the CISA area are shown in Figure 3.5 C and highlight that there is no specific geographical pattern for either condition or a very low prevalence of lymphedema in the area.

Table 3.6: Prevalence of loiasis, onchocerciasis and LF by sex and age class.

		Prevalence							
		Loa loa		**Onchocerciasis.**		**Lymphatic filariasis**			
						Lymphoedema		Hydrocele	
	Age class	*N*	%	*N*	%	*N*	%	*N*	%
Total	15 to 19	167	1.8	-	-	254	0.8	--	--
	20 to 29	312	1.3	214	1.4	439	0.2	--	--
	30 to 39	275	2.9	199	3.5	356	0.6	--	--
	40 to 49	242	2.1	149	5.4	292	0.0	--	--
	50 to 59	269	1.5	164	7.9	318	0.3	--	--
	> 60	293	2.4	196	9.2	332	0.6	--	--
	Chi-square	ns		p<0.001		ns		--	--
Male	15 to 19	80	2.5	-	-	116	0.0	116	0.0
	20 to 29	110	0.0	86	1.2	157	0.6	157	0.6
	30 to 39	92	3.3	65	6.2	118	0.8	118	0.8
	40 to 49	86	2.3	51	3.9	108	0.0	108	1.9
	50 to 59	95	1.1	51	7.8	116	0.0	116	3.4
	> 60	125	1.6	87	6.9	142	0.7	142	8.5
	Chi-square	ns		Ns		ns		p<0.001	
Female	15 to 19	87	1.1	-	-	138	1.4	--	--
	20 to 29	202	2.0	128	1.6	282	0.0	--	--
	30 to 39	183	2.7	134	2.2	238	0.4	--	--
	40 to 49	156	1.9	98	6.1	184	0.0	--	--
	50 to 59	174	1.7	113	8.0	202	0.5	--	--
	> 60	168	3.0	109	11.0	190	0.5	--	--
	Chi-square	ns		p<0.001		ns		--	--

Table 3.7: Summary of clinical LF distribution measured by the presence of lymphedema and hydrocele.

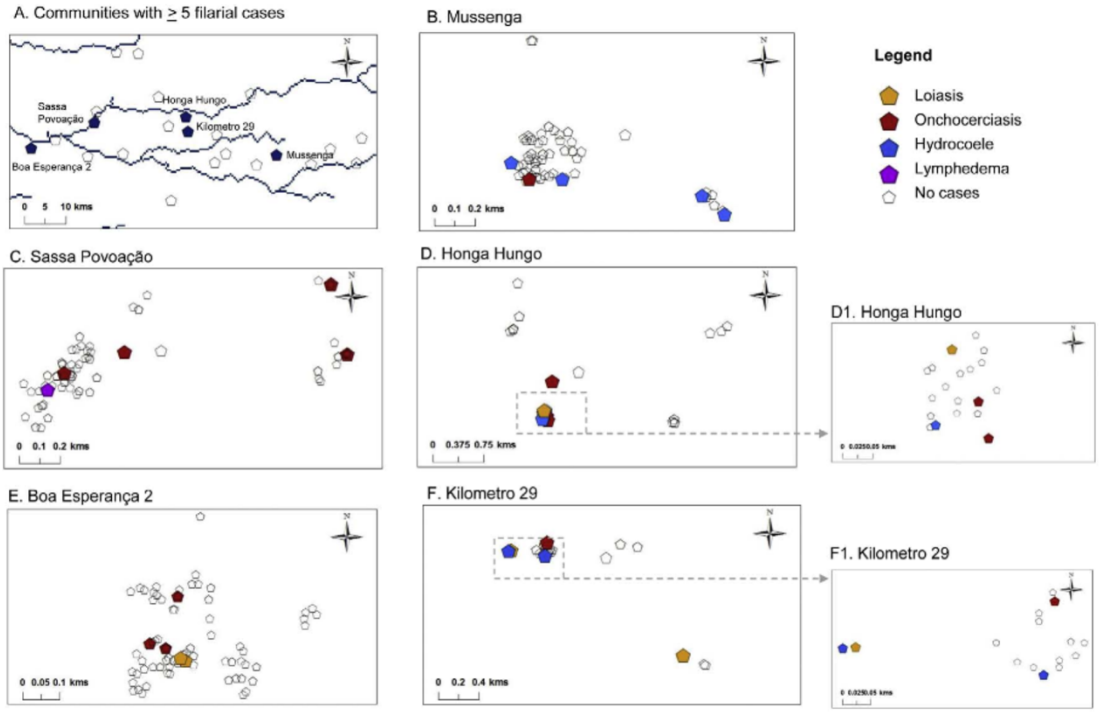
Municipality	Village	*N* examined	Positives Lymphodema	% Positive Lymphodema	*N* male	Positives hydrocele	% Positive hydrocele
Dande	Açucareira Sede	99	0	0.0	24	0	0.0
Caxito	Sassa Povoação	101	1	1.0	32	0	0.0
	Boa Esperança 2	100	0	0.0	30	0	0.0
	Bunba	41	0	0.0	17	0	0.0
	Honga Hungo	46	0	0.0	15	1	6.7
	Icau Centro	24	0	0.0	8	1	12.5
	Jungo	99	0	0.0	33	0	0.0
	Kilometro 29	27	0	0.0	10	2	20.0
	Mabubas	125	0	0.0	37	0	0.0
	Mazaza	25	1	4.0	15	0	0.0
	Muceque Teba	34	0	0.0	21	2	9.5
	Muculo	52	0	0.0	24	1	4.2
	Quilengues	32	0	0.0	16	0	0.0
	Santa Ambuleia	65	2	3.1	32	0	0.0
	Caprédio	9	0	0.0	4	0	0.0
	Lifune Napasso Kicabo	53	0	0.0	14	1	7.1
	Catuta	36	0	0.0	7	0	0.0
	Cherú	48	1	2.1	16	1	6.3
	Coragem	100	0	0.0	29	0	0.0
	Kacamba	25	0	0.0	14	2	14.3
	Mussenga	98	0	0.0	43	4	9.3
	Três Casas	92	0	0.0	34	1	2.9
	Vida e Sacrificio	49	0	0.0	20	0	0.0
Total		**1380**	**5**	**0.4**	**495**	**16**	**3.2**
Ambriz	Capulo	42	0	0.0	5	0	0.0
	Tabi	149	1	0.7	46	2	4.3
Total		**191**	**1**	**0.5**	**51**	**2**	**3.9**
Pango-Aluquem	Cazuangono	99	0	0.8	0	0	0.0
Dembos- quimbaxe	Coqueiros	117	1	0.9	56	0	0.0
Bula Atumba	Ibundo	116	0	0.0	60	0	0.0
Nambuangongo	Muxaluando	104	1	1.0	45	2	4.4
TOTAL	**-**	**2007**	**8**	**0.4**	**761**	**20**	**2.6**

3.6.5 Micro-mapping co-distributions

Overall, there was no distinct geographical pattern of the presence or absence of the different filarial diseases in each of the CISA study communities. Four communities were found to co-endemic for all three filarial diseases, and four communities had none. The presence of loiasis cases was found in two villages, onchocerciasis cases alone in one community, and LF cases alone in two communities. The presence of loiasis and onchocerciasis cases was found in four communities, and loiasis and LF cases alone in two communities, while the presence of onchocerciasis and LF cases was found in four communities.

The communities of Boa Esperança 2, Honga Hungo, Kilometro 29, Mussenga, Sassa Povoação were found to have >5 filarial cases and were located across the study area (Figure 3.5 A). The results from the micro mapping are presented in the following Figures 3.5 B-F and 3.7 and highlight the 'within-community' distribution of each filarial disease. Only one individual from one household in Boa Esperança 2 community was found to have clinical conditions related to two cases (loiasis and onchocerciasis) while all other communities reported the presence of cases from different households, indicating the absence of co-infection.

Figure 3.7 Micro-mapping filarial in high-risk communities.



3.7 Discussion

This is the first survey in the Municipality of Dande, province of Bengo, Angola, to determine the presence of the filarial infections using rapid field methods based on key disease specific clinical conditions. The approach builds on the well-established RAPLOA-LF-REMO survey method, which have been used extensively across Central and West Africa to define prevalence distributions and direct treatment strategies (Noma *et al.*, 2002; Takougang *et al*., 2002; Zouré *et al.*, 2014). To-date, only one integrated RAPLOA-REMO survey in neighboring DRC has been reported, which found significant differences in co-endemicity across the country (Tekle *et al*., 2011). Bordering Angola, the Bas Congo region in DRC had reported SAEs (WHO, 2004a, 2004b), and the availability of loiasis, onchocerciasis and LF data, enabled simple maps and models to be developed to highlight the specific high-risk areas (Kelly-Hope *et al*., 2015, 2014). This is particularly important for Angola, as the current study in Bengo province was conducted in a previously defined high-risk area (Zouré *et al*., 2011), where the filarial diseases were found to be present.

The rapid integrated filarial 'RAPLOA-LF-REMO' clinical survey method used here in Angola also included a new micro-mapping approach to determine prevalence distributions at a fine geographical scale. Study communities were mapped on a scale of ~5-15km apart, which helped to highlight the wide variability in prevalence of all three infections in the relatively small CISA area (Costa *et al*., 2012). However, due to some small community sample sizes, and a highly mobile population with some potentially 'at risk individuals' absent from the study, accurate estimates of risk were compromised. This fact highlights how risk and population dynamics can change over time, and that there is a need for up-to-date information and assessments before treatment strategies are implemented. Notwithstanding these limitations the overall prevalence was low (defined as meso-to-hypo endemic), with no obvious spatial patterns found, the communities with a higher risk of one or more diseases were readily identifiable. This has provided important preliminary information to the national programs as a 'first step' in understanding the local filarial epidemiology and will help to investigate community risk in more detail as a 'second step' by assessing serological and parasitological prevalence rates, and other potential risk factors including the main vectors of loiasis (*Chrysops* spp) and LF. It will also help to determine if alternative intervention strategies are required (Kelly-Hope *et al*., 2017, 2015).

For loiasis, a significant difference was found between the current survey data with the RAPLOA modelled maps from 2010 (Zouré *et al.*, 2011). The reason for this significant difference is unclear but may be related to the timing and spatial resolution of data collected as previous surveys were only conducted in a few villages between 2003-2008 (Zouré *et al*., 2011). It may also be related to levels of deforestation, tree cover change and recent seasonal migration patterns as many people were not available for the survey, and/or urbanization changes in the area (Costa *et al*., 2012; Censo, 2014; Hansen *et al.*, 2013; World Resources Institute, 2017). Further investigation is needed to better determine *L. loa* prevalence, as it may be underestimated. If the *L. loa* risk is found to be meso-endemic, then the risk of SAEs is also likely to be higher. However, the proportion of individuals with high *L. loa mf* loads (>30,000 ml) in such meso-endemic areas is unknown as only a few studies have been conducted, and primarily focused on the relationship between RAPLOA and *mf* rates in high transmission areas (Addiss *et al* 2003; Boussinesq *et al*, 2001; Schluter *et al*, 2016; Takougang *et al*, 2002; Wanji *et al*, 2012).

For onchocerciasis and LF, there was more correlation between the current survey results and modeled levels of endemicity. However, it is important to note that higher onchocerciasis prevalence was found in drug naïve communities outside the defined CDTi area and coincided with communities with a high prevalence of loiasis. A better understanding of the risks and benefits of extending the current CDTi boundaries or whether alternative strategies including doxycycline (Molyneux *et al*, 2003), and/or vector control for both *Simulium* spp and *Chrysops* spp are required (Kelly-Hope *et al*, 2017). An extensive review of *Chrysops* spp suggests that various forms of vector control or new repellent approaches to deter day biting vectors may reduce risk and thus *L. loa mf* loads, which could be a novel intervention in low onchocerciasis transmission areas (Kelly-Hope *et al*, 2015, 2017). Such hypo-endemic onchocerciasis areas are now priority for ESPEN (WHO, 2015), who could provide further strategic, operational and technical support for the implementation and systematic monitoring of safe and effective strategies.

For LF, the few clinical cases verified by medical officers helped to confirm that they were not inguinal hernias. There may be more and/or missed hydrocele cases in the community given the mobility of the population and social sensitivities of exposing legs and genitalia. However, it is likely that the well-monitored HDSS operating in this region would have identified the magnitude of the problem already. While the hydrocele rates in low endemic areas may not be a reliable predictor of prevalence compared with highly

endemic communities (Eigege *et al*, 2003; Gyapong *et al*, 1998), the data indicate that transmission is low, which is in accordance with historical data (Casaca 1966). A random sample of night bloods to detect microfilaremia may have helped to confirm the low LF prevalence and could be included in the more detailed 'secondary step' serological assessments in the future. Nonetheless, this initial clinical survey, suggests that LF transmission may be readily interrupted with the WHO recommended alternative strategy of albendazole twice yearly plus vector control (Kelly-Hope et al, 2017, WHO, 2012).

Further for LF, collaborative links with the national malaria control program will be essential to help increase bed net coverage, which is very low with only around one third of households owning an ITN (Cosep Consultoria Consaude and ICF International, 2011). Morbidity management and disability prevention may be readily addressed through home-based lymphoedema care, and surgery for the few men identified with hydrocoele (WHO, 2013a, 2013b). This epidemiological pattern of low LF prevalence in *L. loa* endemic area has been found elsewhere in DRC and Chad (Bakajika *et al*, 2014; Bregani *et al*, 2007; Tekle *et al*, 2011), and supports the idea that elimination may be more easily achieved in these co-endemic areas than previously thought. This LF-loiasis pattern also supports the idea of competitive exclusion of filarial parasites in Africa (Molyneux *et al*, 2014).

3.10 Conclusion

A better understanding of the extent and intensity of serological and parasitological co-infections is essential, and how risk and populations may have changed since the original loiasis mapping is required for the scale-up of safe and effective treatment strategies (Molyneux *et al.*, 2014). It is feasible that the rapid integrated clinical survey method presented here could be conducted across a larger geographical region in selected 'data naïve' co-endemic areas as an initial risk mapping model. This will highlight the range of co-endemic patterns in the different regions of the country, and provide a broader perspective of the potential resources, specific investigations and technical expertise that may be needed. This is important, as the Angolan LF elimination program will face several challenges in implementing and monitoring the impact of several intervention strategies across the country. It will require significant collaboration, and human and financial support from international partners and stakeholders over the next couple of years in order to accelerate the national targets and global goals (WHO, 2015).

CHAPTER FOUR – MOLECULAR AND SEROLOGY BASED ASSAYS FOR DETECTION OF FILARIAL DNA IN POPULATION OF MUNICIPALITY OF DANDE, BENGO

To treat a filarial condition based on mass drug administration (MDA), it is essential to know where the disease is, and which population is at greater risk. Understanding how different measures of infection are related to residual transmission is important for making programmatic decisions about stopping MDA. Furthermore, the possibility of existing adequate tools to assess infection state will be of extremely relevance to elimination programs, either for the M&E outcome or for the decision of when to cease treatment (Thiele *et al*, 2016). Rapid antigen tests and molecular techniques are more sensitive than mf detection through microscopy, and therefore provide the additional advantage of daytime blood sampling in areas where the parasite is nocturnally periodic (Mladonicky *et al*, 2009)

The Republic of Angola is widely endemic for filariasis and the national neglected tropical disease (NTD) program is scaling up MDA activities to interrupt transmission with the aim of eliminating onchocerciasis and lymphatic filariasis (LF), taking the co-endemicity of loiasis and risk of serious adverse events (SAEs) into account. To better determine the prevalence of the three infections in co-endemic area, an epidemiological survey was conducted across 22 communities using a combination of clinical, serological and molecular diagnostic methods to detect *Loa loa, Onchocerca volvulus* and *Wuchereria bancrofti*. Information on length of residency, vector knowledge, MDA history and bet net were also collected.

A total of 1616 individuals (38.1% male: 61.9% female), with an average age of 43 years, were examined. For *L. loa*, 62% (*n*=100/1616) individuals were found to have eyeworm, based on the rapid assessment procedure for loiasis (RAPLOA) surveys, an 11.5% (*n*=178/1543) based on nested PCR analyses of venous blood. *L. loa* prevalence in long-term residents (>10 years) and older individuals (>60 years) were significantly higher, and older men with eyeworm were better informed about *Chrysops* vectors. For *O. volvulus*, 4.7% (*n*=74/1567) individuals were found to be positive by enzyme-linked immunosorbent assay (Ov ELISA), with only three individuals reporting to have ever taken ivermectin. For *W. bancrofti*, no infections were found using the antigen-based immunochromatographic test (ICT) and real-time PCR analysis; however, 27 individuals presented with lymphatic filariasis (LF) related clinical conditions (lymphoedema = 11, hydrocoele = 14, both = 2). Just under half (45.5%) of the participants owned a bednet, with majority (71.1%) sleeping under it the night before. Our approach of using

combination diagnostics reveals the age-prevalence of loiasis alongside low endemicity of onchocerciasis and LF. Future research foci should be on identifying opportunities for more cost-effective ways to eliminate onchocerciasis and to develop innovative surveillance modalities for clinical LF for individual disease management and disability prevention.

The data from this chapter has been published in *Parasite Epidemiology and Control* journal, on a paper entitled as 'Clinical, serological and DNA testing in Bengo Province, Angola further reveals low filarial endemicity and opportunities for disease elimination' as a sequence of Brito *et al* (2017) paper mentioned in the previous chapter, but the latter will include the molecular and serological diagnostics (Appendix 11). My main contribution was within the fieldwork as technical supervisor, as laboratory main technician for real-time PCR and nested PCR performance, data analyst and participant at scientific conferences and local newspapers (Appendix 13 and 14). Dr. Pedro Van-Dúnem has contributed as the main NTD coordinator through National Directorate of Public Health, Angola MoH. Miguel Brito contributed as project research coordinator. António Martins as head of Caxito General Hospital. Russell Stothard as help to develop the manuscript. Dr Thomas, Dr. Robert and Dr. Benjamin have contributed for the overall implementation of the project by performing the diagnostic of onchocerciasis through the Ov16 test at the College of Public Health, University of Florida. Dr, Louise has supported this project from the implementation to elaboration of the maps through ArGIS 10 software.

4.1 Polymerase Chain Reaction

The polymerase chain reaction (PCR) technique was initially described in 1985, allowing an *in vitro* amplification of a specific DNA fragment through a cyclic mechanism involving denaturation, hybridization and elongation of the DNA strand based on thermostable DNA polymerase (Saiki *et al*, 1988).

The nested PCR technique which usually yields the sensitivity and specificity within diagnostic laboratory settings, has as principles, the use of amplicons products from a previous PCR assay as a template for a second PCR based on specific primers that are usually not the same from the first PCR primer set. The visualization of the amplification

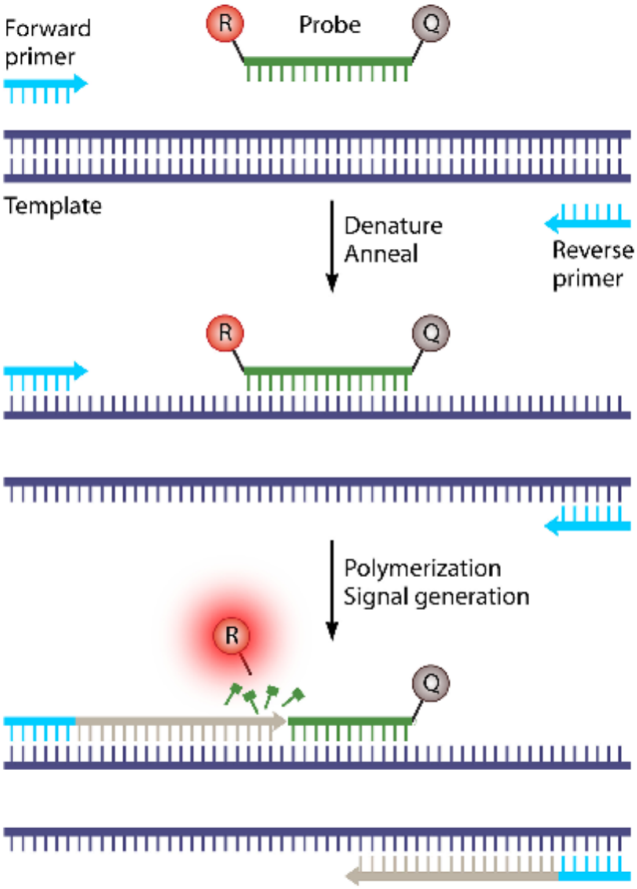
products in a conventional PCR is based on ethidium bromide or alternative dye succeeding agarose gel electrophoresis. The predicted size of the PCR products is what dictates its specificity (Verweij *et al*, 2014).

The main advantages of nucleic acid-based methods such as PCR assays, among numerous of them include high sensitivity, specificity, reduced risk of contamination, simpler standardization of diagnostic procedures, and, monitoring possible ongoing of drug resistant parasite strains (Osei-Atweneboana *et al*, 2012). Furthermore, DNA samples can also be stored and used for genetic characterization and molecular typing, providing a valuable tool for surveys and surveillance studies (Weiss, 1995). As disadvantages, the pre-condition for trained personnel and highly cost machinery are a handicap for their fitness within low-resource settings (Notomi *et al*, 2015).

4.1.1 Real-Time Polymerase Chain Reaction

During the amplification process of this technique, the amplicons are measured in real-time, where the most commonly used probe is the "Taqman" probe, in which the 5'-to-3' exonuclease activity of *Taq* polymerase cleaves the hybridized probe during the elongation phase of the amplification reaction (Klein D, 2002; Espy *et al*, 2006). Within this process, the fluorescent molecule at the 5' end of the probe is separated from the quencher molecule at the 3' end of the probe, leading to a fluorescent signal that can be quantified after each amplification cycle as taken place as seen on Figure 4.1.

Figure 4.1: Taqman probe-based real-time PCR chemistry.



Source: Verweij *et al*, 2014

4.2 Serological diagnostics approaches for lymphatic filariasis

The diagnostic needs of the GPELF aims to eliminate filariasis as a public health problem by the year of 2020, and therefore, it has been relied traditionally on antigen detection as a method for identifying active infections and mapping areas to be targeted for MDA, such as the BinaxNOW Filariasis card test (Gass *et al*, 2012; WHO, 2011a). This immunochromatographic card test (ICT) has been majorly used in populations suspected of carrying *W. bancrofti* parasite and has been performed since 2000 (Weil *et al*, 2013; Weil *et al*, 1997). As advantages of this ICT tool, it can be performed anytime of the day or night (Bakajika *et al*, 2014). However, recent evidence indicates that the sensitivity of ICT might be highly variable due to the narrow time window that might lead to false-positives, age, sex, presence or absence of living adult worms, as well as microfilarial density (Gounoue-Kamkumo *et al*, 2015; Weil *et al*, 2013; Simonsen *et al*, 2004).

The Alere Filariasis Test Strip is the known next generation filarial antigen tool that has been performed since 2013 and has been refined to upgrade on the card test by reporting better sensitivity, specificity, more extended shelf life and lower costs, in Central Africa (Republic of Congo and Democratic Republic of Congo) and West Africa (Liberia and Côte d'Ivoire) settings (Weil *et al*, 2013; Pion *et al,* 2015; Chesnais *et al*, 2014; Beng *et al*, 2020). The Og4C3 antigen ELISA test targets circulating filarial antigen (CFA) in peripheral blood composed by adult filarial worms in the lymphatics and has been reported to be more sensitive compared to the conventional microfilarial detection (Lau *et al*, 2014; Njenga *et al*, 2007). However, as limitations this assay requires a highly structure laboratory setting with well trained staff (Simonsen *et al*, 2008).

As for antibody tools available, there are the novel effective tools for LF control known as Wb123, Bm14 and Wb-SXP-1 assays, which are specific and sensitive recombinant antigen that are used as a marker for filarial infection specifically to certify the cutoff of LF transmission (Kubofcik *et al*, 2012; Kelly-Hope *et al* 2018).

4.3 Molecular diagnostics approaches for lymphatic filariasis

Molecular diagnostic techniques include majorly PCR assays used to detect filarial DNA in human blood samples and molecular xenomonitoring (MX) used to detect parasite DNA in pooled mosquitoes or human blood by PCR (Weil, 2007).Pilotte *et al* (2013) performed a Taqman based real-time PCR analysis able to identify both *W. bancrofti* and *Brugia malayi* DNA extracted from human bloodspots and vector mosquito pools, therefore reporting higher sensitivity, lower costs and reduced labor work for LF surveillance in co-endemic settings in comparison to singleplex assays such as conventional PCR.

Loop-mediated isothermal amplification (LAMP) assay has been widely used to detect various filarial nematodes including *W. bancrofti* and *O. volvulus* and *L. loa*, due to its friendly use in low-resource settings. LAMP is based on a simple visual detection of turbidity produced when magnesium pyrophosophate is precipitated, following fluorescence via an intercalating dye (Tomita *et al*, 2008). Furthermore, its single-step reaction has been reported to amplify several copies of the targeted DNA within an hour, even when a considerable quantity of non-target DNA is observed (Notomi *et al*, 2000). Moreover, the *Bst* DNA polymerase routinely applied in LAMP are more receptive to inhibitors detected in clinical specimens and vectors to which easily dodge while performing a PCR (Alhassan *et al*, 2014).

Recently, a colorimetric test for diagnosis of filarial infection and vector surveillance using non-instrumented nucleic acid LAMP (NINA-LAMP), has been developed by Poole *et al* (2017), where the capacity of distinct strand displacing DNA polymerases has been assessed jointly with the pH sensitive dyes. Therefore, as a result, the efficiency, integrity and adaptability of this assay have showed fitness for monitoring the progress of filarial control programs.

4.4 Serological diagnostic approaches for onchocerciasis

The most developed and advanced serological assays as markers for exposure to onchocerciasis is the IgG4 response to the *O. volvulus* marker Ov16 antibody test that is expressed by the larval stages (L3 and L4) of the parasite; and the OV luciferase immunoprecipitation system assay (LIPS) (Golden *et al*, 2016; Mathison *et al*, 2019). Although this antigen Ov16, is able to target exposure of *O. volvulus* and its infection while the prepatent phase takes place, however, this assay is not able to discriminate within ongoing infection and past exposure to this parasite (Kelly-Hope *et al,* 2018). Burbelo *et al,* 2009, have developed a faster and more distinct assay for diagnosis of *O. volvulus* infection by using four mutated versions previously described *Onchocerca*-specific antigens through LIPS assay. Therefore, targeting antibodies in all four *O. volvulus* antigens, which has easily discriminated *O. volvulus*-infected samples from the uninfected ones, suggesting that this assay is a plausible point-of-care (POC) detection of onchocerciasis (Burbelo *et al,* 2009). Currently, there are no antigen detection test commercially available for onchocerciasis for programmatic use (Kelly-Hope *et al*, 2018).

The minimally invasive SD Bioline Onchocerciasis/LF IgG4 rapid test have been assessed as an integrated surveillance means for elimination of LF and onchocerciasis in Mali, and therefore, results suggest a promising alternative for interruption of transmission in children population (Dolo *et al*, 2019).

4.5 Molecular diagnostic approach for onchocerciasis

Currently, no distinct molecular assay is available for the routine clinical diagnosis of *O. volvulus*, but nonetheless, a few experiments have been performed through the years including conventional PCR, real-time PCR, and PCR-ELISA for the detection of this nematode. As for instance, a challenging PCR assay of the *cox*-1 gene has been performed in Australian clinical setting using a nodule sample where a worm was detected through microscopy (Crowe *et al*, 2018). Furthermore, Thiele *et al* (2016) developed a novel proposal to a real-time PCR with melt curve analysis (qPCR-MCA) based on a single-step reaction, able to detect and identify the nematode mf in skin snip samples, therefore, suggesting a higher sensitive rate compared to conventional PCR and O-150 PCR-ELISA.

As a result, this new approach suggests a timesaving, lower contamination risk and higher sensitivity assay.

PCR of the skin snips samples suggests higher sensitivity; however, it still requires sampling of the skin snips (Morales-Hojas *et al*, 2001). Screening tests using skin snip samples is difficult to perform at bigger scale as a result of the labor-intensive nature of the process itself, the invasiveness, and as a local disease burden decreases, a lowered acceptability from the community to be subjected to this type of process (Awadzi *et al*, 2015).

4.6 Serological diagnostic approaches for *Loa loa*

The capacity to diagnose *L. loa* infection promptly and precisely continues to be a difficult task. After a few attempts with serologic testing by immunoblotting and enzyme-linked immunosorbent assays (ELISA) with crude reporting low precision due to cross-reactivity with other filarial conditions and strongyloidiasis; an alternative antigen, known as LISXP-1, has been used on antigen-based immunoassays, therefore reporting a significantly strong sensitivity and specificity status (Akue *et al*, 1998; Egwang *et al*, 1989; Pedram *et al*, 2017).

Burbelo *et al* (2008) uses LIPS assay for *L. loa* with a recombinant fusion proteins antigen in order to assess antibody responses which has been reported to be readily, sensitive, specific and high throughput. Therefore, this research has shown that LIPS assessing anti-IgG response against LISXP-1 generates significantly high levels for differentiating *L. loa*-infected patients from controls with 100% for both sensitivity and specificity, with a considerable reduced level of cross-reactivity with a few *O. volvulus* and *W. bancrofti*-infected patients sera (Burbelo *et al*, 2008)

The LoaScope, the most recent mobile cell phone-based video microscopy device, has been developed as a POC assay that provides ready and rigorous quantification of *L. loa* mf within 2 minutes after a finger prick (D'Ambrosio *et al*, 2015; Boussinesq *et al,* 2018). This device has been considered potential for onchocerciasis and LF programs in *L. loa* endemic areas, as a way to quickly facilitate the safe administration of ivermectin in Africa context, where the literature has reported high densities of *L. loa* mf such as in

Nigeria, Mali and Senegal (D'Ambrosio *et al*, 2015; Kamgno *et al,* 2017). Nonetheless, this device is not as rigorous when estimating densities lower that <150 mf/ml of blood due to sampling constraints, and inadequate of manufacturing outspread using (D'Ambrosio *et al,* 2015).

4.7 Molecular diagnostics approaches for *Loa loa*

Definitive diagnosis of loiasis can be done by the identification of the adult worm in the eye of after its removal from under the skin or by morphological identification of the microfilariae in blood smears, but these are low throughput methods inadequate for mapping purposes. Molecular methods such as loop-mediated isothermal amplification and quantitative polymerase chain reaction (qPCR) assays are credible alternatives to microscopy-based techniques since they combine a high degree of sensitivity and specificity with high throughput capabilities (Drame *et al*, 2014). However, molecular methods remain impractical for rapid testing at the point-of-care and are relatively expensive.

Nuchprayoon *et al*, (2005) reported an assay system that uses a polymerase chain reaction-restriction fragment length polymorphism (RFLP-PCR), targeted the internally-transcribed spacer 1 (ITS1) region of the ribosomal RNA gene, based on the *Ase I* restriction enzyme digestion that discriminates between five species of filarial nematodes: *W. bancrofti, B. malayi, Brugia pahangi, Dirofilaria inmitis* and *Dirofilaria repens*. This suggests the utility of this PCR in the differential detection of other filariae as *L. loa* and *M. perstans* that sympatrically co-exist in West and Central Africa (Jiménez *et al*, 2011).

The quantitative PCR (qPCR) has reported to be very sensitive, however, it is time consuming, highly costly and not suitable for rapid testing at the point-of-care (POC) (Fink *et al*, 2011).

4.8 Serological diagnostic approach for Mansonelliasis

Despite the fact that the literature does not present efficient antigen- or antibody-detecting immunological assay for diagnosis of mansonelliasis infections, both assays have been designed for onchocerciasis and LF infections, where cross-reactivity with mansonelliasis sera have been tested (da Silva *et al*, 2017; Shelley *et al*, 2001; Wanji *et al*, 2016)

4.9 Molecular diagnostic approach for Mansonelliasis

Molecular diagnosis is highly performed to target and identify mf in peripheral blood, skin biopsies, and adult worms in other tissues, and therefore, it has been proven to be both highly sensitive and specific (Medeiros *et al*, 2015). Succeeding the improvement of filarial parasite-diagnostic PCR assays, significant uncertainties in regard 'old species' and 'new species' distributions have been elucidated among filarial species, including within *Mansonella* species (da Silva *et al*, 2017).

The nested PCR developed in 2010 by Ta-Tang *et al* was able to target any form and life stage of filariae in human host or vector based on the species size, through amplification of the filarial parasite ribosomal ITS1 DNA using universal filariae PCR primers. This assay is performed alongside with gel electrophoresis and/or Sanger sequencing, and therefore, permits the classification of unknown species such as *M. perstans* variant Deux (Ta-Tang *et al*, 2016; Ta-Tang *et al*, 2010). Overall, the literature has showed that most of the assays so far performed are able to discriminate *M. perstans* or *M. ozzardi* from filarial parasites from other species, but unable to discriminate *M. perstans* from *M. ozzardi* (Ta-Tang *et al*, 2010; Mourembou G, 2015). The main limitations of the PCR assay remain the fact that it requires highly trained personnel and expensive infrastructure, which are not suitable indicators for low-resource settings (Ta-Tang *et al,* 2010).

Although further assays such as PCR-RFLP do not permit the classification of new filarial species, it has the advantage of demanding less complicated infrastructures to support them and can differentiate a broad spectrum of filarial species using primers through PCR and/or RFLP assays as complements (Jimenez *et al,* 2011). As disadvantages it still

requires access to PCR reagents and PCR device, which can be a constraint condition for filarial parasite epidemiological research (Jimenez *et al*, 2011).

Jimenez et al first described the DNA-detecting LAMP filarial parasite assay in 2016, although it was not designed specifically for detection of *Mansonella* parasites. However, Poole *et al* (2019) have developed a new LAMP RDT for *Mansonella* spp. based on species-specific DNA repeat sequences proposing a highly sensitive biomarker for the latter parasite.

4.10 Methodology

4.10.1 Study site and sampling

The study was conducted in the Centro de Investigacao em Saúde de Angola/Health Research Centre of Angola (CISA) Health Demographic Surveillance System (HDSS-Dande), area of Dande Municipality, in the northwestern province of Bengo, Angola, as a follow on from Brito *et al*. (2017). In this current study, 22 communities across the CISA area were selected on the basis of their proximity to the rivers and vegetation to help target communities that may perhaps be at higher risk of onchocerciasis and loiasis.

In each community, at least 100 individual aged $\geq$ 15 years were approached and invited to participate in the survey by the field teams through house-to-house visitation. Following consent, a short questionnaire was administered in either Portuguese or Kimbundo, the official and local languages, respectively. Demographic information was collected on the individual's age, sex, length of residency and on clinical clinical indicators for loiaisis (eye worm) and LF (lymphoedema, hydrocoele). In addition, individuals were asked if they had: i) seen the loiasis vector shown to them in a photograph, to better understand if the *Chrysops* spp. was in the area; ii) taken MDA for onchocerciasis as some communities were in a CDTI area defined by APOC and iii) a mosquito net and had used it the night before.

To determine prevalence of filarial infection through serological and molecular methods, 200 μL blood from each individual was collected by venous puncture and stored in EDTA tubes (Figure 4.2). In the field during the survey, 100 μL blood was used for LF detection

using a rapid diagnostic test, and the remaining samples were kept in cooler boxes, before reaching the laboratory and where two dried blood spots were prepared on Whatman 3M filter paper and the kept at 4ºC. The remaining 100 µL blood was used for both LF real-time PCR and *L. loa* nested PCR.

Figure 4.2: Blood collection performance by CISA health technician

4.10.2 Prevalence

Loa loa: To determine the prevalence of *L. loa,* first the rapid assessment procedure for loiasis (RAPLOA) survey method was used, which is based on an individual's history of eye worm. A restricted definition was defined as individuals answering positively to a three question survey including: i) their experience;, ii) recognition of an eye worm in a photograph and; iii) a recent episode of eye worm lasting between 1-7 days (Takougang *et al*., 2002; Zouré *et al*., 2011).

Second, molecular biology methods were used to detect *L. loa*. Genomic DNA was obtained from the whole blood EDTA tubes and extracted using the QIAamp® DNA Blood Kit (Appendix 8). Nested PCR for *L. Loa* detection was adapted from Jimenéz *et al* (2011). Briefly, a first PCR, targeted a 475 bp region of the ribosomal Internal Transcribed Space (ITS) DNA region common to different nematode species was

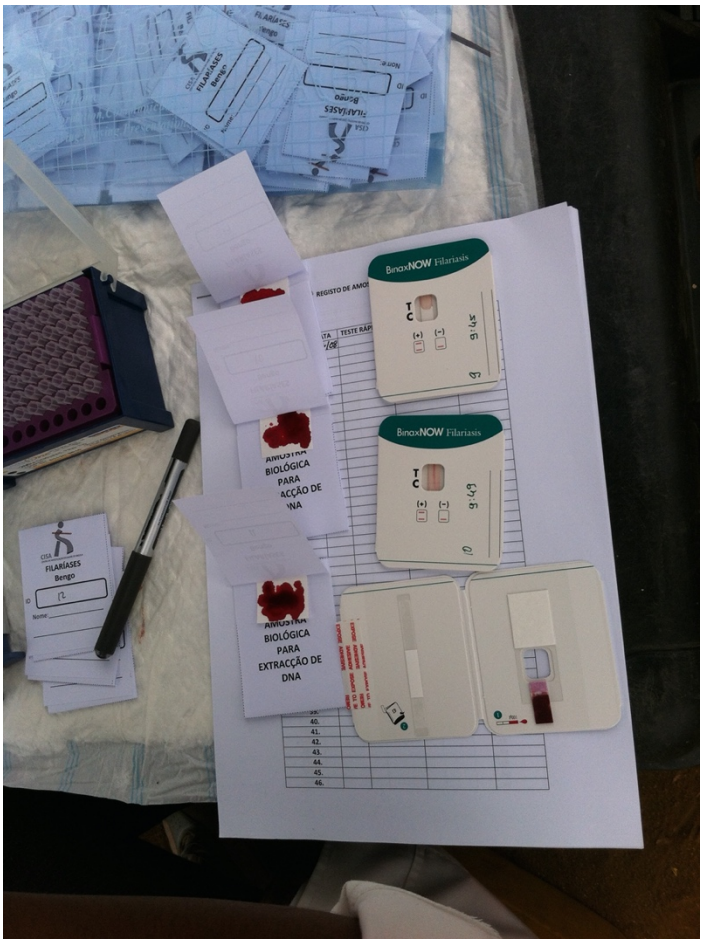
performed and visualized in 2.0% agarose gel electrophoresis. Positive samples were used in a second PCR with *L. loa* specific primers giving 143 bp fragment, visualized in 2.0% agarose gel electrophoresis, confirming the *L. loa* infection. *L. loa* positive control was kindly provided by Professor Samuel Wanji, University of Buea, and Cameroon.

Onchocerciasis: To determine prevalence of the onchocerciasis parasite *O. volvulus*, dried blood spots were exported to the University of South Florida, US for analysis of *O. volvulus* antigens by an ELISA method. Blood spots were eluted and examined for the presence of IgG4 antibodies recognizing the Ov16 antigen by ELISA, following standard procedures as described in (Oguttu *et al.* (20146). Data on the presence of nodules using the REMO method was not used in the study, so we were unable to compare REMO survey data with the ELISA result (Brito *et al.,* 2017). This is acknowledged as a limitation of the study.

Lymphatic filariasis: To determine the prevalence of LF, first, the presence of main clinical conditions including limb lymphoedema (tissue swelling or thickening), and hydrocoele (scrotal swelling) was identified by the field team and verified by a local medical officer. No severity of the condition was recorded. Second, the prevalence of *W. bancrofti* antigen was determined using the rapid diagnostic immunochromatographic test (ICT) card (BinaxNOW Filariasis, (Alere, Portland, ME) with 100µL blood (Figure 4.3). The ICT pad contains a gold-labelled polyclonal anti-filarial antibody that binds to filarial antigen from the blood. Tests were conducted during the survey using the blood collected. At the time of the survey in 2014, the ICT diagnostic was the standard recommended test for determining LF endemicity (WHO, 2011), however, since then several studies have shown that there is a cross-reactivity problem with the ICT diagnostic in communities with high *L. loa* prevalence, which can lead to false positives (Bakajika et al, 2014; Pion et al, 2016). To account for possible cross-reactivity in the ICTs, the prevalence of *W. bancrofti* DNA was determined using Genomic DNA extracted from the whole blood EDTA tubes using the Qiamp DNA Blood kit (Appendix 7). Real-time PCR using specific primers and a Taqman probe was used to detect *W. bancrofti* following Rao *et al*., (2006), with thermal cycling conditions on a Biorad CFX connect real time system instrument (Biorad). Amplification controls included water (no DNA template) as a negative control, and gDNA from *W. bancrofti* positive control was kindly provided by Professor Thomas R. Unnasch, University of South Florida, USA.

The preparation for real-time and nested PCR for detection for *W. bancrofti* and *L. loa* parasites respectively was performed at Pediatric Hospital David Bernardino molecular laboratory, in Luanda. PCR products were detected by agarose gel electrophoresis.

Figure 4.3: Blood spots and ICT processing (Appendix 6).



4.10.3 Data analysis and mapping

All survey data were entered into Microsoft Excel 2010 by the CISA team, and statistical software program IBM SPSS statistical version 21 was used for analysis. First, the prevalence for each community was quantified and mapped using ArcGIS 10.7 (ESRI, Redland CA). Second, the prevalence by length of residency (<5, 5-9, > 10 years), sex (male, female), and age group (15-19, 20-29, 30-29, 40-49, 50-59, ≥60) were summarized and statistical differences examined using the chi-square test (*p*-value significant <0.05). Finally, data were examined overall and by sex and age groups (above/below mean) to assess the relationship between i) *L. loa* eyeworm and infection and the knowledge of the *Chrysops* vector ii) onchocerciasis and history of MDA and iii) LF and bed net ownership and usage.

4.10.4 Ethics and consent

The study was approved by the Angolan National Ethics Committee, administrative approval from the Ministry of Health of Angola and the Liverpool School of Tropical Medicine Research Ethics Committee (Protocol 14.022). Written informed consent was obtained from each individual and was orally explained if they were illiterate. For those who refused to participate, no further questions were asked and no information was recorded. All people identified with clinical conditions or infections were informed by the survey team or local community health worker and referred to the local health clinic for advice on self-care and treatment.

4.11 Results

The field survey was conducted in August 2014. In total, 22 communities, including 1616 individuals (10 to 168 per community) were surveyed across the CISA area of Dande Municipality (Table 4.1). In the majority of communities, it was difficult to reach the targeted enrollment of 100 individuals, as people were either not present in the community or busy at work. Overall, there were 615 males (38.1%) and 1001 females (61.9%) included in the survey, with an average age of 43 years, ranging from 15 to 90 years. The distribution of the communities and prevalence rates are shown in Figure 4.4 A-D.

Table 4.1 Summary of loiasis, onchocerciasis and LF serological and molecular filarial prevalence by community

Village	*Loa loa*				Onchocerciasis		LF			
	No.	**RAPLOA %**	**No.**	**PCR %**	**No.**	**ELISA %**	**No**	**Clinical†**	**ICT**	**PCR**
Acucareira Sede	168	6.0	166	8.3	168	3.0	168	1.8	0.0	0.0
Boa Esperanca 2	97	7.2	96	8.3	97	0	97	3.1	0.0	0.0
Catuta	65	10.8	63	0.0	64	17.2	65	3.1	0.0	0.0
Coragem	93	9.7	92	2.2	92	2.2	93	1.1	0.0	0.0
Honga Hungo	74	6.8	74	1.4	73	1.3	74	1.4	0.0	0.0
Icau Centro	38	5.3	36	50.0	35	8.6	38	2.6	0.0	0.0
Icau Wanda	16	18.3	16	0.0	16	18.8	16	6.3	0.0	0.0
Jungo	59	5.1	56	5.4	59	1.7	59	0.0	0.0	0.0
Kicola	84	3.6	82	28.0	81	1.2	84	1.2	0.0	0.0
Kilometro 29	78	3.8	43	0.0	72	0.0	78	1.3	0.0	0.0
Kixiquela	60	6.7	44	18.2	58	0.0	60	0.0	0.0	0.0
Lifune Nepasso	45	0	44	4.5	45	35.6	45	4.4	0.0	0.0
Mabubas Sede	110	6.4	110	0.0	105	2.9	110	0.9	0.0	0.0
Muculo	58	5.2	58	5.2	57	5.3	58	0.0	0.0	0.0
Mussenga	88	8.0	85	1.2	88	9.1	88	0.0	0.0	0.0
Paranhos	71	7.0	69	66.7	66	3.0	71	4.2	0.0	0.0
Rio Seco	71	5.6	70	1.4	51	13.7	71	1.4	0.0	0.0
Santa Rosa	10	10.0	10	0.0	10	0.0	10	0.0	0.0	0.0
Sassa Povoacao	148	6.8	147	24.5	147	0.7	148	2.0	0.0	0.0
Sorilo	49	4.1	49	2.0	49	0.0	49	0.0	0.0	0.0
Sosso	55	3.6	53	20.8	55	0.0	55	3.6	0.0	0.0
Tres Casas	79	3.8	78	0.0	79	8.9	79	1.3	0.0	0.0
Total	**1616**	**6.2**	**1543**	**11.5**	**1567**	**4.7**	**1616**	**1.7**	**0.0**	**0.0**

Note: Grey shade indicates above average prevalence measures
† Includes limb lymphoedema and/or hydrocoele (scrotal swelling)

Table 4.2: Summary of loiasis, onchocerciasis and LF by length of residency

Length of residency	RAPLOA		L. loa		Oncho		LF	
	N	%	N	%	N	%	N	%
< 5 years	401	3.0	364	12.4	380	3.4	401	0.7
5 to 9 years	421	6.2	386	8.8	401	5.7	421	0.5
≥ 10 years	791	7.8	793	12.5	777	4.9	791	1.4
Chi-square	p=0.012		Ns		Ns		Ns	
Total	1616	6.2	1543	12.0	1567	4.7	1613	0.9

Figure 4.4: The distribution of the communities and prevalence rates of the filarial infections.

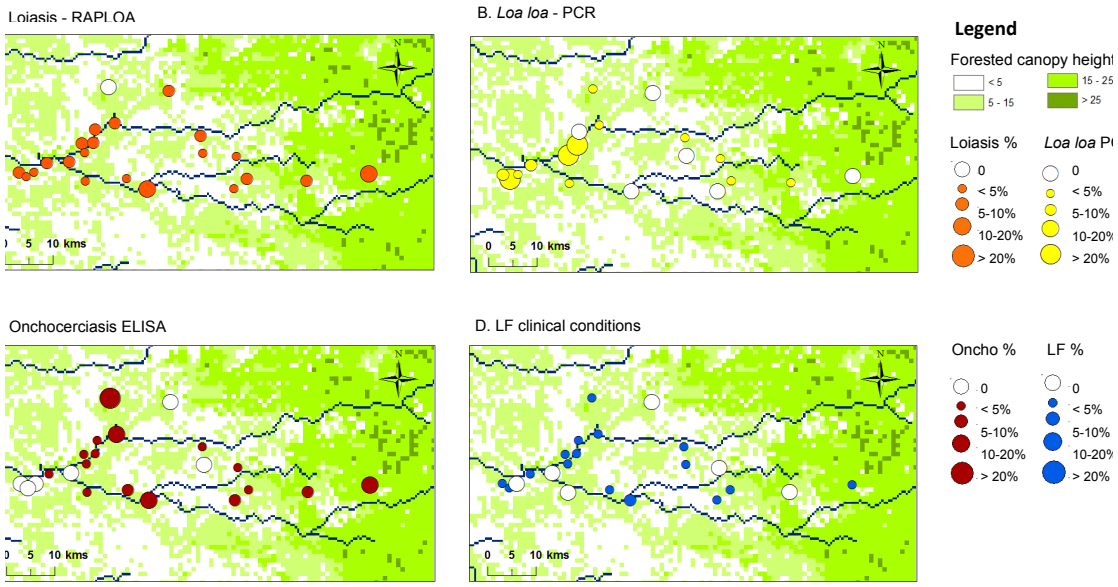


Table 4.3: Prevalence of loiasis, onchocerciasis and LF by age classe and sex

		RAPLOA		L. loa PCR		Onchocerciasis		LF clinical†	
	Age class	N	%	N	%	N	%	N	%
Overall	15 to 19	190	2.6	78	21.9	176	2.3	190	1.1
	20 to 29	313	3.2	301	15.6	305	5.6	313	0.3
	30 to 39	247	4.0	235	11.5	239	5.4	247	0.0
	40 to 49	249	5.6	235	7.2	241	4.1	249	2.0
	50 to 59	307	7.8	294	7.5	300	5.0	307	1.6
	> 60	310	11.9	300	8.7	306	4.9	310	1.0
	Chi-square	p=0.000		p=0.000		Ns		Ns	
Male	15 to 19	86	2.3	81	22.2	77	3.9	86	2.3
	20 to 29	103	3.9	100	18.0	98	4.1	103	0.0
	30 to 39	90	4.4	86	8.1	85	4.7	90	0.0
	40 to 49	96	6.3	94	9.6	91	6.6	96	4.2
	50 to 59	124	4.8	120	5.8	118	4.2	124	3.2
	> 60	116	8.6	112	7.1	114	6.1	116	1.7
	Chi-square	Ns		p=0.001		Ns		Ns	
Female	15 to 19	104	2.9	97	21.6	99	1.0	104	0.0
	20 to 29	210	2.9	201	14.4	207	6.3	210	0.5
	30 to 39	157	3.8	149	13.4	154	5.8	157	0.0
	40 to 49	153	5.2	141	5.7	150	2.7	153	0.7
	50 to 59	183	9.8	174	8.6	182	5.5	183	0.5
	> 60	194	13.9	188	9.6	192	4.2	194	0.5
	Chi-square	P=0.000		p=0.002		Ns		Ns	

Note: † Includes limb lymphoedema and/or hydrocoele (scrotal swelling) in overall measure and for males

Loiasis

For RAPLOA, 6.2% of 1616 individuals surveyed were found to have eye worm based on the restricted eye worm definition in 21 communities (Table 4.1). The highest prevalence rates were found in Icau Wanda (18.3%) and Catutua (10.8%), while none were reported from Lifune Nepasso (0.0%) Figure 4.4A. Prevalence increased signficantly with length of residency from 3.0% in individuals living <5 years to 7.8% in individuals living ≥ 10 years in the community (Chi-square=10.962, *p*=0.012) (Table 4.2). Overall, there was no signficant difference between male 5.2% and female 6.8% prevalence rates. However, significant differences by age group overall (Chi-square=30.1 *p*=0.000) and among females (Chi-square=28.66, *p*=0.000) were found, with the highest prevalences in the >60 year age group (Table 4.3). When asked about the main *Chrysops* spp. vector, 11.2% (n=179) of individuals recognized the fly when presented with a photograph. Overall, males (12.8%), individuals aged >43 years (27.3%) and those with eye worm (23.4%) were twice as likely to recognize the fly than the related subgroups (females 9.2%; aged <43 years 13.9; no eye worm 11.4%). Males with eye worm were signficantly more likely to recognise the *Chrysops* vector in comparison with those with no eyeworm history (Table 4.4).

For *L. loa* nested PCR, 11.5% of 1543 individuals tested were found to be positive in 16 communities (Table 4.1). The highest rates were found in Icau Centro (50.0%), and Paranhos (66.7%), and no positives found in Catuta, Icau Wanda, Kilometro 29, Mabubas Sede, Santa Rosa and Tres Casas (Figure 4.4B). Prevalence did not signficantly differ with length of residency (Table 4.2) or by sex (males 11.3%; females 11.7%). However, signficant differences by age groups were found overall (Chi-square=36.708, *p*=0.000) and among males (chi-square= 22.051, *p*=0.001) and females (chi-square= 19.517, *p*=0.002), with the highest prevalences in the 15-19 year age group (Table 4.3). Taking infections status into account, when asked about the main *Chrysops* spp. vector, no signficant differences were found by sex and age (Table 4.4). Overall, the relationship between the RAPLOA and nested PCR was not significant with only eight RAPLOA positive individuals also positive by PCR.

Table 4.4 Summary of the knowledge of *Chrysops* vector overall and by age and sex

Village	Have you seen this fly?	RAPLOA		L. loa PCR	
		N†	%	N††	%
Overall	Yes	179	11.2	168	9.5
	No	1301	5.1	1251	12.2
	Chi-square	p=0.001		Ns	
Male	Yes	94	12.8	62	11.3
	No	466	3.6	477	17.2
	Chi-square	p=0.000		Ns	
Female	Yes	85	9.4	106	8.5
	No	835	5.9	774	9.0
	Chi-square	Ns		Ns	
<43 yrs	Yes	20	10.0	62	11.3
	No	747	9.2	477	17.2
	Chi-square	Ns		Ns	
≥43 yrs	Yes	66	27.3	106	8.5
	No	647	13.9	774	9.0
	Chi-square	p=0.004		Ns	

† 1480 included as 136 answers missing
†† 1419 included as 120 answers missing

Onchocerciasis

For onchocerciasis, 4.7% of 1567 individuals tested were found to have be positive in 16 communities (Table 4.1). The highest prevalence rates were found in Lifune Nepasso (35.6%) and Icau Wanda (18.8%), and no positves from Boa Esperança 2, Kilometro 29, Kixiquela, Santa Rosa, Sorilo and Sosso (Figure 4.4C). Prevalence did not signficantly differ with length of residency or by sex (males 5.0%; females 4.6%) overall (Table 4.2). Similarly, no significant differences by age group overall or among males and females were found (Table 4.3). Three individuals (0.2%) from Coragem (female, 59 years), Honga Hungo (male, 41 years, positive ELISA) and Lifune Nepasso (male, 21 years) reported they had taken a drug for onchocerciasis, 68.8% reported they had not and the remaining individuals did not know.

Lymphatic filariasis

For LF, none of the 1616 individuals surveyed were found to be positive for LF antigen or by real-time PCR (Table 4.1). However, 1.7% was found to have lymphoedema and/or hydrocoele in 16 communities (Table 4.1). In total, 11 cases of lymphoedema (0.68%; male=6 cases, 5 females), 14 cases of hydrocoele in men (0.87%) and two men with both conditions (0.12%). The highest number of cases was identified in Sassa Povoação (3 lymphoedema; 1 hydrocoele), Açucareira Sede (1 lymphoedema, 2 hydrocoele) and Boa Esperança 2 (3 hydrocoele), Sosso (2 lymphoedema, 1 hydrocoele) and Catuta (1 lymphodema and 1 hydrocoele), Lifune Nepasso (1 lymphodema and 1 hydrocoele). One lymphodema case was found in Coragem, Icau Centro, Kilometro 29, and Rio Reco and one hydrocoele case found in Honga Hungo, Icau Wanda, Kicola, Mabubas Sede, and Três Casas. Prevalence did not signficantly differ with length of residency or for lymphoedema by sex (males 1.2%; females 0.6%) (Table 4.2). Similarly, no significant differences by age group overall or among males and females were found (Table 4.3).

Overall, 735 individuals (45.5%) reported they owned a bed net, 860 (53.2%) did not and 21 (1.2%) did not answer the question. There were signficant differences between males (50.1%) and females (43.6%) and between younger (43.1%) and older (49.3%) individuals (Chi-square= 6.28, p=0.012), but not between individuals affected and not affected by LF clinical condtions. With respect to bednet usage, 71.1% of those who owned a bed net reported they slept under it the previous night. Overall, there were signficant differences between males (75.2%) and females (68.2%)(Chi-square= 4.151, p=0.04) in bed net usage, but not by age or clinical condition.

4.11 Discussion

Our survey used a combination of clinical, serological and molecular diagnostics to confirm the low levels of filarial endemicity across the CISA study area within Dande Municipality (Brito *et al*, 2017). This will help to inform the Angola NTD program on appropriate, safe and better-tailored intervention strategies locally, taking the widespread loiasis prevalence into account. This is particularly important for onchocerciasis, which was found to be hypo-endemic here, as previously reported (Brito *et al,* 2017). As a result, ivermectin MDA is not recommended due to the risk of SAEs (Zouré *et al*, 2011). Currently, no alternative drugs are available for these hypo-endemic onchocerciasis hotspot areas, and only a few individuals indicated that they had taken ivermectin, so the SAE risk is relatively unknown. However, the CISA area may be suitable for the new Test-and-Not-Treat (TNT) strategy that includes the use of the Loascope to help make a diagnosis and direct the most appropriate treatment regimen depending on the individual's infection status (Kamgno *et al,* 2018; Boussinesq *et al*, 2018). This area was previously considered in RAPLOA studies to be a high-risk area for SAEs (Takougang *et al,* 2002). Therefore, further confirmation of the onchocerciasis distribution through skin snipping or use of other diagnostics may be necessary (Kelly-hope *et al*, 2018). Before or during an alternative strategy such as TNT is conducted.

The prevalence of loiasis using the RAPLOA method was found to be widespread, and higher than in the previous survey by Brito *et al* (2017). The reason for this difference in prevalence is unclear, but may be due to different populations being sampled, seasonality and people being away for work at different times of the year. This is a mobile population that frequently visit the town Caxito (Rosário *et al*, 2019). The difference between loiasis and the *L. loa* nested-PCR prevalence was also surprising with little correlation between the tests and higher *L. loa* PCR prevalence in younger age groups. The implications of this and how it relates to *L. loa* mf intensity and risk SAEs are unclear as this study was limited and did not include parasitological analysis; however, it may suggest that the positive results in younger population indicate recent transmission of *L. loa* in the area. Furthermore, it may be that younger people who are infected may be new or mobile residents and not exposed enough to experience eyeworm, which was more evident in longer term and older residents. The higher risk of eyeworm in positive and older

individuals, especially males, may be related to their occupation and visits to forested areas as they were more likely to recognize the *Chrysops* vector, also known as the readily identifiable Red Fly in Africa (Kelly-Hope *et al*, 2017). More frequent forest visits have been associated with an increased risk in loiasis (Mischlinger *et al*, 2018; Brant *et al*, 2018), and using local knowledge to determine risk zones in communities and workplaces may also help to identify vector habitats. Currently, no data exists on the *Chrysops* vector in Angola (Kelly-Hope *et al*, 2017), however *C. dimidiate* and *C. longicornis* have been implicated (Hawking, 1974), which may have different ecological niches to other parts of Central Africa.

The lack of LF serological and molecular positive individuals and the few clinical cases further indicates that this a non-endemic or very low endemic area for LF, which is in accordance with historical data and recent mapping surveys from 2005 and 2010, and as such may not require MDA. It is also possible that the lymphoedema and hydrocoele cases are not related to LF, and different clinical algorithms may be needed in areas of uncertainty (Deribe *et al*, 2018). It is important that Angola's NTD program uses this data to inform WHO and help shrink the map by reducing the number of endemic municipalities throughout the country (Casaca, 1966; Brito *et al,* 2017). This will save time and resources and consider more appropriate surveillance strategies for low prevalence areas (Riches *et al*, 2020; Kelly-Hope *et al*, 2017), especially in loiasis co-endemic areas where is increasing evidence of low LF prevalence (Wanji *et al,* 2019; Kelly-Hope *et al*, 2018). Engagement with the national malaria control program can help to increase the bed net coverage in the area will be critical as this can reduce *W. bancrofti* transmission (Rebollo *et al,* 2015; Berg *et al*, 2012; Bockarie *et al*, 2008), and is a WHO recommended alternative strategy in loiasis co-endemic areas (WHO, 2012; WHO, 2011a). Overall, the number of patients was low and health workers need to be trained to provide care to patients, including home-based self-care for lymphoedema, with subsequent referral if needed for surgical management of hydrocele, as it can improve patient economic and quality of life outcomes (WHO, 2013; WHO, 2019; Betts *et al*, 2020).

4.13 Conclusion

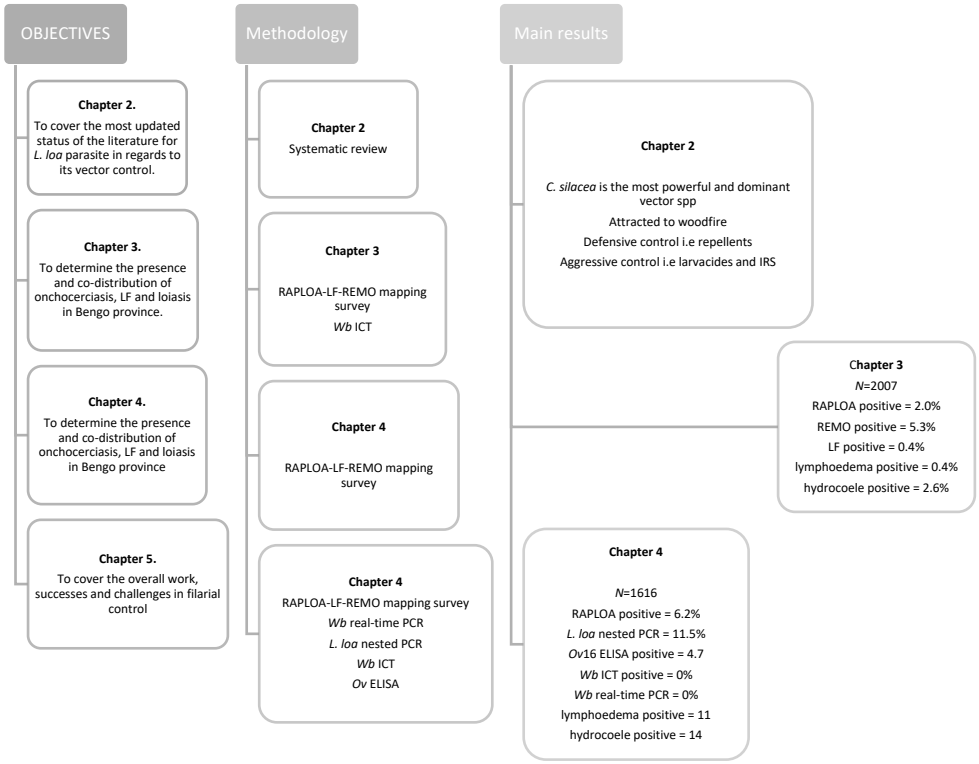
This study highlights an integrated approach including how an alternative range of molecular and serological diagnostic tools can better define the filarial prevalence across the CISA study areas. The reason for different geographical distributions may be related to environmental factors, however, Molyneux *et al* (2014) has suggested the possibility of competitive exclusion where if the three human filaria are present then their interactions might reduce the intensity of all infections due to factors such as niche separation, periodicity, immunity, geographical distribution of the parasites, ecological barriers. Nonetheless, focus now needs to be on devising opportunities to implement a practical and cost-effective strategy to eliminate onchocerciasis and conduct innovative surveillance for LF in the area. All of this will, of course, require collaboration between program, the community, national partners and international stakeholders (Colebunders *et al,* 2019).

CHAPTER FIVE: OVERALL WORK

5.1 Study rationale

The study of the prevalence of filariasis in Bengo province was chosen because it is still a public health concern nationwide (Figure 5.1). Understanding lymphatic filariasis-loiasis-onchocerciasis co-distribution at a micro-level, delineating in relation to forested areas and how people move in and around these areas, may provide insights into where SAEs are more likely to occur, and if the Test-and-not-Treat (TNT) strategy should be used to help minimize risk.

Figure 5.1: Summary of the main findings related to the study objectives and methodology



5.1.1 Specific Objectives and Findings:

Chapter 2 covered the literature assessment for *L. loa* parasite in regard to its insect vector transmission characteristics that have been targeted for vector control providing insights into the potential for integrated vector management (Figure 5.2). Most of the articles identified were from the 1950-1960s, being the *Chrysops silacea* the most competent and dominant vector species. This vector has been described to be the most attracted to wood fire due to dissemination of odorous particles rather than carbon dioxide (CO_2) involved in the smoke in the canopy. Main vector targeted measures proposed to impact on *L. loa* transmission included defensive such as personal repellents, household screening, clearing vegetations, while the aggressive control measures included adulticides, larvicides and indoor residual spraying (IRS). Long ago, control depended mainly on the regime of insecticides and clearing of vegetation neighboring the residences, but contemporarily, repellents, trappings and destruction of the canopy are the best-proposed options around residencies, carefully balanced against their detrimental impact on local biodiversity

The usage of integrated RAPLOA-LF-REMO survey, based on key clinical manifestations of filarial diseases, which has helped to assess the filarial prevalence maps and predict areas at highest risk of SAEs in Angola (Figure 5.2). LF filarial surveys have been performed in Angola in 1966 and then from 2000, while RAPLOA was performed between 2003 and 2011 (ESPEN, 2017). As for this study, none of the reported cases for lymphoedema tested positive with ICT for *W. bancrofti* antigen as also observed in Beng *et al* (2020) in Cameroon setting, although FTS was applied instead. The presence of clinical disease, especially lymphedema, has been considered to be a poor indicator for LF in Angola. Another possible explanation for the presence of bilateral elephantiasis in the community is caused by podoconiosis, since its occurrence have been reported in the past in Angola and, from environmental predictions, may overlap within the study area thereby confounding the burden of LF (Chandler *et al*, 2020; Sime *et al*, 2018; Deribe *et al*, 2020). The REMO methodology was first performed in Angola in 2002 and afterwards in 2011, covering a total of 956 villages (Angola NTDs, 2017).

For loiasis, a significant difference was found between the current survey data with the RAPLOA modelled maps from 2010 (Zouré *et al.*, 2011). The main reasons for this may be due to the timing, spatial resolution of data collected, environmental changes and recent migration; as previous surveys were only conducted in a few villages between 2003-2008 (Zouré *et al*., 2011; Censo, 2014; World Resources Institute, 2017). Overall, RAPLOA-LF-REMO survey alone is not as effective as it can lead to misdiagnosis or missing diagnosis of filarial infections. Investing in fitting mapping of LF in *L. loa* endemic areas may not just help to prevent unneeded MDA for LF elimination, but it will also help to diminish the map of LF distribution.

The assessment and description of the prevalence and co-distribution of filarial parasites, namely *L. loa, O. volvulus* and *W. bancrofti*, in Bengo province based in serological and molecular methods. Available information in the current or historical geographical distribution of LF in Angola is scarce. The country has a population of about 24.3 million residents and few of them have actually been properly tested for LF. In a couple studies performed from 2000 to 2016, over 16.316 individuals from 16 provinces were tested for circulating filarial antigen by ICT and positivity rates ranged from 0.0-98.0% in Angola (ESPEN, 2017), however, positive tests were not confirmed by other tools to diagnose *W. bancrofti* infection, as also reported in Cameroon by Wanji *et al* (2019) and Kelly-Hope *et al* (2018). Currently, apart from this research, there are no literature data available in regards the molecular diagnostic of filarial infection in Angola region as a baseline point, specifically real-time, conventional and nested-PCR approaches. For this reason, it has been fundamental to apply such techniques, not only just for reference but also to compare its accuracy and precision with further parasitic and serological diagnostics in the Angolan setting, and in Africa in general. Results of the molecular analysis by PCR confirmed the presence of *L. loa* and the absence of *W. bancrofti* as well as seen in Cameroon by Beng *et al* (2020) and Wanji *et al* (2019), which suggests that the studied region in Angola is hypoendemic.

5.2 Success in filarial control

The development of new, more-sensitive diagnostics is now broadening our knowledge of infection prevalence and of the risk of re-infection (Osei-Atweneboana *et al*, 2012). For example, the PCR methods have the advantage that no fresh samples are needed to make a correct diagnosis, as the DNA from damaged parasites in peripheral blood is detectable for years by this technique, while the morphology is usually lost (Osei-Atweneboana *et al*, 2012).

An integrated mapping exercise that has been implemented by ESPEN, which includes Angola data, allows the identification of filarial cases across the country, which will help to ensure effective implementation of morbidity management and disability prevention services for these filarial diseases. As an example, Kebede *et al* (2018) has pioneered this strategy in Ethiopia, therefore identifying more than 25,000 cases of elephantiasis across few districts known to be endemic for both LF and podoconiosis.

GIS technology has enlightened a new approach for analyzing digital map created by earth observing satellite sensors and for regulating evaluation of spatial and temporal environments (Hay, 2000). Therefore, by using this approach, it is possible to undermine the onus of the filarial diseases by reproducing data that allow the implementation of strategies and resources towards the general population to take conscious actions, and the health actors to reform the surveillance, prevention and control of filarial diseases (Ratmanov *et al*, 2013; Giardina *et al*, 2014).

5.3 Challenges in filarial control

The urgent issue regarding the filarial co-distribution between *L. loa* and *Onchocerca* species, stands for the fact of lack of studies and evidence of how many people are co-infected, therefore inhibiting the use of ivermectin MDA as it might cause severe complications if patients carry heavy microfilaremia (Kamgno *et al*, 2017). Recent advances in point-of-care screening may help to limit the risk through implementation of a ‘test-and-not-treat’ strategy that will prevent ivermectin delivery to individuals with high-risk *L. loa* infection (Kamgno *et al*, 2017).

The target populations sampled earlier in the literature were focused on the adult population, and while positive findings in this group are validated for identifying infection in the population, it is possible that the younger population is a sentinel of recent transmission, specifically children, the same group now recommended for assessment guiding the decision to stop MDA.

The earlier defined justification of a sampling strategy to identify villages in regard to blackfly breeding sites along fast-flowing rivers remains valuable, but it is not enough for onchocerciasis elimination mapping. Adapting strategies to account for co-endemic filarial infections is vital since in *Loa*-endemic settings, it is not enough to determine whether or not onchocerciasis is endemic. Coordinated mapping for *L. loa* infection must be done as efficiently as possible so that an appropriate, safe plan for MDA treatment can be designed. For elimination mapping to be carried out safely and efficiently, national program expects the WHO to establish strategies to address onchocerciasis co-endemicity with LF and/or loiasis based on best evidence and consensus.

Further, challenges for effective control of NTDs include finalizing coordinated mapping of NTDs, updating NTDs morbidity data, effectively implementing large-scale MDA, improving the performance of community-directed treatment with ivermectin projects, extending campaigns of deworming with ivermectin and praziquantel throughout Angolan territory.

5.4 Changes in parasite transmission

The ecology of vectors species and intermediate host species is mostly likely to change as global climate changes and other anthropogenic landscape changes are taking place in the meantime. As a result of these changes either expansion or limitation of the filarial species presence might occur in certain regions. On the other hand, population migration and increasing of human host population density also have a major impact in the success of pathogen transmission (King, 2019).

5.5 The national movement towards elimination

Angola has recently mapped filarial diseases that have been widely distributed, therefore affecting large numbers of people, some of whom live in remote locations that are extremely difficult to access (WHO, 2017; ESPEN, 2017). As a result, the increase in data collection from sites, with geo-referencing, and with molecular tools give a more detailed view on transmission and where to target control efforts.

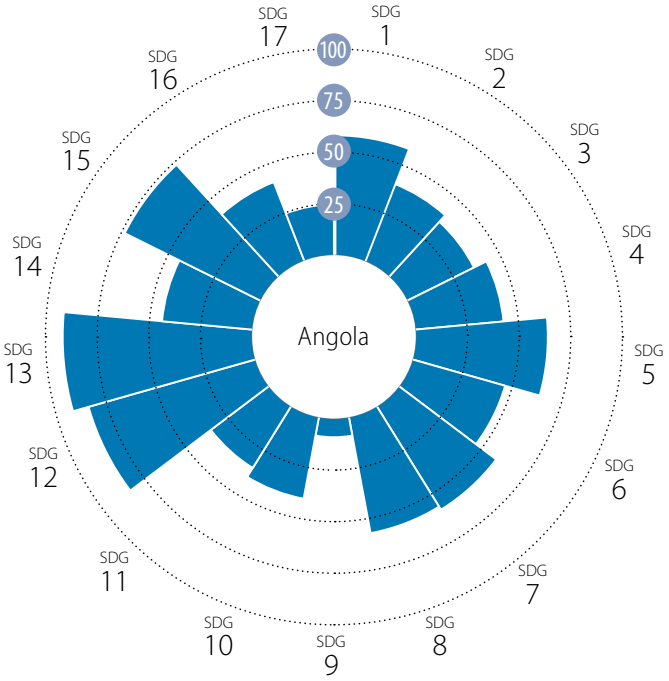
The crucial correlation between NTDs and SDGs have been lately pointed out in a way that symptoms from filarial infections affect the SDG 1-4 by limiting people from doing their daily routine (Addisu *et al*, 2019; Bangert *et al*, 2017). Furthermore, the highest predominance of filarial infections most commonly arises in remote and rural areas while the community from urban settings and socioeconomically stabilized areas are exempted, therefore, impeding SDG 10 which covers the reduction within and among countries (Addisu *et al,* 2019; Bangert *et al*, 2017).

The 2019 SDG Index and Dashboards Report provides an evaluation of the actual status of where African countries stand in regard to SDGs and their improvement in respect to the goals, but also how African governments are applying strategies to conquer them (SDG, 2019). The 2019 SDG Index ranks 52 African countries based on the 97 indicators across all the 17 goals. Such scoring positions a country between the worst (0) and best (100) outcomes. In the year of 2019, Angola ranked with a score of 49.26 meaning that the country is 49% of the way towards conquering the SDGs.

According to the SDG performance, Angola fell into the Cluster 3 category entitled 'Middle of the Pack' which is considered the largest cluster out of the five, as well as the most diverse (Figure 5.2). Therefore, this group has been classified as having the best regional performance in terms of SDG 12 and SDG 13. On the other hand, the major challenge identified is poverty, innovation and infrastructure, and inequality. Achievement on SDG 8 for this group is close to the top. As a result, Angola and the other 20 countries that belong to this cluster must certify that they prosper their progress in a way to minimize poverty and promote human welfare, without unbalanced environmental sustainability (SDG, 2019).

One of the aims of SDG 3 is to 'end the epidemics' of all conditions including NTDs. Currently the coverage of preventive chemotherapy for NTDs is 21.4%, the UHC tracer index is 43.2%, and the subjective wellbeing in 3.8% (average ladder score 0-10). This could actually be achieved by implementing a comprehensive, holistic strategy of community-based, large-scale preventive chemotherapy and individual treatments supported by vector control delivered in tandem with universal health coverage (SDG, 2019).

Figure 5.2 Angola average performances by SDG.



Source: SDG, 2019.

The most important factor at the international level is the recognition by supporting program that collaborate towards the onchocerciasis elimination mapping that has been set for 2020, 2025 and 2030, however, none of these goals can be met if the extent of onchocerciasis infection stands unsure (WHO, 2012c).

At country level, the Angolan Ministry of Health through the National NTD Control Program, with technical support from WHO, carried out a data review and validation of five key NTDs namely LF, onchocerciasis, schistosomiasis, geohelminthiasis and trachoma (WHO, 2019). This process will therefore allow a national observation of the

health and socio-economic impact of such diseases through monitoring, prevention, control and elimination. With this achievement, it is expected the expansion of this database throughout the country as a way to determine the areas eligible for preventive chemotherapy or mass treatment of anthelminthic drugs based on a better visualization of the distribution and prevalence of the diseases. Angola national programs must examine the usefulness of information for each municipality that are both missing data on onchocerciasis and are ivermectin naïve in order to evaluate if any can be ruled out from additional assessment because of their epidemiological unsuitability for onchocerciasis transmission. Preventive treatment of filarial infections requires activities additional to the distribution of medicines, to ensure a holistic approach to the causes and effects of the diseases. The broader strategy would include preventive chemotherapy, vector ecology and management, water sanitation, and, morbidity management. The next step would be the implementation, which would include capacity building, delivery and logistics, planning and coordination; and data management and analysis (Rebollo *et al*, 2015).

Advocacy must be promoted in regards the increasing awareness of filarial NTDs and their impact on affected communities; community engagement, education, promoting integrated management schemes and their potential benefits to society and donors. In terms of local research management, this project promoted the assessment of common clinical and laboratory diagnostic platforms for filarial infections, which were practical in the fieldwork. Furthermore, mapping was also used to identify their overlapping distribution to allow an integrated coordinated control and treatment activities. Further local research should be managed in regard to piloting of integrated approach in one or several regions in Angola. Other than that, understanding community resilience and program factors that strengthen community participation would be a major benefit.

5.6 Limitations

The study has several limitations which include:

1. The low enrolment rate of the communities within Bengo province due to highly mobile population, which is, considered a common issue, therefore resulting in a possible underestimation of the filarial infections and morbidity estimates as low or non-adherers. The same pattern was observed in a similar study performed in Zaire province (Appendix 12). This fact highlights how risk and population

dynamics can change over time, and that there is a need for up-to-date information and assessments before treatment strategies are implemented.

2. The pre-control epidemiological data were not available for the communities in Dande Municipality; therefore, it was not possible to observe and compare the impact of MDA intervention on the epidemiology of the diseases at community level.
3. This study did not cover all the suspected filarial endemic communities from the Municipality of Dande. Further limitation includes the lack of blackfly catches in the different regions in Bengo and Zaire provinces. Although this reflects a lack of biting at the sampling time in these localities, the result may not reflect true absence of simuliids and of any associated transmission.

5.7 Future Work

5.7.1 Surveillance approach

It is imperative to comprehend the dynamics of transmission at low prevalence in order to notify surveillance for detecting elimination and resurgence for NTDs, as a way to promote and certify the guidance, designs are generated further data. Therefore, it has been proposed by the literature regular training of health extension workers and primary health care workers on the screening and management of the different conditions (WHO, 2018b). WHO has developed a manual to aid training of frontline health workers in recognition of the signs and symptoms of NTDs (WHO, 2018b).

5.7.2 Potential future focus and research

As there is no updated of the Angolan entomological profile data in the literature since pre-colonial period in regards the indices of onchocerciasis transmission, it should also be considered the study of *O. volvulus* infection based on *Simulium* biting infectivity, and infective rates a 'limitation' phenomenon, for comparative analysis, as seen in Ghana and Nigeria (Amuzu *et al*, 2010). As at least 11/18 provinces from Angola have been reported to be endemic for onchocerciasis, where CDTi has been taking place from 2005 until 2016, it would be of most value to evaluate the manifestation and biting patterns of *S.*

damnosum complex, as a way to interrupt the transmission cycle of the disease in vulnerable human populations (ESPEN, 2017).

5.7.3 Disease burden approach

Although the province of Bengo, was classified as hypo-endemic for onchocerciasis, many other provinces in Angola, including Uíge, Cuanza Norte and Cuanza Sul, have been classified as endemic, where therefore, CDTi is taking place (ESPEN, 2017). In such regions, the contribution of community knowledge, attitudes and perceptions towards onchocerciasis and its influence on participation and acceptability of CDTi should be investigated, as this data is unknown in the Angola context. Therefore, a review on historical and current about onchocerciasis in the whole country should be considered as a way to understand the current status of the situation and plan for future challenges in order to fight this disease better in Angola. A recent studied performed in Tanzania by Mushi *et al* (2020) have reported inadequate levels of knowledge, negative attitudes and perceptions in regards CDTi which suggests a continuous transmission of onchocerciasis. Furthermore, as the literature as suggested common co-infection between onchocerciasis, loiasis and *M. perstans*, novel studies should be performed to establish the geographic range and risk factor among these conditions in suspected endemic regions (Schulz-Key *et al*, 1993; Wanji *et al,* 2003; Van Hoegarden *et al*, 1987; Noireau *et al*, 1989).

Although the physical and psychosocial matters in regards disfigurement caused by NTDs are well documented in the general literature, there is a significant lack of data at population level in regards the activity limitation and social impact in Angolan context (Yanik *et al*, 2004; Williams *et al*, 2011). A few studies have used the WHO Disability Assessment Schedule survey in their analysis in patients with podoconiosis, which is a disease with similar aetiology, suggesting a significant high score (Barlett *et al,* 2015; Caprioli *et al*, 2020). Another interesting approach that should be considered for Angola context is to address the stigmatization and disability impact of LF symptoms including hydrocele and elephantiasis, in regards the quality of men's lives as reported in Betts *et al* (2020), Eneanya *et al* (2019) and Ton *et al* (2015); and for loiasis as seen in Gabon on a study by Veletzky *et al* (2020).

As an alternative to pharmaceutical worming approach, the research and evaluation of anti-filarial biomolecules extracted from medicinal plants should also be considered for

future work, mainly in Angola context, where pharmaceutical therapy is often inaccessible or unavailable in rural settings; and where people have resource to alternative medicines to treat themselves which may or may not have beneficial impacts (Edwige *et al*, 2018; Costa *et al,* 2013). As mentioned in the Chapter 1, a couple of plants are used to treat *L. loa* infections, and among those at least 6 plants are available in Angola, including *Costus lucanusianus K Schum* locally known as 'mukisu', *Senna occidentalis (L) Sink* locally known as 'nhoca-nhoca', *Portucala olerecea L* locally known as 'mbembe', *Nicotiana tabacum L.* locally known as 'tabaco', and, *Cissus quadrangularis* locally known as 'dilengue' (Costa *et al,* 2013; Edwige *et al*, 2018).

5.7.4 Sociodemographic approach

Although the filarial survey from the current study didn't include participants aged below 15 years old, the younger class should be included in future mapping studies, as according to WHO, the younger and pediatric population could be considered to be sentinels, therefore indicating patent or past infections, as it was reported in younger individuals infected with onchocerciasis in Wanji *et al* (2015) and Mladonicky *et al* (2009), where reported positive responses regarding ICT in younger children (5-14 years old) suggested recent filarial exposure or infection. Furthermore, WHO (2016a) also recommends that Ov16 serology assessment in population younger than 10 years of age should be estimated whether MDA should be cautiously interrupted.

5.7.5 Diagnostic approach

The FTS is the most recent filarial antigen test that was implemented to upgrade on the ICT for mapping of LF (Beng *et al*, 2020). The use of the LoaScope based Test and Not Treat strategy would be of most use to identify and exclude individuals from ivermectin treatment for *L. loa* at risk for SAEs which might help increase adherence for treatment (Kamgno *et al*, 2017).

5.7.6 Data management approach

As a way to become more environmentally friendly, data generated in the field could be collected electronically using smart phones or tablets and uploaded to a center server coded, instead of using paper forms. As an example, Magpi, Epi Info and SurveyCTO

software have been majorly used to collect field data due to their high data quality, accuracy and laboring time reduction (Oswald *et al*, 2020).

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APPENDIX

Appendix 1: Approval letter from Ethics Committee

REPÚBLICA DE ANGOLA
MINISTÉRIO DA SAÚDE
COMITE DE ÈTICA

Parecer sobre o protocolo de estudo designado "**Estudo das Filaríases na Província do Bengo, Angola",** submetido a este Comité pelo **Dr. Miguel de Brito**, investigador coordenador do Centro de Investigação em Saúde de Angola.

Na apreciação do protocolo em referência o Comité constatou que se trata de um estudo que tem como objectivo descrever a distribuição e o nicho ecológico dos vectores e paralelamente, actualizar e criar mapas referentes a distribuição da Filaríase linfática e da Oncocercose na província do Bengo, aplicando para o efeito a metodologia proposta pela Organização Mundial de Saúde.

O Comité verificou que no estudo, o Termo de Consentimento Livre e Esclarecido contempla todas as informações necessárias sobre a pesquisa e sobre os aspectos éticos.

Os resultados obtidos permitirão avaliar a estratégia de prevenção recomendada pela OMS para regiões de coendemicidade de Oncocercose e Filaríase , pelo que não há inconveniente que se realize no país.

LUANDA, AOS 14 DE AGOSTO DE 2014.-

A COORDENADORA DO COMITÉ

Dra. Filomena Gomes da Silva
"BIOLOGA"

Appendix 2: Consent letter in Portuguese

ESTUDOS DAS FILARÍASES NA PROVÍNCIA DO UÍGE E ZAIRE, ANGOLA

CONSENTIMENTO INFORMADO

Introdução e objectivo da pesquisa:
Os parasitas filariais (vermes) são transmitidos aos seres humanos através de uma variedade de vectores de insectos que podem causar doenças clínicas significativas para os indivíduos infectados. Actualmente, existe um esforço a nível global para eliminar essas doenças nas áreas endémicas com medicamentos seguros e eficazes. No entanto, é importante compreender a origem das doenças e se existe algum factor em particular que aumente o risco no individuo e/ou comunidade. Portanto, este estudo tem como objectivo determinar se a filaríase linfática (elefantíase), oncocercose (cegueira dos rios), loíases (verme do olho) e mansonelloisis estão presentes na província do Uíge e Zaire, Angola e quais os factores que poderão influenciar as sua transmissão na comunidade.

Riscos e análises:
O procedimento não causará nenhum dano ao participante apesar do desconforto que poderá sentir durante a picada da agulha, que será uma dor momentânea enquanto uma pequena quantidade de sangue é retirada e colocada num e no papel de filtro que será usado para testar a filaríase linfática, loíase, oncocercose e mansonella. Os resultados das filaríases serão analisados no laboratório do Centro de Investigação em Saúde de Angola (CISA), e se decidir, poderá obter o resultado no espaço de 3 mêses após do inquérito pela equipa do CISA que guardará os resultados confidencialmente.

Custos para os participantes incluídos na pesquisa:
A sua participação nesta pesquisa não custará nada.

Benefícios:
Os resultados irão identificar a presença de doenças na sua comunidade e factores que podem aumentar o risco da infecção. No caso de haver resultados positivos para filaríase linfática (elefantíase) e oncocercose (cegueira do rio) e loíase, o resultado será enviado confidencialmente para a direcção provincial de saúde e para o Ministério da Saúde para iniciarem o tratamento e gestão de qualquer morbidade relacionada, incluindo aconselhamento, se necessário.

Voluntariado:
A sua participação nesta pesquisa é totalmente voluntária. Se optar por não participar, isso não vai afectar a sua decisão de não participar, não afectará a sua saúde de qualquer forma.
Esta estudo foi aprovada pelo Ministério da Saúde de Angola.

Se tiver quaisquer perguntas, comentários ou reclamações sobre o estudo, por favor, entre em contacto com o Cisa, Centro de Investigação em Saúde de Angola, entidade responsável pela dinamização do estudo, através do número de telefone 234290220.

Janeiro de 2016

CONSENTIMENTO INFORMADO

ESTUDOS DAS FILARÍASES NA PROVÍNCIA DO BENGO, ANGOLA

Eu, _____(nome do Inquiridor), forneci as explicações acima à pessoa seleccionada para o estudo.
Assinatura(inquiridor):_____

Eu, _____(nome do inquirido) recebi oralmente as explicações sobre a informação que consta neste termo de consentimento, compreendi o vosso pedido, aceitando e autorizando minha participação no mesmo.

Assinatura ou impressão digital:_____

Data: __ / __ / __

Appendix 3: Consent letter in English

STUDIES OF FILARÍASES IN THE PROVINCE OF BENGO, ANGOLA

INFORMED CONSENT

Introduction and purpose of the research:
Filarial parasites (worms) are transmitted to humans through a variety of insect vectors that can cause significant clinical illnesses for infected individuals. Currently, there is a global effort to eliminate these diseases in endemic areas with safe and effective medicines. However, it is important to understand the origin of the diseases and whether there is any particular factor that increases the risk in the individual and / or community. Therefore, the objective of this study is to determine if lymphatic filariasis (elephantiasis), onchocerciasis (river blindness), loiasis (eyeworm) and mansonelloisis are present in the province of Bengo, Angola and what factors may influence its transmission in the community.

Risks and analysis:
The procedure will not cause any damage to the participant despite the discomfort he may feel during the needle bite, which will be momentary pain as a small amount of blood is withdrawn and placed in one and the filter paper that will be used to test lymphatic filariasis, loiasis, onchocerciasis and mansonella. The results of the filariasis will be analyzed in the laboratory of the Center for Research in Health of Angola (CISA), and if you decide, you can get the result within 3 months after the survey by the CISA team that will save the results confidentially.

Costs for participants included in the survey:
Your participation in this survey will cost you nothing.

Benefts:
The results will identify the presence of diseases in your community and factors that may increase the risk of infection. In the case of positive results for lymphatic filariasis (elephantiasis) and onchocerciasis (river blindness) and loiasis, the result will be sent confidentially to the local medical center and to the Ministry of Health with your permission to start treatment and management of any morbidity, including counseling if necessary.

Volunteering:
Your participation in this research is entirely voluntary. If you choose not to participate, this will not affect your decision not to participate, it will not affect your health in any way.
This study was approved by the Ministry of Health of Angola.

If you have any questions, comments or complaints about the study, please contact Cisa, the Angola Health Research Center, the entity responsible for promoting the study, by calling 234290220.

January de 2014

INFORMED CONSENT
STUDIES OF FILARÍASES IN THE PROVINCE OF BENGO, ANGOLA

I, _____(interviewer's name), gave all the explanations above to the person selected for the study.
Signatura(interview):_____

I, _____(interviewee's name) received verbally the explanations in regards to the information contained in this consente form, I understood your request, accepting and authorizing my participation in the same.

Signature or digital print: _____

Date: __ / __ / __

Appendix 4: RAPLOA-LF-REMO survey in Portuguese

MAPEAMENTO EPIDEMIOLÓGICO RÁPIDO DE FILARÍASES

QUESTIONÁRIO **Bloco 1|__| 2 |__| 3 |__| 4 |__|**

Data:|__|__|/|__|__|/2014. Inquiridor - |__|__| Bairro |__|__|__|

Comuna |__|__|__|__|__|__|__|__|__| **(A preencher no CISA)**

Comentários

____

____

____

____

____

____

QUESTIONÁRIO 1

1. Agregado familiar |__|__|__|-|__|__|-|__|__|__|__|-|__|__|
Bairro Sector AF ID (preencher no CISA)

2. Nome |__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|
|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__| **3.** Nome de Casa |__|__|__|__|__|__|__|__|__|__|__|__|__|

4. Data de Nasc. |__|__|/|__|__|/|__|__|__|__| **5.** Sexo: M |__| F |__| **6.** Há quanto tempo vive no bairro |__|__| anos

ATENÇÃO – Colocar esta questão sem mostrar nenhuma imagem.

7. LOA1 –Alguma vez sentiu ou viu vermes a andarem na parte branca do seu olho? SIM |__| NÃO |__|

8. LOA2–Alguma vez sentiu ou viu no seu olho algo igual ao que se mostra na imagem 1? (se **NÃO** passa para **10**) SIM |__| NÃO |__|

9. LOA3–A última vez que verificou esta condição, o verme permaneceu no olho menos de 7 dias seguidos? SIM |__| NÃO |__|

ATENÇÃO – Não colocar esta questão. O Inquiridor deve preencher **SIM**, **se a resposta às questões 7, 8 e 9 for SIM**. Deve preencher **NÃO** se, **PELO MENOS**, **uma das respostas às questões 7, 8 e 9 for NÃO.**

10 História do verme do olho (se **NÃO** passa para **12**) SIM |__| NÃO |__|

11. Se sim, é mais frequente numa determinada época do ano? Cacimbo |__| Chuva|__| Ambas |__| Não Sabe |__|

12. FL1 - Tem inchaço na perna idêntico ao apresentado na imagem 2a? SIM |__| NÃO |__|

ATENÇÃO – colocar esta questão APENAS a HOMENS.

13. FL2 - Tem inchaço no escroto (bolsa dos testículos) idêntico ao apresentado na imagem 2b? SIM |__| NÃO |__|

14. ONCO 1 - Tem caroços debaixo da pele em alguma parte do corpo, como se mostra na imagem 3? SIM |__| NÃO |__|

15. VG 1 – Conhece a doença que se apresenta na imagem 4 (Verme da Guiné)? SIM |__| NÃO |__|

(se a resposta for **NÃO**, **termina questionário e agradece**)

16. VG 2 - Viu alguém com esta doença (Verme da Guiné) recentemente? SIM |__| NÃO |__|

17. VG3 – Se viu, refira quando (mês e ano) e onde (bairro/província)

|__|__|/|__|__|__|__|. Local |__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|
mês ano Bairro ou Província ou País

Appendix 5: RAPLOA-LF-REMO survey in English

RAPID EPIDEMIOLOGICAL MAPPING OF FILARIASIS

SURVEY **Block 1|__| 2 |__| 3 |__| 4 |__|**

Date:|__|__|/|__|__|/2014. Interviwer- |__|__ Neighborhood |__|__|__|

Commune |__|__|__|__|__|__|__|__|__|__| (**To be filled in by CISA**)

Comments____
____
____
____
____

SURVEY 1

1. Family Aggregate |__|__|__|-|__|__|-|__|__|__|__|-|__|__|
Neighborhood Sector FA ID (To be filled in by CISA)

2. Name |__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|
|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__| **3.** Nickname |__|__|__|__|__|__|__|__|__|__|__|__|__|

4. D.O.B. |__|__|/|__|__|/|__|__|__|__| **5.** Sex: M |__| F |__| **6.** How long have you been living in this neighborhood |__|__| yes

ATTENTION – Ask this question without displaying any image.

7. LOA1 –Have you ever felt or saw worms walking in the white part of your eye? YES |__| NO |__|

8. LOA2– Have you ever felt or seen in your eye something like the one shown in picture 1? (if **NO** jump to **10**) YES |__| NO |__|

9. LOA3– The last time you checked for this condition, the worm remained in your eye less than 7 days in a row? YES |__| NO |__|

ATTENTION – Do not put this question. The Inquirer must complete **YES, if the answer to questions 7, 8 and 9 is YES.** Must complete **NO** if**, ONLY, one of the answers to questions 7, 8 and 9 is NO.**

10 History of the worm of the eye (if **NO** jump to **12**) YES |__| NO |__|

11. If so, is it more frequent at a particular time of year? Cacimbo |__| Raining|__| Both |__| Don't know |__|

12. LF1 - Has swelling in the leg identical to that shown in figure 2[a]? YES |__| NO |__|

ATTENTION – Put this question ONLY to MEN.

13. LF2 - Has swelling in the scrotum (testis pouch) identical to that shown in figure 2b YES |__| NO |__|

14. ONCHO 1 - Does it have lumps under the skin on any part of the body, as shown in figure 3? YES |__| NO |__|

15. VG1 – Do you know the disease that appears in image 4 (Worm of Guinea)? YES |__| NO |__|

(if the answer is **NO, finish questionnaire and thank**)

16. VG2 - Did you see anyone with this disease (Guinea Worm) recently? YES |__| NO |__|

17. VG3 – If so, mention when (month and year) and where (neighbordhood/province)

|__|__|/|__|__|__|__|. Local |__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|

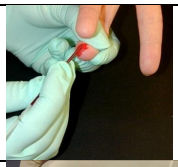
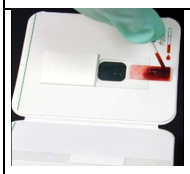
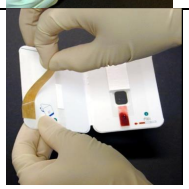
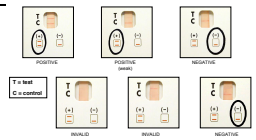
Appendix 6: ICT Protocol

The ICT card test has been shown to be a useful and sensitive tool for the detection of *Wuchereria bancrofti* antigen and is being used widely by lymphatic filariasis elimination Programs. Although the test is relatively simple to use, adequate training is necessary to reduce inter-observer variability and to reduce the misreading of cards, which can lead to false positive results.

Basic Guidelines

i. Cards are currently known to have a limited shelf life at ambient temperatures (3 months at 30°C) but longer shelf life when stored at 4°C (approximately 9 months). Cards should NOT be frozen.
ii. 100 microliters of blood should be collected by finger prick into a calibrated capillary tube coated with an anticoagulant (EDTA or heparin). Alternatively, finger prick blood can be collected into a microcentrifuge blood collection tube coated with either EDTA or heparin.
iii. Before beginning field surveys, two cards from each lot of cards should be tested using a weak positive control that can be obtained from the Filariasis Research Reagent Repository Center (www.filariasiscenter.org). When using this control, the test line can be very faint. DO NOT use cards that are negative when tested with the control.
iv. When transporting cards for use in the field, a cool box is not required. However, care should be taken not to expose cards to extreme heat for prolonged periods of time.
v. Cards must be read using adequate lighting. Faint lines can be difficult to see when lighting is not adequate. This is especially important when reading cards at night.

Test procedure

	1. Remove card from pouch just prior to use.		2. Collect 100uL blood by finger prick using a calibrated capillary tube. DO NOT add blood directly from the finger to the card.
	3. Add blood sample slowly to the white portion of the sample pad. DO NOT close the card before the sample migrates to the pink portion of the sample pad (takes approximately 30 seconds after adding blood).		4. Remove adhesive liner and close card. Start timing. DO NOT read cards of the plasma have not flowed ALL the way down the strip as a false positive result can occur.
	5. Read test results 10 minutes after closing card. DO NOT read cards at any time other than 10 minutes as false positive readings may occur.		6. Test interpretation

Appendix 7. DNA Extraction Protocol and real-time PCR for *Wuchereria bancrofti*

QIAmp genomic DNA kits enable rapid and efficient purification of high-quality genomic DNA from a diverse variety of sample materials for a blood range of downstream applications. The QIAmp genomic DNA kits provide reliable DNA purification, fast procedures and easy handling; ready-to-use DNA free of contaminants and enzyme inhibitors; high DNA recovery from a wide range of sample sources, and, no phenol-chloroform extraction or time-consuming alcohol precipitation.

This protocol was carried out manually using a centrifuge. QIAmp kits utilize the selective binding properties of the unique QIAmp silica membrane to isolate pure DNA. After lysis in an optimize buffer and adjustment of DNA binding conditions, the sample is loaded directly onto a QIAmp spin column. DNA is bounded to the silica membrane, and contamination removed in 2-wash steps.

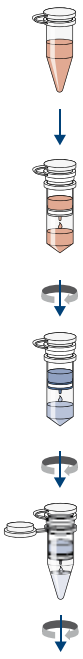
QIAmp genomic DNA kit components

Note: This protocol is for isolation of genomic DNA from 1-100 ul of whole blood treated with EDTA, citrate, or heparin-based anticoagulants.

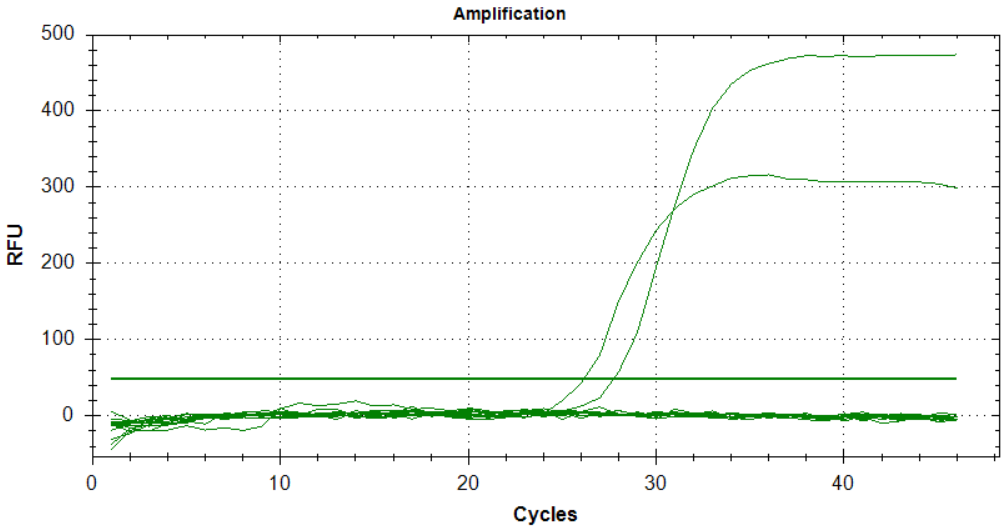
1. Pipet 200ul whole blood into a 1.5 microcentrifuge tube.
2. Add 10ul Proteinase K.
3. Add 200ul buffer AL, close the lid, and mix pulse-vortex (15s).
4. Incubate at 56 C for 10min
5. Briefly centrifuge the 1.5ml tube to remove drops from the inside of the lid
6. Add 200ul ethanol (96-100%), close the lid, and mix thoroughly by pulse-vortexing for 15s.
7. Briefly centrifuge the 1.5ml tube to remove drops from the inside of the lid.

8. Carefully transfer the entire lysate from step 8 to the QIAmp Mini Elute Column without wetting the rim, close the lid, and centrifuge at 6000xg (8000rpm) for 1 min.
9. Carefully open the QIAmp Mini Spin column and add 500ul buffer AW1. Close the cap and centrifuge at 6000xg (8000rpm) for 1 min. Place the QIAmp Mini spin column in a clean 2ml collection tube and discard the collection tube containing the filtrate.
10. Carefully open the QIAmp Mini spin column and add 500ul buffer AW2 without wetting the rim. Close the cap and centrifuge at full speed (20,000xg; 14,000 rpm) for 3min.
11. Recommended: Place the QIAmp Mini spin column in anew 2ml collection tube and discard the old collection tube with the filtrate. Centrifuge at full speed for 1min.
12. Place the QIAmp Mini spin column in a clean 1.5ml microcentrifuge tube and discard the collection tube containing the filtrate. Carefully open the QIAmp Mini Spin column and add 200ul buffer AE or distilled water. Incubate at room temperature (15-25 oC) for 1 min, and the centrifuge at 6000xg (8000rpm) for 1 min.

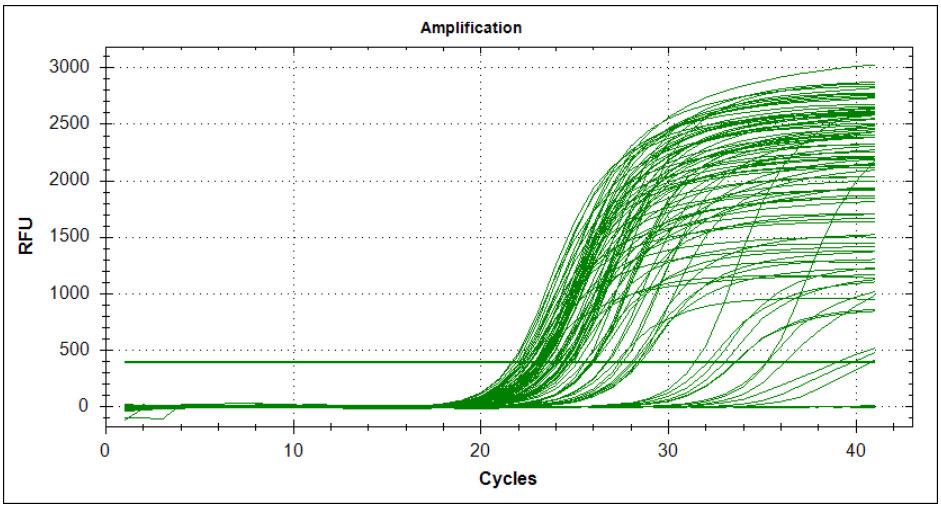
QIAmp blood procedure



SYBR Green real-time PCR amplification. It shows that all samples were amplified as all of them have human DNA.



Taqman real-time PCR amplification plot demonstrating detection of positive control for *W. bancrofti* parasite in all runs. It shows that only the gDNA from *W. bancrofti* microfilaremia produced signals in both positive controls. It did not detect any presence of the parasite in the remaining samples.



Appendix 8. Nested PCR protocol for *Loa loa*

Preparation of 10uM working solution of primers

(ITS-1 F, ITS1 R, *Lloa*F1, *Lloa*F2 primers)

1.Defrost the stock to 100 solution of primer

2. Vortex for 3s

3. Centrifuge 30s at full speed

4. Label an eppendorf tube with primer name, dilution date, and concentration (10 µM)

5. Pipette 90 concentration (10 primer name, dilutcentrifuge at 6000xg (ufto be diluted)

6. Pipette 10 µl of the primer to 100 □M

7. Vortex for 3s

8. Centrifuge 30s at full speed

9. Use immediately or freeze at -20oC

Primer - 3 species (*Loa loa* 475bp, *Mansonella* 484bp, *Wuchereria bancrofti*, 482bp)

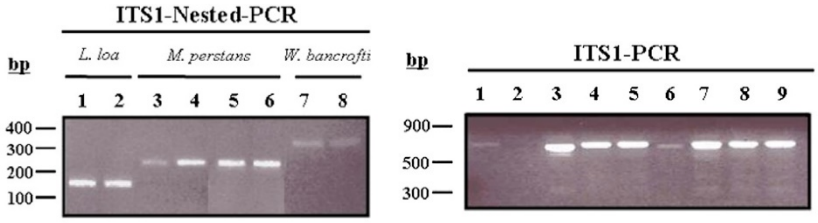
ITS1-F 5'-GGTGAACCTGCGGAAGGATC-3'

ITS1-R 5'-CTCAATGCGTCTGCAATTCGC-3'

***Loa loa* (fragment of 143 bp)**

LlF1:5'-GATGATGATATATGATGAAG-3'

LlR1: 5'-TTAAGCTATCGCTTTATCTTC-3'



I - AMPLIFICATION OF THE ITS1 FRAGMENT COMMON TO ALL 3 SPECIES (PCR-1)

1- FILL IN THE PCR REGISTRATION SHEET

2 - DEFROST THE PRIMERS (STOCKS OF 10UM), AND THE PCR MIX (2X - ALREADY CONTAINS ENZYME, DNTPS, BUFFER AND MGCL2)). AFTER DEFROSTING VORTEX FOR 3S AND CENTRIFUGE.

3- DEFROST THE SAMPLES TO BE AMPLIFIED

4- IDENTIFY THE PCR TUBES (0.2 ML) WITH THE RESPECTIVE NUMBER. (DO NOT FORGET THE WHITE OF THE PCR AND THE WHITE OF THE EXTRACTION, AND THE POSITIVE CONTROL)

5- PREPARE THE MASTER MIX OF THE PCR (PREPARE FOR THE N SAMPLES PLUS THE BLANKS) IN THE PREPARATION ROOM

Reagents	Per sample	N samples
Mix PCR (2X)	12.5 µL	X N
ITS-1 F	0.5 µL	X N
ITS-1 R	0.5 µL	X N
H_2O	9.5 µL	X N

Note: Put the water as last and mix the reagents by pipetting up and down about 3 or 4 time

6- PLACE 23 ML MASTER MIX IN EACH PCR TUBE,

7- PIPETTE 2 ML OF DNA INTO THE CORRESPONDING PCR TUBE (AMPLIFY THE EXTRACTION BLANK AND PLACE A PCR BLANK WHERE WATER IS ADDED) AND ADD THE POSITIVE CONTROL.

8- CLOSE THE TUBE TIGHTLY AND PLACE IN THE THERMOCYCLER WITH THE FOLLOWING PROGRAM:

Cycles	Function	Temperature	Duration
1	Pre-denaturation	95 °C	10min
40	Denaturation Annealing Extension	94 °C 58 °C 72 °C	30s 30s 45s
1	Final extension	72 °C	10min
1	Hold	4 °C	∝

9- AFTER FINISHING THE PCR RUN THE SAMPLES IN 2% AGAROSE GEL AND PHOTOGRAPH. 10- STORE THE IDENTIFIED SAMPLES AT -20 C

II – 2% AGAROSE GEL ELECTROPHORESIS FROM PCR-1 PRODUCT (LOA LOA 475BP, MANSONELLA 484BP, WUCHERERIA BANCROFTI, 482BP FRAGMENTS)

1- Fill in the sheet of agarose gel

2- Weigh 2 g of agarose and add 100 ml of TBE1X buffer. (Or recast the agarose gel by placing the gel used inside the erlenmayer and proceed to step 3. NOTE - the gel should not be reused more than 3 times)

3. Heat in the microwave until it boils (about 90 sec) and allow to cool to about 60oC.

4- Add 5 µl of RedSafe.

5- Pour the gel into the holder placed in the electrophoresis vial, and place the combs necessary to form the wells; wait 10-15 mins to solidify.

6- Prepare "drops" of application buffer, on a piece of Parafilm

7- Mix 5 µl of each PCR sample with one drop of application solution and apply to a well. NOTE - these samples will be re-amplified so use a new tip for each sample to avoid contamination between samples)

8- Turn on the power and run the samples for 30 min at 120V.

9- View the gel on a UV transilluminator (254 nm) and photograph.

10- Record the number / name of the gel photograph on the agarose gel sheet.

III – AMPLIFICATION OF THE SPECIFIC FRAGMENT OF *LOA LOA* (PCR-2)

1- FILL IN THE PCR REGISTRATION SHEET

2 - THAW THE PRIMERS (STOCKS OF 10UM), AND THE PCR MIX (2X - ALREADY CONTAINS ENZYME, DNTPS, BUFFER AND MGCL2). AFTER THAWING VORTEX (3 SECONDS) AND CENTRIFUGE.

3- DEFROST THE SAMPLES TO BE AMPLIFIED, WHICH ARE THE PCR PRODUCTS OF THE ITS1 PRIMERS.

4- ADD 100 ML OF STERILE WATER (DILUTION 1: 5) TO EACH PCR-1 TUBE (ALWAYS CHANGE THE TIP TO AVOID CONTAMINATION)

5- IDENTIFY THE PCR TUBES (0.2 ML) WITH THE RESPECTIVE NUMBER. (DO NOT FORGET THE WHITE OF THE PCR AND THE WHITE OF THE EXTRACTION, AND THE POSITIVE CONTROL)

6- PREPARE THE MASTER MIX OF THE PCR (PREPARE FOR N SAMPLES PLUS WHITES) IN THE PREPARATION ROOM

Reagents	Per sample	N sample
Mix PCR (2X)	12.5 µL	X N
ITS-1 F	0.75 µL	X N
ITS-1 R	0.75 µL	X N
H_2O	9 µL	X N

NOTE: PUT THE WATER AS LAST AND MIX THE REAGENTS BY PIPETTING UP AND DOWN ABOUT 3 OR 4 TIMES

7- PLACE 23 ML MASTER MIX IN EACH PCR TUBE,

8- PIPETTE 2 ML OF DNA (1: 5 DILUTION OF PCR-1) INTO THE CORRESPONDING PCR TUBE (DO NOT FORGET THE PCR BLANK)

9- COVER THE TUBE WELL AND PLACE IN THE THERMOCYCLER WITH THE FOLLOWING PROGRAM:

Cycles	Function	Temperature	Duration
1	Pre-Denaturation	95º C	10 min
40	Denaturation Annealing Extension	94º C 58º C 72º C	30 s 30 s 45 s
1	Final Extension	72º C	10 min
1	Hold	4º C	∞

10- AFTER FINISHING THE PCR RUN THE SAMPLES IN 2% AGAROSE GEL AND PHOTOGRAPH.

11- STORE THE IDENTIFIED SAMPLES AT -20oC

IV – 2% AGAROSE GEL ELECTROPHORESIS OF PCR-2 PRODUCT

(*LOA LOA* 143 BP FRAGMENT)

1- FILL IN THE SHEET OF AGAROSE GEL

2- WEIGH 2 G OF AGAROSE AND ADD 100 ML OF TBE1X BUFFER. (OR RECAST THE AGAROSE GEL BY PLACING THE GEL USED INSIDE THE ERLENMAYER AND PROCEED TO STEP 3. NOTE - THE GEL SHOULD NOT BE REUSED MORE THAN 3 TIMES)

3. HEAT IN THE MICROWAVE UNTIL IT BOILS (ABOUT 90 SEC) AND ALLOW TO COOL TO ABOUT 60oC.

4- ADD 5 ML OF REDSAFE.

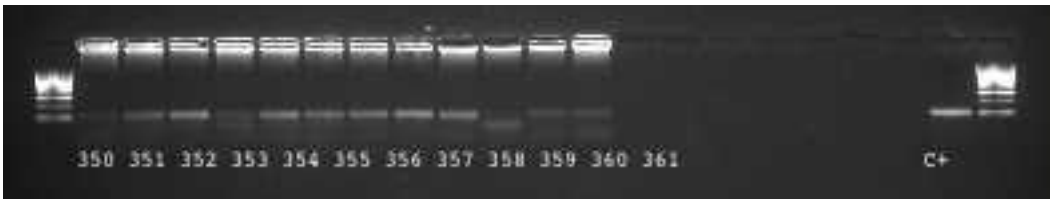
5- POUR THE GEL INTO THE HOLDER PLACED IN THE ELECTROPHORESIS VIAL, AND PLACE THE COMBS NECESSARY TO FORM THE WELLS; WAIT 10-15 MINS TO SOLIDIFY.

6- PREPARE "DROPS" OF APPLICATION BUFFER, ON A PIECE OF PARAFILM

7- MIX 5 ML OF EACH PCR SAMPLE WITH ONE DROP OF APPLICATION SOLUTION AND APPLY TO A WELL

8- TURN ON THE POWER SUPPLY AND RUN THE SAMPLES FOR 30 MIN AT 120V.
9- VIEW THE GEL ON A UV TRANSILLUMINATOR (254 NM) AND PHOTOGRAPH.
10- RECORD THE NUMBER / NAME OF THE GEL PHOTOGRAPH ON THE AGAROSE GEL SHEET.

Nested PCR *L. loa* amplification products in 2% agarose gel electrophoresis. First and last wells show the molecular hyperladder. C+ stands for the *L. loa* positive control. The positive bands in the gel electrophoresis were taken as those appearing at 188bp, where the size of positive control is expected to appear (samples 350-358, 360-361). The negative results were taken as those samples, which do not show a band at the expected zone of 188bp (sample 359).



The sensitivity and specificity of nested-PCR assay was found to be 98% and 100% respectively. Thus, the positive predictive value (PPV) and the negative predictive value (NPV) for PCR for *L. loa* are 99% and 98% respectively (Table 4.3). The sensitivity and specificity of ELISA assay was found to be 91% and 99% respectively. Thus, the PPV and NPV for ELISA for *O. volvulus* is 99% (Table 4.3).

Sensitivity and specificity of PCR and ELISA assays.

Assay	Sensitivity	Specificity	PPV	NPV	Accuracy
PCR	98%	100%	99%	100%	100%
ELISA	91%	99%	98%	99%	99%

Appendix 9.'*Loa loa* vector *Chrysops spp*. Perspective on research, distribution, bionomics, and implications for elimination of lymphatic filariasis and onchocerciasis'

Kelly-Hope *et al. Parasites & Vectors* (2017) 10:172
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Parasites & Vectors

REVIEW Open Access

Loa loa vectors *Chrysops* spp.: perspectives on research, distribution, bionomics, and implications for elimination of lymphatic filariasis and onchocerciasis

Louise Kelly-Hope[1*†], Rossely Paulo[1,2†], Brent Thomas[1], Miguel Brito[2,3], Thomas R. Unnasch[4] and David Molyneux[1]

Abstract

Background: Loiasis is a filarial disease caused *Loa loa*. The main vectors are *Chrysops silacea* and C. *dimidiata* which are confined to the tropical rainforests of Central and West Africa. Loiasis is a mild disease, but individuals with high microfilaria loads may suffer from severe adverse events if treated with ivermectin during mass drug administration campaigns for the elimination of lymphatic filariasis and onchocerciasis. This poses significant challenges for elimination programmes and alternative interventions are required in *L. loa* co-endemic areas. The control of *Chrysops* has not been considered as a viable cost-effective intervention; we reviewed the current knowledge of *Chrysops* vectors to assess the potential for control as well as identified areas for future research.

Results: We identified 89 primary published documents on the two main *L. loa* vectors *C. silacea* and *C dimidiata*. These were collated into a database summarising the publication, field and laboratory procedures, species distributions, ecology, habitats and methods of vector control. The majority of articles were from the 1950–1960s. Field studies conducted in Cameroon, Democratic Republic of Congo, Equatorial Guinea, Nigeria and Sudan highlighted that *C. silacea* is the most important and widespread vector. This species breeds in muddy streams or swampy areas of forests or plantations, descends from forest canopies to feed on humans during the day, is more readily adapted to human dwellings and attracted to wood fires. Main vector targeted measures proposed to impact on *L. loa* transmission included personal repellents, household screening, indoor residual spraying, community-based environmental management, adulticiding and larviciding.

Conclusions: This is the first comprehensive review of the major *L. loa* vectors for several decades. It highlights key vector transmission characteristics that may be targeted for vector control providing insights into the potential for integrated vector management, with multiple diseases being targeted simultaneously, with shared human and financial resources and multiple impact. Integrated vector management programmes for filarial infections, especially in low transmission areas of onchocerciasis, require innovative approaches and alternative strategies if the elimination targets established by the World Health Organization are to be achieved.

Keywords: *Loa loa*, Loiasis, Tropical eye worm, *Chrysops*, Vector control, Lymphatic filariasis, Onchocerciasis, Neglected tropical diseases, NTDs, Africa, Integrated vector management, Bionomics

Appendix 10.' Rapid integrated clinical surveys to determine the prevalence and co-distributions patterns of lymphatic filariasis and onchocerciasis in a *Loa loa* co-endemic: The Angolan experience'

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Rapid integrated clinical survey to determine prevalence and co-distribution patterns of lymphatic filariasis and onchocerciasis in a *Loa loa* co-endemic area: The Angolan experience

Miguel Brito[a,b,1], Rossely Paulo[a,c,1], Pedro Van-Dunem[d], António Martins[a], Thomas R. Unnasch[e], Robert J. Novak[e], Benjamin Jacob[e], Michelle C. Stanton[c], David H. Molyneux[c], Louise A. Kelly-Hope[c,*]

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ABSTRACT

The Republic of Angola is a priority country for onchocerciasis and lymphatic filariasis (LF) elimination, however, the co-distribution of the filarial parasite *Loa loa* (loiasis) is a significant impediment, due to the risk of severe adverse events (SAEs) associated with ivermectin used in mass drug administration (MDA) campaigns. Angola has a high risk loiasis zone identified in Bengo Province where alternative interventions may need to be implemented; however, the presence and geographical overlap of the three filarial infections/diseases are not well defined. Therefore, this study conducted a rapid integrated filarial mapping survey based on readily identifiable clinical conditions of each disease in this risk zone to help determine prevalence and co-distribution patterns in a timely manner with limited resources. In total, 2007 individuals from 29 communities in five provincial municipalities were surveyed. Community prevalence estimates were determined by the rapid assessment procedure for loiasis (RAPLOA) and rapid epidemiological mapping of onchocerciasis (REMO) together with two questions on LF clinical manifestations (presence of lymphoedema, hydrocoele). Overall low levels of endemicity, with different overlapping distributions were found. Loiasis was found in 18 communities with a prevalence of 2.0% (31/1571), which contrasted to previous results defining the area as a high risk zone. Onchocerciasis prevalence was 5.3% (49/922) in eight communities, and LF prevalence was 0.4% for lymphoedema (8/2007) and 2.6% for hydrocoeles (20/761 males) in seven and 12 communities respectively. The clinical mapping survey method helped to highlight that all three filarial infections are present in this zone of Bengo Province. However, the significant difference in loiasis prevalence found between the past and this current survey suggests that further studies including serological and parasitological confirmation are required. This will help determine levels of infection and risk, understand the associations between clinical, serological and parasitological prevalence patterns, and better determine the most appropriate treatment strategies to reach onchocerciasis and LF elimination targets in the loiasis co-endemic areas. Our results also suggest that the utility of the earlier RAPLOA derived maps, based on surveys undertaken over a decade ago, are likely to be invalid given the extent of population

Appendix 11.'Clinical, serological and DNA testing in Bengo, Angola. Further reveals low filarial endemicity and opportunities for disease elimination'

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Clinical, serological and DNA testing in Bengo Province, Angola further reveals low filarial endemicity and opportunities for disease elimination

Rossely Paulo [a,b,*,1], Miguel Brito [a,c,1], Pedro Van-Dunem [d], António Martins [a], Robert J. Novak [e], Benjamin Jacob [e], David M. Molyneux [b], Thomas R. Unnasch [e], J. Russell Stothard [b], Louise Kelly-Hope [b]

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ABSTRACT

The prevalence of *Loa loa*, *Onchocerca volvulus* and *Wuchereria bancrofti* infections in an under-surveyed area of Bengo Province, Angola, was determined by surveying 22 communities with a combination of clinical, serological and DNA diagnostics. Additional information was collected on participants' duration of residency, access to mass drug administration, knowledge of insect vectors and use of bednets. A total of 1616 individuals (38.1% male: 61.9% female), with an average age of 43 years, were examined. For L. *loa*, 6.2% (n = 100/16616) individuals were found to have eyeworm, based on the rapid assessment procedure for loiasis (RAPLOA) surveys, and 11.5% (n =178/1543) based on nested PCR analyses of venous blood. *L. loa* prevalences in long-term residents (>10 years) and older individuals (>60 years) were significantly higher, and older men with eyeworm were better informed about *Chrysops* vectors. For *O. volvulus*, 4.7% (n = 74/1567) individuals were found to be positive by enzyme-linked immunosorbent assay (Ov 16 ELISA), with only three individuals reporting to have ever taken ivermectin. For *W. bancrofti*, no infections were found using the antigen-based immunochromatographic test (ICT) and real-time PCR analysis; however, 27 individuals presented with lymphatic filariasis (LF) related clinical conditions (lymphoedema = 11, hydrocoele = 14, both = 2). Just under half (45.5%) of the participants owned a bednet, with the majority (71.1%) sleeping under it the night before. Our approach of using combination diagnostics reveals the age-prevalence of loiasis alongside low endemicity of onchocerciasis and LF. Future research foci should be on identifying opportunities for more cost-effective ways to eliminate onchocerciasis and to develop innovative surveillance modalities for clinical LF for individual disease management and disability prevention.

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[1] These authors contributed equally to this work.

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Appendix 12. Epidemiology of filariasis in Zaire province, Angola, ASTMH Abstract Book from 66th Annual Meeting

EPIDEMIOLOGY OF FILARIASIS IN ZAIRE PROVINCE, ANGOLA

Célio C. Njinga[1], Filipa Vaz[1], Rossely C. Paulo[2], Pedro Van Dunem[3], Miguel Brito[4]

[1]CISA, Luanda, Angola, [2]Liverpool School of Tropical Medicine/CISA, Luanda and Liverpool, Angola, [3]National Coordinator at Public Health Department, Luanda, Angola, [4]Lisbon School of Health Technology, Lisbon, Portugal

Filariasis are known to be endemic in Angola and contributes to outpatient morbility and mortality of the country with over 12 million people at risk of infection. As so, little is known about the geographical distribution and co-endemicity of three filarial parasites such as *Onchocerca volvulus, Wuchereria brancrofti, and Loa loa.* Hence, it is important to understand the distribution in co-endemic areas with multiple species of Filariasis with the assess of the prevalence of lymphatic filariasis (LF), onchocerciasis and loiasis.The 6 municipalities of Zaire were surveyed and 7 communes were selected based on the geographical location-river proximity and swampy areas of plantation or forest. The random sampled respondents were interviewed using the Rapid assessment procedure (RAPLOA) for *Loa Loa* and the Rapid Epidemiological Mapping of onchocerciasis (REMO) surveys to assess LF clinical signs (hydrocele and/or Lymphedema). A total of 157 respondents took part in the survey. The nested Polymerase Chain Reaction (PCR), followed by electrophoresis and visualization with staining with ethidium bromide was performed to detect *Loa loa* infection. For *Wuchereria bancrofti* (LF), Real-Time PCR with specific Taqman probes, Dried Blood Spot (DBS) samples was used. In addition, for each subject an onchocerciasis rapid test and dried blood spot (DBS) was performed between 8 a.m. - 3 p.m. The results of molecular testing were combined with conventional epidemiological approaches to determine the spatial-temporal distribution of loiasis and population-level risk factors for reported diseases. Moreover, the nested PCR assay showed 0% prevalence for loiasis. Still, the prevalence for LF is also found to be of 0%, according to the Real-Time PCR. Conversely, with the rapid tests for onchocerciasis infection, it was found that males have higher prevalence (6.3%) compared to females (3.8%). The molecular biology did not find co-endemecity but it provided insights for the importance for innovative approaches and alternative strategies for filarial infections required for the elimination targets established by the World Health Organization.

Appendix 13. Conference poster publication

IV Science and Technology National conference poster (Luanda, 2015)

Spatial clustering of filariasis and risk factors analysis in Bengo Province, Angola

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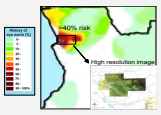
Background

The implementation of lymphatic filariasis (LF) and onchocerciasis elimination programmes in areas where *Loa loa* is co-endemic is problematic because individuals with heavy *L. loa* infections may develop severe adverse events when treated with ivermectin. This has implications for mass drug administration campaigns and alternative strategies may be required. One of the highest *L. Loa* risk areas is Dande Municipality, Bengo Province in Northern Angola (Figure 1: Insert shows study area).

Figure 1. RAPLOA distribution



The aim of this study was to determine the distribution of filariasis and associated risk factors in Bengo Province using rapid field survey methods including clinical observations, serology and molecular analysis. RapidEye (5m resolution) satellite data was also employed to provide the foundation for empirical information on vector and parasite populations in relation to key environmental drivers.

Methods

Study site: The study was conducted the Centro de Investigação em Saúde em Angola (CISA) area in Dande Municipality. In total, 22 villages across peri-urban and rural areas were surveyed. In each village, up to 100 individuals were assessed in August-Sept 2014

Filariasis

i) *L. loa* - the rapid assessment procedure for loiasis (RAPLOA) based on eye worm history was used and blood collected for molecular analysis with Real Time PCR

ii) LF - the rapid diagnostic ICT card was used to detect LF antigen, and questions related to morbidity (presence of limb lymphoedema and hydrocele)

iii) Onchocerciasis – Blood spots were collected to detect antigen using the Ov16 ELISA

Risk factors

Surveyed individuals were asked about intervention usage and local vectors

i) Bed net ownership and usage

ii) Ivermectin consumption

iii) Chrysops spp. identification

High resolution RapidEye image and environmental analysis and modelling on digital elevation, river flow and forest cover was conducted with geographical information system (GIS) and Ecognition image analysis software.

Figure 2. Villages and households georeferenced to RapidEye Image

LF Results

For LF: A total of 1616 individuals were surveyed. Few LF clinical cases were found with 13 lymphoedema (0.8%) and 16 hydrocele cases (1.7%) identified. The serological survey found all rapid diagnostic tests ICTs were negative, which was also confirmed with PCR laboratory analysis. Regarding interventions, a low bed net ownership (46.1%) was reported, with only 33.8% sleeping under a net the previous night.

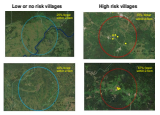
Loa loa Results

For Loa loa: Overall, *L. loa* prevalence was 6.2% with positive individuals found throughout the study area (yellow dots – Figure 3). Village prevalence ranged from 0 to 18% suggesting a 'hypo-endemic' area. Molecular analysis of samples is underway.

Figure 3. Loa loa positive distribution

For RAPLOA, there was no significant difference by sex (males 5.2%, female 6.8%) however a significantly higher prevalence in older individuals was found (<40 yrs 3.3%; >40yrs 8.7%).

Figure 4. Loa negative/positive villages



Of particular note was the lack of *L. loa* in six of the villages within this small area (blue circles – Figure 3). Differences between *L. loa* positive and negative villages were examined in relation to forest cover, which is a potentially important habitat characteristic for *Chrysops* spp.

Using the high resolution RapidEye image and a specialized image exaction method (multi-resolution segmentation) (eCognition) and GIS software(ArcGIS) . the proportion of dense forest cover within a 2.5km buffer of each village centre was compared.

Figure 5. Forest cover differences

L. loa negative villages were found to have less forest cover (25-40%) than positive villages (57-67%) as shown by the two village examples presented in Figures 4 and 5, which may explain prevalence differences.

When asked about the main *Chrysops spp.* vector, 12% of individuals recognized the fly when presented with a photograph. Overall, males and individuals >40 years of age were twice as likely to recognize the fly, whereas individuals with loiasis (by RAPLOA), were 2.5 times more likely, and most significantly males >40 years of age with loiasis were 4.24 times more likely, to recognize the fly than the related subgroups (Table 1).

Table 1. Knowledge of *Chrysops* spp. in local area when shown a photograph

Variable combination	Odds Ratio	95% CI	Z statistic	P- value
Male (female)	1.98	1.45 to 2.71	4.262	P < 0.0001
Older age>40 yrs (younger age)	1.92	1.38 to 2.68	3.867	P < 0.0001
Male older age >40 yrs (male younger age)	2.06	1.28 to 3.30	2.98	P = 0.0029
Loa +ve (*Loa* –ve)	2.54	1.39 to 4.0	3.185	P = 0.0014
Loa +ve older age >40 yrs (*Loa* –ve older age)	2.24	1.26 to 4.0	2.730	P=0.0063
Male *Loa* +ve (male *Loa* –ve)	3.87	1.76 to 8.39	3.417	P = 0.0006
Loa +ve male older age >40 yrs (*Loa* –ve male older age)	4.24	1.68 to 10.67	3.061	P = 0.002

Onchocerciasis Results

For onchocerciasis: Overall sero-prevalence was 4.7% with village prevalence ranging from 0 to 35%. Only a few selected household clusters were spatially distinct with high sero-prevalence (>20%), suggesting a 'hypo-endemic' area.

Figure 6. Oncho positive distribution

No significant difference by sex (males 5.0%, female 4.5%) or age group (<40 yrs 4.6%; >40yrs 4.8%) and little or no evidence of ivermectin consumption was found.

Figure 7. Hypo-endemic breeding sites

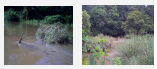
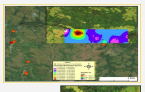


Figure 7 shows examples of potential breeding sites of *Simulium* spp (orange square – Figure 6) in this 'hypo-endemic' study area.

The hypo-endemic onchocerciasis riverine areas appear to have distinct characteristics of trailing vegetation in fast flowing water, which caused ripples and oxygenated water for the vector to breed. This differs to the Pre-Cambrian signatures found in hyper-endemic area elsewhere and modelled.

Figure 8. Interpolates *Simulium* spp. signature along riverine location



Analysis of previous models and RapidEye image help to define a potentially new signature habitat of vegetation (chlorophyll A). The potential *S. damnosum* s.l. breeding sites was interpolated to produce a risk map along the river (Figure 8).

Figure 9. Interpolated layer around breeding sites

The lag was extended and a layer created of the interpolated signature on RapidEye data, and to help visually detect soils around the immature, flood–prone larval habitat (Fig 9).

Discussion

Filariasis is endemic at different prevalences and spatial patterns throughout the region, and the presence of *L. loa* suggests that alternative strategies for the elimination of LF and onchocerciasis are required.

These may include the scaling up vector control with key information on the best targets directed by local community knowledge given their potential ability to identify and micro-map *Chrysops* spp. habitats and high risk areas.

Acknowledgements

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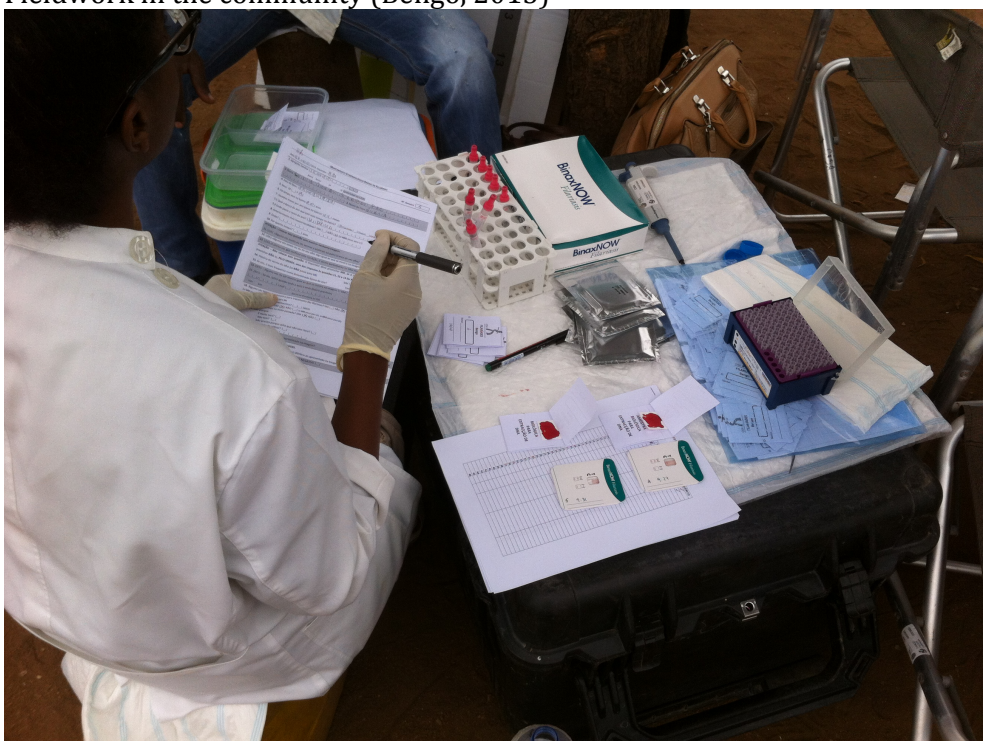
Fieldwork in the community (Bengo, 2015)



Appendix 14. Local scientific newspaper giving credit to CISA in regards the NTDs study (Sol journal, 2015)

Local houses at the community (Bengo, 2015)

Local scientific newspaper giving credit to CISA in particular the NTDs study (Sol journal, 2015)

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Foco

SAÚDE PRECISA DE MAIS ESTUDOS

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Os estudos científicos no sector da saúde ainda são reduzidos em Angola, mas esse quadro tem vindo a mudar e pode trazer uma nova luz para doenças até agora negligenciadas. Miguel Brito, coordenador do Centro de Investigação em Saúde, disse ao *SOL* que, mesmo durante a guerra, as instituições **«não baixaram os braços».**

Um dos principais desafios do sector da saúde em Angola está relacionado com os estudos da investigação científica, que ainda são insignificantes. Mas aos poucos, fruto do desenvolvimento do país nos últimos anos, o quadro tem vindo a inverter, o que pode ajudar na diminuição dos elevados índices de doenças negligenciadas em várias regiões do território nacional.

A falta de recursos humanos qualificados e de capital financeiro que possam garantir uma investigação de qualidade em saúde são os dois principais 'calcanhares de Aquiles' do sector. «São os fundos que permitem que as instituições contratem um número mínimo de quadros qualificados, **que visa permitir que haja investigação de qualidade»**, acrescentou ao *SOL* Miguel Brito, coordenador do Centro de Investigação em Saúde de Angola (CISA).

No entanto, o responsável também defende que os projectos desta área devem ser auto-sustentáveis, sobretudo em alturas de crise como a que o país atravessa. Miguel Brito lembrou que, mesmo durante a fase de guerra civil, as instituições que se dedicavam às actividades de investigação no sector da saúde em Angola «**não baixaram os braços**». Desenvolviam as suas actividades, embora em dimensão reduzida devido à insegurança e insegurança.

Número de estudos abaixo da procura

Com a mudança de paradigma social e político a situação inverteu-se. Contudo, o número de estudos de investigação ainda não atende à procura, considera o coordenador do CISA. Defende que, se Angola ambiciona atingir patamares de países de pesquisas científicas em saúde em África, deve adoptar de forma sistemática e organizada uma política virada para a formação de recursos humanos para sector, seja por via externa ou interna.

«Não podemos esquecer que Angola esteve em guerra durante um longo período e, ainda assim, foram realizados estudos em algumas zonas, independentemente de todos os problemas. Instituto o responsável. Lembrou, porém, que hoje existem mais instituições vocacionadas para a investigação, sem que isso origine um aumento de estudos e projectos.

Isso deve-se em muito à falta de profissionais especializados: «O número de quadros qualificados é irrisório para atender às necessidades, uma vez que a investigação avalia-se pelo número de instituições de qualidade que se dedicam a essa actividade».

Vontade de trabalhar em Angola

Miguel Brito destacou ainda a necessidade de os profissionais, formados em Angola ou no exterior, quererem trabalhar no país. **«É importante que os jovens angolanos que se formaram no sector optem em trabalhar no país, para ajudarem a encontrar soluções para os diversos trabalhos de investigação em saúde existentes,** afirmou na conversa com o SOL.

Apesar da dificuldade em encontrar quadros capacitados, o coordenador do CISA revelou que há também aqueles que têm uma paixão inexorável pela investigação. E alguns só não a fazem mais, ou de todo, por falta de disponibilidade em função das responsabilidades que têm a nível das instituições. Segundo Miguel Brito, isso **«é algo que o próprio país também tem de pensar em reverter».**

O responsável do Centro de Investigação em Saúde de Angola – instituição que funciona no Cazizi desde 2007, ano em que foi criada – entende que os órgãos que se dedicam à pesquisa no ramo da saúde a nível nacional, sejam eles públicos ou privados, não podem esperar que Governo financie todos projectos de investigação.

Defende por outro lado que é obrigação de quem governa financiar, isso sim, a formação dos quadros das instituições em todos os níveis. O objectivo dessa formação passa por conseguir que o país seja mais competitivo nesse sector, em todos os capítulos vocacionados para a investigação científica da origem de diferentes patologias.

Faltam projectos atractivos

Quanto aos investigadores seniores, na visão de Miguel Brito devem criar projectos atractivos e importantes para atrair outros financiadores. E neste âmbito reconhece igualmente que o número de investigadores experientes, com capacidade para orientar projectos nacionais, ainda é pequeno. O ponto positivo é que traz esperanças é que, tal como nos estados, esse número tem vindo a crescer.

O CISA conta com mais de 60 funcionários, e uma parte significativa dos orientadores são investigadores internacionais, devido à referida insuficiência de quadros angolanos.

Miguel Brito deseja inverter esse quadro dentro de dez anos: **«Queremos continuar a manter parcerias em todo mundo, tendo em conta que é sempre bom partilhar técnicas científicas com estrangeiros, mas é fundamental que num futuro próximo tenhamos muitos angolanos seniores para equilibrar o actual cenário».**

Sobre a origem dos investimentos que garantem a sustentabilidade dos projectos que o CISA tem vindo a desenvolver desde 2007, Miguel Brito explicou que são provenientes de três entidades: a fundação portuguesa Calouste Gulbenkian, o Governo angolano, e a cooperação institucional portuguesa para o país.

Quanto à gestão dos recursos nesta altura de crise, o interlocutor disse que o centro tem procurado gerir o que tem, mantendo os olhos postos noutras latitudes: **«Também procuramos parcerias internacionais».**

Técnica do CISA a recolher amostras

Dande, no Bengo, recebeu uma investigação do CISA sobre hipertensão

CISA bastante atento a focos de doença locais

Doenças contagiosas e parasitárias, algumas hereditárias, têm sido um dos destaques do CISA. Estudo recente no Dande detectou hipertensão em 20% das pessoas testadas.

De acordo com coordenador do Centro de Investigação em Saúde de Angola, grande parte das doenças negligenciadas no país não causam mortalidade, mas sim morbilidade elevada, ou seja, atingem uma percentagem muito grande de uma comunidade quando surgem. E esse impacto varia de região para região.

Miguel Brito referou ainda que as doenças mais comuns nesses casos são as que estão associadas **à «falta de diagnóstico, falta de hábitos de higiene e de condições de tratamento de água».**

Em função do desenvolvimento que se tem registado no sector da saúde no país, o responsável acredita que, nos próximos dez anos, a área da investigação dará passos significativos. Garante ter conhecimento de que muitos jovens angolanos estão a formar-se no sector e alguns já começaram mesmo a trabalhar nela.

Confia também que esses jovens profissionais têm vontade de ajudar o país a desenvolver-se na investigação. **«Não vou dizer que daqui a dez anos Angola estará nos** *top* **da investigação em saúde, mas acredito que os números de estudos serão satisfatórios para o desenvolvimento do país»**, avançou Miguel Brito ao SOL.

Doenças contagiosas e hereditárias em destaque

O CISA tem tido como principal foco de actuação os estudos da prevalência de doenças contagiosas e parasitárias, que afectam sobretudo as crianças da faixa etária do ensino pré-escolar.

Também tem estado envolvido nos últimos anos em projectos de investigação de doenças hereditárias, principalmente as hemoglobinopatias – alterações graves no sangue que podem levar à morte ainda nos primeiros anos de vida.

Recentemente, a instituição realizou no município do Dande, província do Bengo – onde estão sediados os seus laboratórios, imediatamente no Hospital Geral do Bengo, na cidade de Caxito – um estudo sobre a prevalência de problemas de hipertensão na população. O teste foi feito a 2.200 pessoas e os resultados indicaram que havia uma prevalência da doença na ordem dos 20%.

José Mauricio