UNIVERSITY OF LIVERPOOL

Investigation of risk factors for better control and surveillance of lymphatic filariasis in Papua New Guinea

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

By

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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.

Signed,

Melinda Susapu

Abstract

Papua New Guinea (PNG) has an estimated population of 7 million inhabitants; of which 5.6 million are at risk of lymphatic filariasis (LF). LF is a debilitating disease caused by nocturnal periodic nematode *Wuchereria bancrofti* and transmitted by *Anopheles* mosquitoes, similar to malaria. LF is targeted for elimination, and PNG is a member of the Global Programme to Eliminate LF, which aims to interrupt transmission through mass drug administration (MDA) and providing patient care to those affected by the clinical conditions of lymphedema and hydrocoele. There is a need to collect and collate more national and published data to understand the risk factors influencing transmission so that control, elimination and surveillance can be targeted. This research project aimed to address some gaps in knowledge and conducted four specific activities including i) a scoping review of research on human prevalence and mosquito vectors in PNG and ii) a field survey to determine *W. bancrofti* antigenemia prevalence and related demographic and environmental risk factors iii) a micro-mapping microfilaria (Mf) survey and iv) entomological survey in an endemic area in Usino Bundi district of Madang Province. The review highlighted human prevalence as high as 48.8% and the significant impact of MDA in selected places. The entomological review found 17 studies on LF, with *An. punctulatus*, *An farauti* and *An. koliensis* identified as main vectors, and impacted by MDA and vector control for malaria, but most entomology was done in one region. The Ag prevalence survey conducted in 398 households across 4 villages found one village at significantly higher risk with 28.9% prevalence (Korona) with most clinical cases, while 2 villages had low prevalence ($\leq$5%) and one village none. Overall Ag prevalence significantly increased with individuals age and was higher in household made of semi-permanent/bush material. Most (>90%) of participants did not know about LF or the LF Programme. The Mf survey of 301 individuals in high risk Korona village found 29.9% Mf prevalence which varied significantly by hamlet; Korona (24.6%; 16.6/µl), Koinduna (31.9%; 21.6/µl) and Tongona (43.3%; 17.3/µl). There was an increasing trend with age, and males (34.5%) had a significantly higher prevalence than females (23.4%), and those participants who reported using mosquito coils/spray for personal protection had a significantly lower prevalence (12.2%) than those who didn't (33.2%). Interpolated maps were able to show a relationship between Mf positives per household and selected risk factors. The entomology field survey found two main LF vectors, *An. punctulatus* (infection rate 14.6%) and *An. farauti* (8.5%), in all hamlets of the high risk village, Korona. The series of studies in this thesis provides key information to the National LF Elimination Programme to help target public health campaigns, and may be used to plan future research studies.

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The CNTD admin staff for the many research assistance I received, Rinki Deb for the help with ethics write-up, Michelle Stanton with help on getting started with ArcGIS and SPSS data analysis, Hannah Betts with SPSS and QGIS, Hayley Mableson for showing me how to format my thesis, and to Louise Melia and Sara Donovan for LSTM travel and logistics, as well as all the others in the CNTD group, who I have not mentioned specifically, I am very much indebted to you for your assistance and patience with me.

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Many thanks to the Department of Health, PNG, my employer the duration of my PhD, very special thanks to my immediate boss at the Department, Mr Leo S. Makita, Program Officer for Malaria & Vector Borne Diseases, for trusting me and giving me unwavering support for the last 7 years, you are truly one of a kind. Not forgetting Dr Sibauk V. Bieb, Executive Manager for Public Health for encouragement to complete my PhD. My colleagues at MSCU, Pauline, Mary, Mama Tara and Rosh, thanks for keeping it real for me in the last 4 years of my PhD.

Lastly, my family; Mummy, Daddy, apas Sandra, Lynette & Lashma, ambo Doddy & family, my son Phillip and niece Samelyn and my beloved Winter, thank you for your prayers, love and support.

Dedication

I would like to dedicate this to the four most influential men in my life;

1) God the Father, the Alpha and the Omega,
2) My Daddy who has nave stopped believing and wanting the best for me,
3) My Son, Phillip Levi, you are my one true inspiration &
4) My Love, Winter for showing me that there is always Hope and for enduring the person I have become

Table of Contents

List of Tables

List of Figures

List of Key Abbreviations

Ag	Antigenaemia
CDC	Centers for Disease Control
CI	Confidence intervals
DALY	Disability Adjusted Life Years
DDT	Dichlorodiphenyltrichloroethane
DEC	Diethylcarbamazine citrate acid
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetraacetic acid
GAELF	Global Alliance to Eliminate Lymphatic Filariasis
GPELF	Global Program to Eliminate Lymphatic Filariasis
IEC	Information, Education, Communication
IRS	indoor residual spraying
ITN	Insecticide treated nets
ITS2	internal transcribed spacer region 2
LF	Lymphatic filariasis
L1	First stage larvae
L2	Second stage larvae
L3	Third stage larvae (infective larvae stage found in mosquitoes)
L4	Fourth stage larvae
LLIN	Long lasting insecticide nets
NGO	Non-Government Organization
NTD	Neglected tropical diseases
MDA	Mass drug administration
Mf	Microfilariae
MIBR	Monthly Infective Biting Rate
PacELF	Pacific Region for the Elimination of Lymphatic Filariasis
PCR	Polymerase chain reaction
PNG	Papua New Guinea
PNGIMR	Papua New Guinea Institute of Medical Research
RFLP	Restriction fragment length polymorphism
TAS	Transmission Assessment Survey
Wb	*Wuchereria bancrofti*
WHA	World Health Assembly
WHO	World Health Organization
WPRO	Western Pacific Regional Office

Chapter One

General Overview

1. Introduction

1.1 Lymphatic filariasis

Lymphatic Filariasis (LF), commonly known as elephantiasis, is a neglected tropical disease (NTD) caused by thread-like parasitic nematodes, and affects 73 countries in the tropical and sub-tropical region of the world (World Health Organization (WHO 2017).

LF is caused by 3 species of parasitic nematodes; the *Wuchereria bancrofti* parasite accounts for 90% of infection worldwide, while *Brugia malayi* and *Brugia timori* are more localised and mainly confined to South East Asia (WHO 2017). The parasitic worms are transmitted by several mosquito species which are found in the *Anopheles*, *Culex*, *Mansonia* and *Aedes* genera (WHO 2013; 2017)

An estimated total population of 1.3 billion are considered to be at risk with more than 120 million people infected and an estimated 40 million suffering from clinical manifestations including limb lymphoedema, genital disease (hydrocoele, chylocele) and acute attacks which are painful and often accompanied with fever. These clinical conditions can be incapacitating and disfiguring for life, making LF one of the leading causes of disability worldwide (WHO 2017; Ramaiah and Ottesen, 2014).

1.2 Global Programme to Eliminate Lymphatic Filariasis

The importance of LF was highlighted a wide range of experience and expertise by the International Task Force for Disease Eradication (CDC 1993), which identified LF as one of several diseases that could be eliminated as a public health problem (WHO 2010). The prime reasons being the main causative agent for LF, *Wuchereria bancrofti* is exclusive to humans as host and the availability of safe and affordable

drug regimens including different combinations of ivermectin, Diethylcarbamazine citrate acid (DEC), albendazole, which have shown evidence to reduce microfilaraemia to very low levels that can interrupt transmission.

In 1997, the 50th World Health Assembly, adopted Resolution WHA50.29, which made a commitment to eliminate LF as a public health problem (WER, 2012). In support of this resolution, the WHO formed the Global Programme to Eliminate LF (GPELF) in 2000, urging all LF endemic Member States to work towards targeting LF elimination by 2020. Since then, escalating pressure and work has been applied to endemic countries to control and lower the spread of LF (WHO 2017).

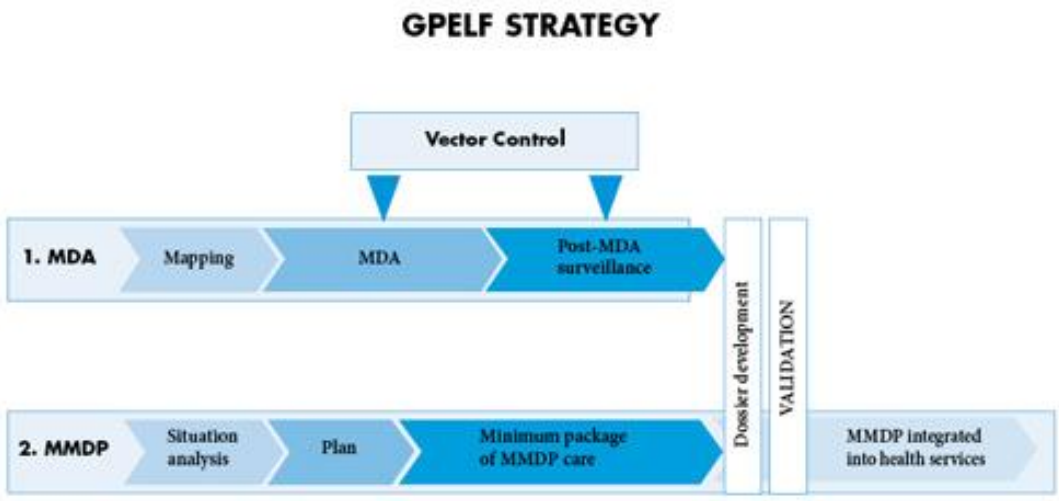
The GPELF based its elimination strategy on two main components;

(1) to stop the spread of infection (interrupting transmission) – through a strategy of mass drug administration (MDA) to at risk populations for at least 5 year

(2) to alleviate the suffering of affected populations (controlling morbidity) – through a strategy of morbidity management and disability prevention (MMDP).

Figure 1.1 highlights the GPELF strategy and the steps within each component that each endemic country programme needs to follow in order to reach elimination.

Figure 1.1 The Global Programme to Eliminate LF Strategy



Source. WHO 2017

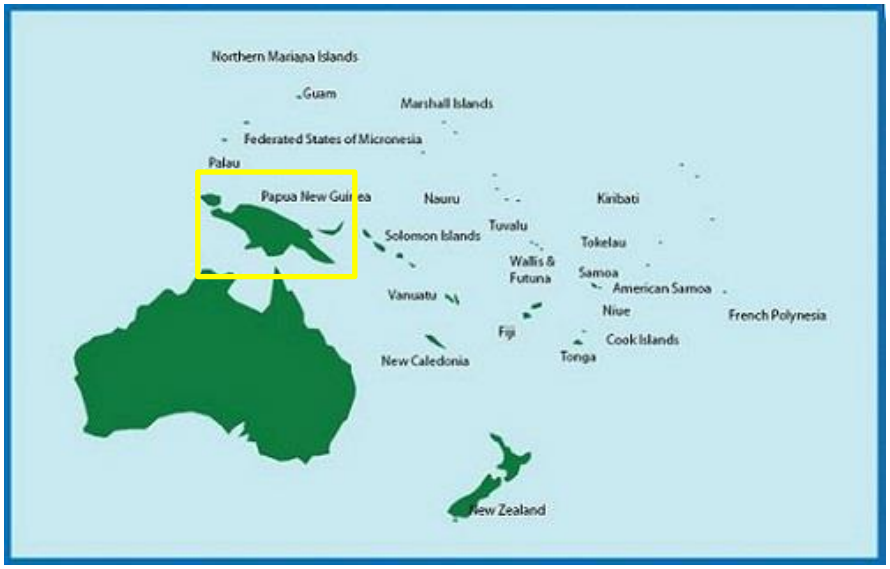
1.3 Lymphatic filariasis in the Western Pacific

The Western Pacific Region is divided into two sub-regions, the Mekong-plus and the Pacific groups, the latter is known as the Pacific Programme for the Elimination of LF (PacELF), which formed in 1999 and made up of 22 island countries as shown in Figure 1.2 (excluding Australia), and has the largest estimated burden in Papua New Guinea (PNG) (highlighted in yellow square).

An estimated 40 million people are at risk of the LF in the Western Pacific Region, which accounts for approximately 3% of the global burden (WHO 2017). The main parasites responsible for the disease in the regions are *W. bancrofti* and *B. malayi* which are transmitted by three mosquito genera, including *Anopheles, Culex* and *Aedes* (WHO 2010). The recommended strategy for the PacELF region was the combination of DEC and albendazole once per year for five years (WHO 2017).

Since the inception of GPELF, the majority of countries in the Western Pacific Region have made good progress in the implementation of MDA and interruption of transmission, with many in the surveillance post-MDA phase. In 2016, WHO were able to announce that 4 countries had successfully eliminated F as a public health problem including Cambodia, Cook Islands, Niue and Vanuatu (WHO 2017).

Figure 1.2 Countries included in the Pacific Programme for the Elimination of LF



Source: http://www.wpro.who.int/southpacific/pacelf/countries/en/

1.4. Lymphatic filariasis in Papua New Guinea

Papua New Guinea has a population of 7.2 million people (PNG National Census 2012), with an estimated 5.6 million at risk of LF, which makes up 90% of the population in the Pacific sub-region requiring MDA (WHO WER 2015). PNG is reported to have one of the highest endemicity levels in the world with community prevalence rates ranging from 10-98% (Graves et al. 2013). LF is caused by *W. bancrofti* and considered endemic throughout the country. The main mosquito vectors include the *Anopheles punctulatus* group, which are similar to those that transmit malaria.

The PNG LF Elimination Programme receives some funding and substantial technical support from WHO Western Pacific Region Office and the PacELF organization. Since the inception of the PNG LF Elimination Programme in 2000, the program has developed a National LF Strategic Plan (2001-2020) to assist with the MDA of at least 85% of the country's population living in endemic districts with plans for the home-care of people leaving with clinical manifestations. However, the plan is quite optimistic, as well as expensive with many regions difficult to access due to rugged terrain, scattered rural populations, poor infrastructure, lack of human resources as well as a lack of effective social mobilisation and MDA compliance among community members.

The PNG National Department of Health is having difficulties in sustaining and generating funds to support the plan, hence the MDA can only cover a few provinces at a time, and the LF Programme is well behind targets and yet to scale up MDA in many regions. With external assistance and support from integrated programs e.g. malaria control program issuing insecticide treated nets (ITNs), several provinces (with an average population size of 120,000 people) have been treated once annually since 2008 without follow-up MDAs. This still poses the threat of re-infection and continuing infection and transmission in the country.

At the start of this research project, there was no comprehensive information collated from studies on LF human infection and the vectors that transmit *W. bancrofti*, which made it very difficult to understand the epidemiology of the disease across the country, and thus implement the best control strategies. Further, to the best of my knowledge, no specific risk factor study on the environment, household infrastructure, what the community understands about LF, preventative measures and the National LF Elimination Programme had been conducted.

1.5 Rational for study, overall aim and specific objectives

Given the status of the PNG LF Elimination Programme, and to better understand risk factors and to help scale up intervention strategies to high risk communities, this thesis aimed to contribute to research in PNG on LF by collating related human prevalence and entomological information, and undertaking specific field studies to examine the prevalence of infection, burden of disease, entomology and potential risk factors in an endemic area of the country, with the aim to improve the control, elimination and surveillance.

Aim and specific objectives

The overall aim of this study is to investigate risk factors associated with LF infection, disease and transmission for better control, elimination and surveillance of LF in PNG.

The specific objectives to achieve this aim are as follow and are presented as individual chapters (3 to 6);

1. To review LF research in PNG, with specific focus on entomology in Madang Province
2. To map *W. bancrofti* antigen (Ag) prevalence and risk factors associated with LF in Madang Province
3. To micro-map and spatially analysis MF prevalence in a highly endemic village in Madang Province
4. To examine the distribution and incrimination of *Anopheles* species in LF transmission in a highly endemic village in Madang Province

1.6 Layout of the thesis

Chapter one provides a general overview of LF, GPELF, the Western Pacific Region and PNG as well as the rational for the topic, it also outlines the main aim and specific objectives, related chapters and provides a brief layout of the thesis.

Chapter two provides a review of the literature on LF in general (history, global burden, parasite periodicity and lifecycle, vectors, clinical manifestations, diagnosis and treatment), GPELF, and a brief background on LF in PNG.

Chapter three provides a scoping review of research conducted in PNG, and is the first research-related objective of the study. The reviews summarises studies on LF human prevalence and the impact of MDA, as well as entomological research, with specific focus on entomology in the proposed study area in Madang Province.

Chapter four is the second research objective and includes the mapping of *W. bancrofti* antigen prevalence and examination of associated risk factors in the Usino Bundi District of Madang Province. Four villages were surveyed using WHO guideline, a semi-structured household questionnaire also used to gather demographic, household and knowledge of disease information.

Chapter five is the third research objective ad includes micro-mapping and spatial analysis of microfilaria (Mf) prevalence in a highly endemic village to detect current infection rates. A more in depth fine scale spatial analysis of an endemic village using night time Mf survey and a further short questionnaire to try to elicit details and specific risk factors associated with within village patterns.

Chapter six is the fourth research objective and examines the distribution and incrimination of *Anopheles* species in LF transmission in a highly endemic village. It specifically identified vector species, their biting patterns, infection rates and relation with positive Mf households within the village.

The last and final chapter seven provides a summary of key findings from each of the research related chapters and lists a number of main recommendations for future programmatic activities and/or scientific research.

Chapter Two

Background and Literature Review

2.1 Lymphatic filariasis general

2.1.1 History of lymphatic filariasis

Human lymphatic filariasis or elephantiasis as it's commonly known is one of the oldest diseases in the world (WHO 2017), with some of its earliest records dating back 4000 years as portrayed in an Egyptian pharaoh sculpture obviously suffering from lymphoedema of the lower limbs (Dean, 2001). The disease is caused by tiny thread-like parasitic nematodes which are transmitted by several genera of mosquitoes.

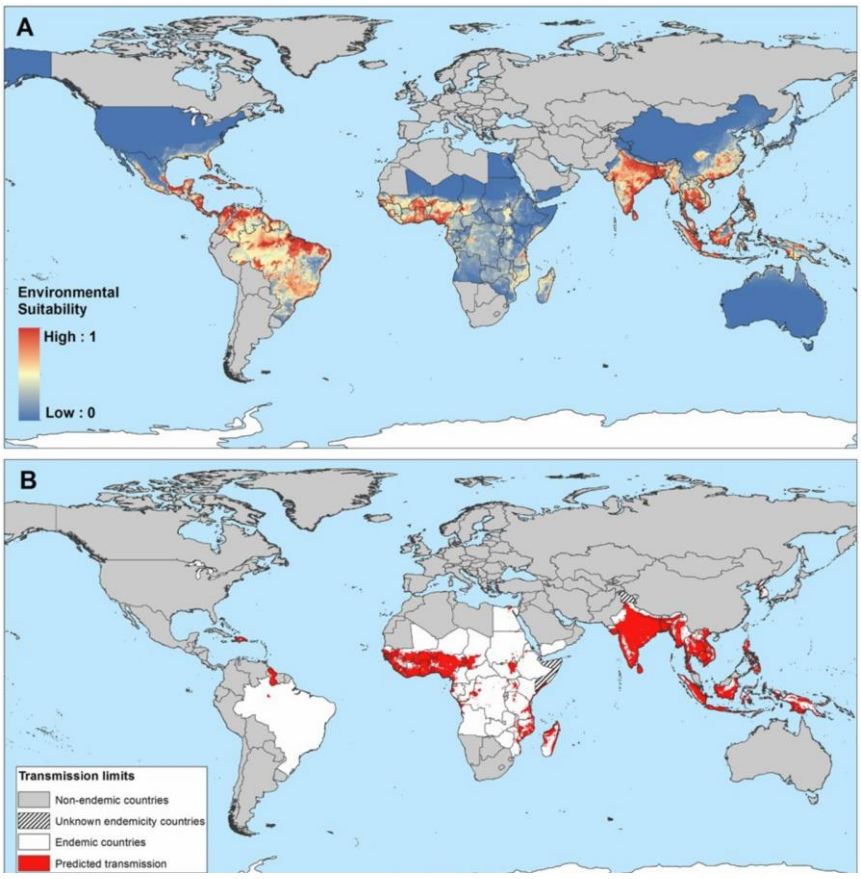
LF was known in ancient times where physicians and medical writers associated the morbidity with stagnant waters around areas where people living with lymphoedema were common. It was not until the 19th century when more concrete proof of association between the clinical manifestations and the parasitic worm was made. Between 1862-1888, scientists and physicians discovered the adult worm in chyluria and hydrocele fluid and blood but were not sure how the disease was transmitted until Patrick Manson in 1887 showed *W. bancrofti* larva development in *Culex quinquefasciatus* (Melrose, 2004).

There are three filarial species responsible for human lymphatic filariasis, *Wuchereria bancrofti* responsible for 90% of the global burden and found throughout the tropics and subtropical countries, while *Brugia malayi* is responsible for 9% of the global burden and mainly found in Asia and *Brugia timori* is confined to Indonesia (WHO 2010, 2017).

2.1.2 The global burden of lymphatic filariasis

LF is a disease associated with poverty and affects most vulnerable countries in the tropics and sub-tropical regions as seen in Figure 1A-B which highlights the environmental suitability and limits of transmission based on pre-intervention data (Cano et al. 2014). At the start of GPELF, the disease was considered to be endemic in 81 countries, however after surveys and further investigations to determine endemic foci in selected low endemic countries, only 73 countries were found to need MDA to control and eliminate LF (WHO, WER, 2009). The WHO estimates that over 1.3 billion people are at risk of infection with approximately 65% residing in the South-East Asia Region, 30% in the African Region and the remainder in the other regions (WHO, 2010). An estimated 120 million people are affected, with 83 million living with lymphatic disability, 15 million with lymphoedema (mainly lower limbs) and about 23 million men with hydrocele.

Figure 2.1: Global environmental suitability (A) and (B) limits of LF transmission



Source: Cano et al. 2014

At the GPELF halfway mark of 2010, in the South-East Asia region, 9 countries were endemic with an estimated 874 million people at risk of infection, which represented the highest number of people at risk of LF infection in a region. An estimated half of infected people (~60 million) and incapacitated physically by LF live in this region. All three human filarial parasites occur and although *C. quinquefasciatus* is the predominant mosquito vector, other genera like *Aedes, Anopheles* and *Mansonia* play a role in parasite transmission in some areas.

In 2010 in the African region, there were 39 endemic countries with an estimated 405 million people at risk of bancroftian filariasis infection. *W. bancroftian* is the only causative agent of LF in the region and is primarily transmitted by *Anopheles* although *Culex* is occasionally responsible for transmission in urban areas in East Africa (WHO 2010).

In the Eastern Mediterranean region, 3 countries are endemic with an estimated 12 million people at risk of bancroftian filariasis infection making up 1% of global population at risk (WHO 2010).

In the Americas, 7 countries are endemic with an estimated 11 million people at risk of infection, also making up only 1% of global population at risk. *W. bancrofti* is the main parasite in the region and *Culex quinquefasciatus* is the main vector of transmission (WHO 2010).

In the Western Pacific region, 23 countries with an estimated 40 million people at risk of infection which accounts for about 3% of global population at risk. *W. bancrofti* and *B. malayi* are responsible for infection and mosquito species from *Anopheles*, *Aedes* and *Culex* are vectors (WHO 2010).

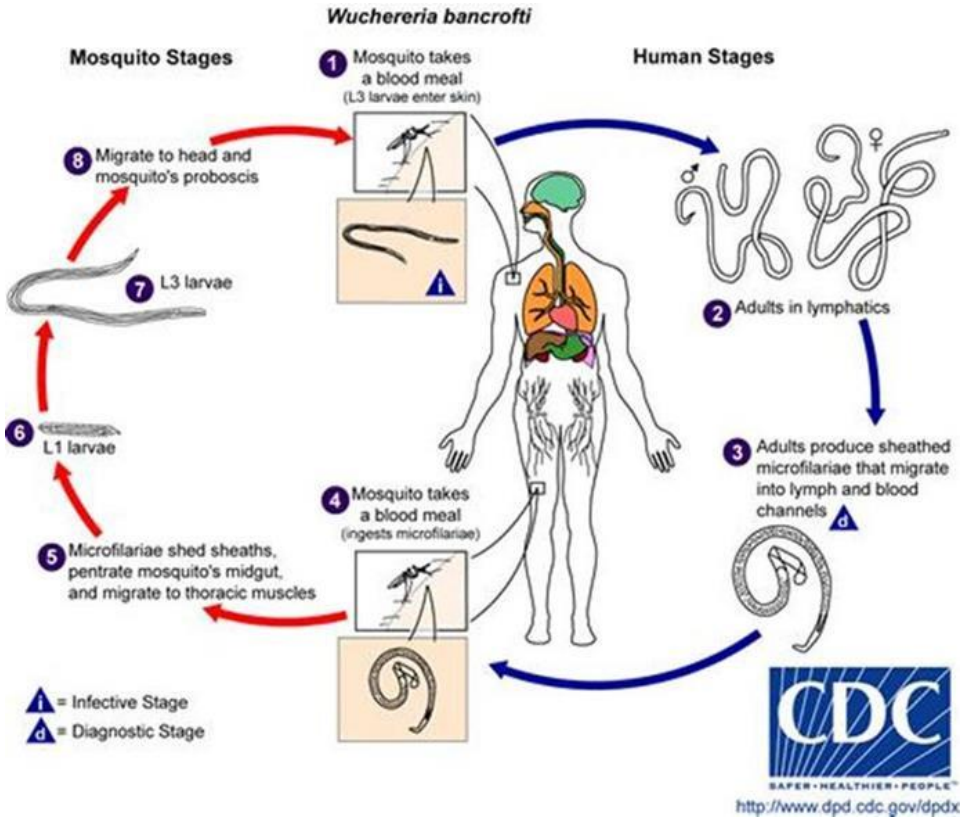
2.1.3 The parasite, its periodicity and life cycle

The three filarial worms have similar life cycles, involving humans and several genera of mosquitoes, the life cycle of *W. bancrofti* is shown in Figure 2.2. The filarial parasitic worm needs an arthropod vector for maturity of their larvae and transmission from one vertebrate host to another (Schacher, 1973, Sasa, 1973). The Mf are the reservoir of filarial infection and transmission. They have developed several adaptations to ensure successful transmission from host to vector. An example of this is the periodicity by which Mf peak in the peripheral blood.

In most endemic areas, including PNG, the lymphatic filariae has a nocturnal periodicity; Mf is absent in blood circulation during the day but if infected appear in large numbers between 21.00 and 02.00, which also coincide with peak biting times of the majority of vectors. During the day, the Mf are in the microvasculature of tissues, especially in the lungs (Eberhard, Roberts et al., 1988). It appears that Mf is able to regulate its periodicity by physiological signals from the host such as oxygen tension in the blood and body temperature (Dean, 2001). There are clear benefits being available in high numbers during a time when the vectors are actively feeding. The periodic pattern of each parasite is important to understand in terms of diagnostics, so that the right tests and tools can be implemented at the right time.

The life cycle of the main parasite *W. bancrofti* is presented over the page and directly sourced from the Centers for Disease Control (CDC). It highlights the human and mosquito stages of the cycle, as well as the infective stage of the parasite and when a suitable diagnostic stage would be best to implement.

Figure 2.2. Life cycle of *Wuchereria bancrofti*



"During a blood meal, an infected mosquito introduces third-stage filarial larvae onto the skin of the human host, where they penetrate into the bite wound 1. They develop in adults that commonly reside in the lymphatics 2. The female worms measure 80 to 100 mm in length and 0.24 to 0.30 mm in diameter, while the males measure about 40 mm by .1 mm. Adults produce microfilariae measuring 244 to 296 µm by 7.5 to 10 µm, which are sheathed and have nocturnal periodicity, except the South Pacific microfilariae which have the absence of marked periodicity. The microfilariae migrate into lymph and blood channels moving actively through lymph and blood 3. A mosquito ingests the microfilariae during a blood meal 4. After ingestion, the microfilariae lose their sheaths and some of them work their way through the wall of the proventriculus and cardiac portion of the mosquito's midgut and reach the thoracic muscles 5. There the microfilariae develop into first-stage larvae 6 and subsequently into third-stage infective larvae 7. The third-stage infective larvae migrate through the hemocoel to the mosquito's prosbocis 8 and can infect another human when the mosquito takes a blood meal 1."

Source: Centers for Disease Control (CDC)

https://www.cdc.gov/parasites/lymphaticfilariasis/biology_w_bancrofti.html

2.1.4 The vectors

It was first demonstrated that some mosquito species were vectors of *W. bancrofti* in 1878 (Scott, 2000). The major mosquito species that transmit the lymphatic filariae varies with geographical, climatic and ecological factors. The principle mosquito vectors that transmit the parasites are found in 4 genera: *Anopheles, Aedes*, *Culex* and *Mansonia* (Sasa 1976, Scott, 2000). These mosquito species have been found to be selective in the species of LF they transmit. For example, *Anopheles* spp. can transmit *W. bancrofti*, *B. malayi*, and *B. timori* but *Culex* spp. transmits *W. bancrofti* only; and *Aedes* spp. and *Mansonia* spp. can transmit *W. bancrofti*, and *B. malayi* (Sasa, 1976; Scott, 2000).

There are also regional differences in vector distributions; South-East Asia – predominately *Culex pipiens* group, with some subgenus *Anopheles;* Africa – predominately *Anopheles gambiae* and *An. funestus* complexes, with selected *Culex* in the urban areas of the East Coast; Pacific – *Aedes*, *Anopheles punctulatus* group, and *Culex quinquefasciatus* and the American – *Culex quinquefasciatus.*

Sampling of adult mosquitoes

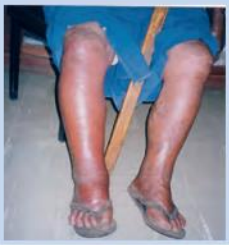
Several methods have been used for the collection of human-biting mosquitoes in endemic areas. Landing (human bait) or light trap catches are commonly used for exophagic and exophilic species including *Aedes, Mansonia* and some *Anopheles* mosquitoes. In areas where endophilic and endophagic mosquitoes like *Culex quinquefasciatus* predominate, specimens may be efficiently collected from the walls of huts and houses by resting or spray collections. Service (1993) has provided a comprehensive review of field sampling methods for adult and larval stages of mosquitoes.

2.1.5 Clinical manifestations and treatment

The main clinical manifestations of LF are not directly fatal but are estimated to account for 2.8 million Disability Adjusted Life Years (DALY's), which does not include mental illness of the patients or other family members who may be negatively affected as well e.g. caregivers (WHO, 2013; WER 2016, Litt et al. 2012)

The main clinical symptoms include i) acute dermatolymphangioadenitis (ADLA): acute inflammation of the skin, lymph vessels and lymph glands ii) lymphoedema (or elephantiasis for more severe forms) of limbs and breast and iii) hydrocoele: collection of excess fluid inside the scrotal sac that causes the scrotum to swell or enlarge. Pictures are shown in Table 2.1 with the recommended treatment for each condition. The stages of severity of leg lymphoedema are commonly classified according to clinical signs as shown and described in Figure 2.3 (Debrah et a. 2006)

Table 2.1. Clinical manifestations and treatment of LF

Clinical manifestation		Treatment	References
Acute dermatolymphangioadenitis		Antibiotics, antipyretics, analgesics	*3,4,16*
Lymphoedema and elephantiasis		Hygiene, antibacterial creams, antifungal creams	*3,4,16*
Hydrocoele		Surgery	*18* and general surgical manuals

Source: WHO 2013 (refs 3, 4,16, 18 = WHO 2003a 2003b, Dreyer 2002, WHO 2002 listed in references) http://apps.who.int/iris/bitstream/10665/85347/1/9789241505291_eng.pdf

Figure 2.3. Stage of the severity of lymphoedema

(A) Non-reversible swelling. (B) Shallow skin folds at the ankle. (C) Alteration of skin texture and formation of knobs (arrowheads). (D) Deep skin folds in addition. (E) Mossy lesion in addition to (D). (F) Patient unable to perform daily tasks (Debrah *et al.*, 2006).

For all conditions, it is important to implement simple basic hygiene measures to reduce the risk of secondary bacterial infections, this is important for ADLAs which may contribute to the progression of lymphoedema to more severe stages. For mild and moderate case of lymphoedema home-based care can greatly improve patients' conditions and include limb washing, wound care, foot care, suitable footwear and exercise. For hydrocoele, the main recommendation is surgery (Addis et al. 1999, WHO 2003a, WHO 2016, 2017).

The disease affects mainly adults however the infection is acquired in early childhood years in most endemic areas. LF is not only a disease of physiological dysfunction that results in widespread disability (Zeldenryk et al., 2011), but creates psychological problems like depression, anxiety and social isolation (Wynd et al., 2007, Litt et al., 2012, Ton et al., 2015). LF is the second most disabling disease worldwide, after depression (WHO, 2010). Although a large portion of the world's population live in endemic areas, it is likely that the majority of them know very little about how the disease affects them and the community and ways to manage it especially in relation to psycho-social issues and stigma (Perera et al., 2007).

2.1.6 Diagnosis of LF in humans

Several diagnostic methods are available to determine infection and disease status of an individual (WHO 2017). Parasitological and immunological diagnosis techniques have been developed over the years, especially the latter with recent advances in technology. Diagnosis can be done by:

1. Detection of microfilariae (Mf) by direct or concentrated techniques
2. Detection of filarial antigens and antibodies
3. Detection of parasite DNA by molecular methods
4. Detection of adult worms
5. Skin tests with filarial antigen

Direct techniques for detecting Mf in capillary blood is useful where only one species of filarial worm is present, as species identification can be difficult with this method. The capillary blood is extracted and placed on slide and observed under

microscope to determine presence of Mf. Thick blood film is another widely used method of direct Mf detection that can be easily employed in the field, a 60µl of blood obtained from the finger is used to make three strips of 20µl each, air dried and stained to detect presence of Mf. Although there are limitations and disadvantages of this method, it is still reliable when done correctly and is also a cheap and affordable diagnostic method.

Membrane filtration and Knott concentration methods are two most common concentrated techniques used to detect Mf. These methods are preferred if there is presumably low Mf density, hence membrane filtration technique is used more often to determine density rather than presence of Mf (WHO Bench Aids, 1997). The Knott concentration method is highly sensitive and is the most widely used method (Melrose, 2004). It can be easily done in the field and taken back to the lab to be check for Mf. Periodicity of the Mf is important to determine suitable time for sample collection, for example the *W. bancrofti* strain in PNG is nocturnal periodic, hence MF surveys are conducted at night between 10pm-2am for reliable results.

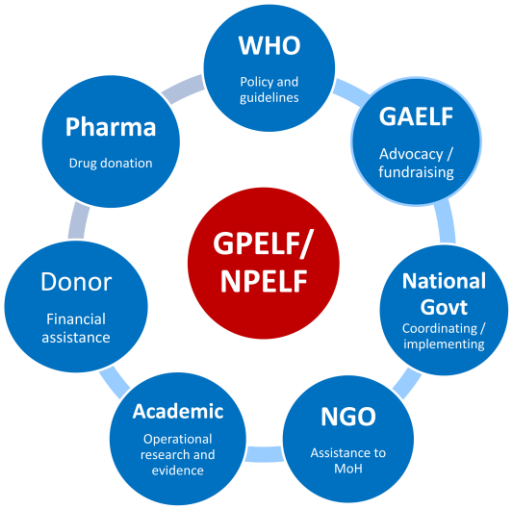
Filarial antigen diagnosis have been developed from raising antibodies against different filarial antigens (Weil et al, 1997). For instance the monoclonal antibody raised against *Onchocerca gibsoni* antigen (the Og4C3 assay) is highly specific for *W. bancrofti* and is able to pick amicrofilaraemic and microfilaraemic infections. Antigen diagnostic tests unlike Mf tests, blood samples can be taken at any time of the day.

The BinaxNOW® Filariasis immuno-chromatographic (ICT) cards (Alere Inc., Scarborough, ME) are rapid tests that are easily and conveniently used in the field with very minimum supervision. The test can be done any time of the day and results are obtained in 10 minutes. The ICT has been used over the past 15 years of the programme in establishing endemic foci in countries, and in surveillance activities (WHO 2011). The ICT has recently been replaced with Filariasis Test Strip (FTS), which is more stable to field conditions and is considered to have a longer shelf life (Yahathugoda et al. 2015).

2.2 The Global Programme to Eliminate Lymphatic Filariasis strategy and progress

The GPELP has progressed well since its inception and is one of the most successful public health programmes in history. It is a public-private partnership with many stakeholders involved including the WHO, member of the Global Alliance to Eliminate LF (GAELF), endemic country governments and LF programmes, NGOs, academia, donors, and pharmaceutical companies as shown in Figure 2.3. GAELF has been very fortunate to received support from two pharmaceutical companies, Merck & Co, GlaxoSmithKline (GSK) who have donated drugs towards this course for the last 14 years, and more recently from Eisai Co. (WHO, 2017; Ichimori et al., 2014).

Figure 2.4. Diagram to highlight the partnership in GPELF



Source. Ichimori et al., 2014

The GPELF strategy has two main pillars which include the following below, but also recommends working in partnership and integrating programmes where possible (Figure 2.4 and 2.5) (WHO 2018; Ichimori et al. 2014)

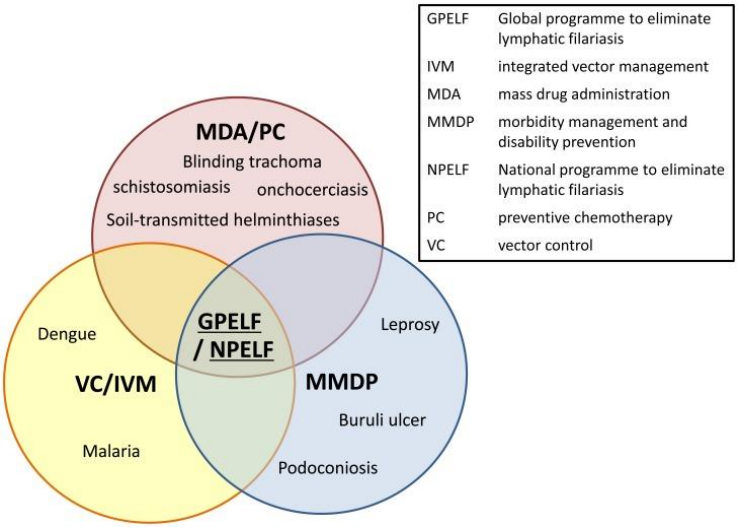
2.2.1 To stop the spread of infection (interrupting transmission), which targets endemic districts with MDA and aims to treat the entire population at risk. The following drug combinations are used and recommended to be implemented for at

least 5 years with a coverage of at least 65% of the total population at risk (WHO 2011; 2017; Ichimori et al. 2014)

- 6 mg/kg of body weight diethylcarbamazine citrate (DEC) + 400 mg albendazole; or
- 150 µg/kg of body weight ivermectin + 400 mg albendazole (in areas that are also endemic for onchocerciasis);
- 400 mg albendazole preferably twice per year (in areas that are also endemic for Loa loa).

Vector control is also considered to be an important supplementary intervention for interrupting transmission given that LF is a mosquito-borne disease (see Figure 1.1, 2.5) (WHO, 2010, 2017). The impact of transmission is most likely highest in places where there are *Anopheles* mosquitoes and vector control for malaria may impact. The role of vector control in GPELF and the importance of working with malaria control programmes has long been raised as an important issue (Manga 2002; Molyneux et al., 2004; Molyneux et al., 2009; Bockarie et al., 2009; Kelly-Hope et al., 2013), especially as there is evidence that intervention such as ITNs and indoor residual spraying (IRS) can help to reduce transmission (van den Berg et al., 2013; Webber, 1977, 1979). However, not many endemic countries have demonstrated LF-malaria links.

Figure 2.5 Opportunities for integrating activities into other disease programmes



Source: Ichimori et al., 2014

The second main pillar of the GPELF strategy is

2.2.2 To alleviate the suffering of affected populations (controlling morbidity), which aims to manage morbidity and prevent disability (MMDP) to help reduce the suffering that people affected may experience. A minimum package of care to manage lymphoedema and hydrocoele is recommended for all endemic countries if they want to show that they are making sure care is provided for people living with these conditions and prevent progression of these clinical manifestations of LF where possible (WHO, 2017). The new MMDP activities focus on i) planning, including patient estimates ii) capacity building to deliver services for MMDP and iii) documentation of services for MMDP.

Table 2.2 The WHO's recommended minimum package of care

MDA or individual treatment to destroy any remaining adult parasites and microfilaria
Surgery for hydrocoele (in W. bancrofti endemic areas)
Treatment for episodes of adenolymphangitis (ADL)
Management of lymphoedema to prevent both progression of disease and episodes of ADL.

2.2.3 Progress on GPELF in MDA and MMDP activities

Overall there has been significant progress in MDA and MMDP scale up of activities with an estimated total of 4.5 billion treatments taken by people living in endemic communities between 2000-2012, which has resulted in an estimated reduction in 9.6 million LF cases, 79 million Mf carriers, 19 million hydrocoele cases and at least 5 million lymphoedema cases (Ramaiah and Ottesen 2014). While this is positive progress, there are still many challenges ahead for some countries, especially in Africa and selected countries in other regions such as India, Indonesia in South-East Asia and PNG in the Western Pacific.

In 2015, the WHO reported that the African region had 35 endemic countries with approximately 395 million people requiring MDA, with a regional MDA coverage rate of 44.5% and 12 countries reporting MMD services available. This compared to the South-East Asia region (9 countries; 500 million people requiring MDA; 72.4% MDA coverage and 6 countries with MMDP services); and the Western Pacific region (22 countries; 25 million people requiring MDA; 46.4% MDA coverage and 9 countries with MMDP services) (Table 2.3 and 2.4) (WHO WER 2016)

Table 2.3 Summary of MDA implementation information by WHO region, 2015

Table 2 **Mass drug administration (MDA) implemented for lymphatic filariasis (LF) by WHO region, 2015**
Tableau 2 **Administration massive de médicaments (AMM) contre la filariose lymphatique par Région OMS, 2015**

WHO region – Région OMS	No. of LF endemic countries – Nbre de pays d'endémie	Estimated population requiring MDA – Estimations de la population nécessitant l'AMM	No. of countries initiated MDA – Nbre de pays ayant commencé l'AMM	No. of countries implemented MDA in 2015 – Nbre de pays ayant mis en œuvre l'AMM en 2015	No. of countries stopped MDA nationwide – Nbre de pays ayant arrêté l'AMM au niveau national	Total population estimated to be covered by MDA – Estimations de la population totale couverte par l'AMM	Total population reported to have ingested drugs as part of MDA – Population totale ayant ingéré les médicaments dans le cadre de l'AMM	Programme coverage (%) – Couverture par le programme (%)	Regional coverage (%) – Couverture régionale (%)
African – Afrique	35	395 318 019	26	20	2	212 700 125	176 520 205	82.99	44.49
Americas – Amériques	4	10 603 965	4	3	0	8 488 882	5 427 895	63.94	51.19
Eastern Mediterranean – Méditerranée orientale	3	13 393 890	3	0	2	0	0		
South-East Asia – Asie du Sud-Est	9	501 123 697	9	6	3	459 756 377	362 592 014	78.87	72.36
Western Pacific – Pacifique occidental	22	25 102 138	21	6	11	17 388 483	11 645 055	66.79	46.39
Total	**73**	**945 541 709**	**63**	**39**	**18**	**698 333 867**	**556 185 169**	**79.64**	**58.82**

Table 2.4 Summary of MMDP data reported to WHO in 2015

Table 3 **Summary of morbidity management and disability prevention data ever reported to WHO**
Tableau 3 **Synthèse des données relatives à la prise en charge de la morbidité et à la prévention des incapacités notifiées à l'OMS, toutes périodes confondues**

WHO region – Région	No. of LF endemic countries – Nbre de pays d'endémie de la FL	No. of countries reporting lymphedema patients – Nbre de pays notifiant des cas de lymphœdème	No. of lymphoedema patients reported – Nbre de cas de lymphœdème notifiés	No. of countries reporting hydrocoele patients – Nbre de pays notifiant des cas d'hydrocèle	No. of hydrocele patients reported – Nbre de cas d'hydrocèle notifiés	No. of countries reported MMDP services – Nbre de pays déclarant des services de prise en charge de la morbidité et prévention des incapacités	No. of countries monitoring MMDP by implementation unit* – Nbre de pays surveillant les services de prise en charge de la morbidité et prévention des incapacités au niveau des unités de mise en œuvre*
African – Afrique	35	11	45 463	11	72 548	12	2
Americas – Amériques	4	4	8 482	3	3 181	3	–
Eastern Mediterranean – Méditerranée orientale	3	2	1 306	1	18	3	2
South-East Asia – Asie du Sud-Est	9	8	954 203	7	433 674	6	8
Western Pacific – Pacifique occidental	22	16	4 572	16	840	9	6
Total	**73**	**41**	**1 014 026**	**38**	**510 261**	**35**	**18**

* Considered if reported data indicates number of implementation units with known cases or where service was provided. – Pris en compte si les données notifiées indiquent le nombre d'unités de mise en œuvre où des cas ont été identifiés ou des services ont été dispensés.

2.3 Lymphatic filariais in Papua New Guinea

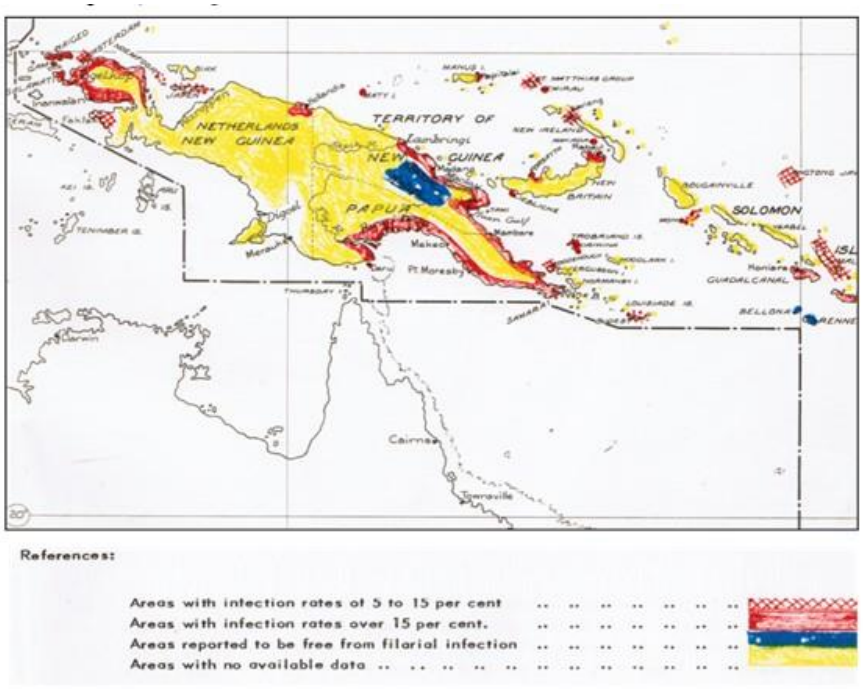
2.3.1 Historical distribution

LF is a major infectious disease and public health problem in PNG. Evidence of the disease was first recorded in the 17th and 18th centuries when Europeans made contact with island communities (Laurence 1989). A review of the global dispersal of Bancroftian filariasis, Laurence (1989) placed the early infection of humans by *W. bancrofti* somewhere in South-east Asia about 3000 years ago. The sea-faring Malay speaking people moved eastwards into the Pacific carrying the filarial parasite with them and New Guinea island was the first in the pacific region to come in contact with the parasite. The parasite, which was originally transmitted by *Anopheles and Aedes* mosquitoes in South-east Asia was easily adapted to transmission by the common human-biting *Anopheles punctulatus* group of mosquitoes. It was reported that the only parasite in PNG was *W. bancrofti* with no reported *Brugia malayi* or *Brugia timori* in PNG or the western half of the island of New Guinea governed by Indonesia. (Bockarie *et al,* 2000; Bryan *et al.,* 1986; Melrose *et al.* 2000).

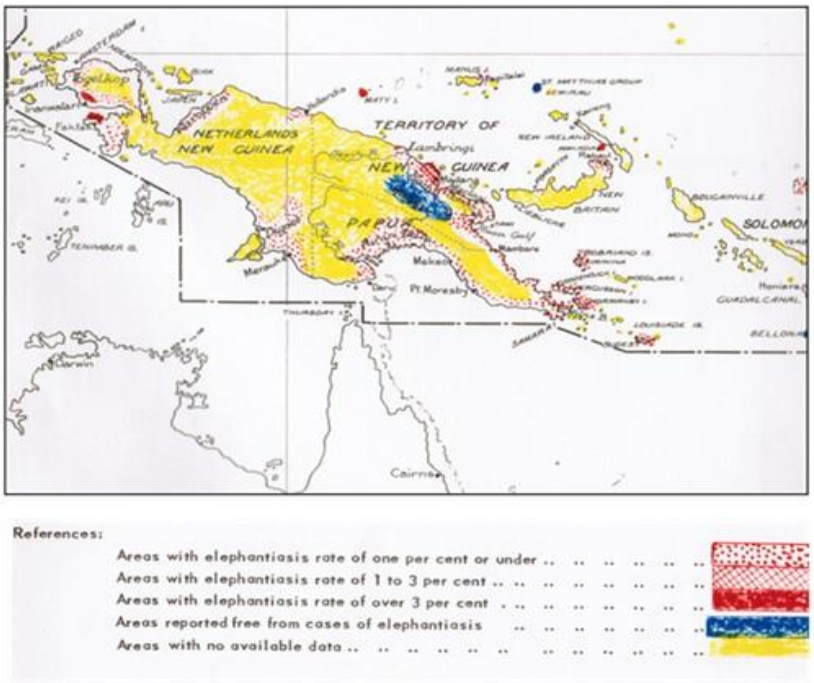
A historical review by Iyengar (1954) highlights the distribution and epidemiology of filariasis, MF and elephantiasis prevalence maps are shown in Figure 2. 6A, B and highlight the MF infections in Western, New Ireland and New Britain Provinces and with MF prevalence high rates in Morobe Province and rates up to 55% reported in Milne Bay Province. A further review on *W. bancrofti* infection and disease in PNG by Alexander (2000), highlighted studies in the East Sepik Province yielded community-based prospective data on filarial infection and disease (Alexander *et al*., 1999; Bockarie *et al*., 1998; Kazura *et al*., 1997; King *et al*., 2001; Tish *et al*.; 2001). Chronic disease and acute disease were considered high with all combinations of the three main clinical manifestations evident but there was not sufficient data to understand patterns. A very high incidence of acute disease was observed in the Dreikikir area of the East Sepik Province where 0.31 episodes per person-year was experienced in the leg alone (Alexander *et al*., 1999). Incidence generally increased with age, except in the breast, where episodes were

Figure 2.6 Historical maps of LF microfilaria and elephantiasis in PNG

A. Distribution and prevlence of microfilaria



B. Distribution of lymphoedema elephantiasis



Source. Iyengar, 1954

concentrated in the reproductive age range. Males had slightly higher incidence than females in the leg and arm. Chronic disease was strongly associated with acute disease incidence in all locations. Microfilaremia had a statistically significant association with acute disease in the leg, arm, and breast, but not the scrotum.

2.3.2 PNG vectors

Vector composition, vector ecology and transmission dynamics of filariasis in the Drekikier area are well documented (Bockarie, Kazura et al. 1996). To date, entomological studies carried out in the Drekikier area and some other areas in PNG have shown that the main vectors of *W. bancrofti* are the members of the *Anopheles punctulatus* complex, which the three main vectors are *A. punctulatus, A. koliensis* and *A. farauti* (Bockarie *et al,* 1996, Bryan, 1986).

Anopheles punctulatus prefers breeding in sun-lit water, road ruts and drains. *Anopheles koliensis* favours subcoastal areas and generally breeds in temporary pools, in grasslands and in pools around the edges of jungles. *Anophele farauti* occurs mainly in the coastal areas and can breed in fresh or brackish water and permanent swamps or temporary *pools. Anopheles koliensis and An. punctulatus* are be equally capable of transmitting W. *bancrofti* (Bockarie *et al,* 1996).

Vectors infections rates tend to be higher in PNG compared to those found in *Anopheles* mosquitoes in other regions of the world. Village-specific infection rates reported for biting catches of *An. punctulatus* sl in the Dreikikir area of the East Sepik Province ranged from 2% to 11.7% and infective rates from 0.4% to 3.5%. The 34 infective larvae of *W bqncrofti* observed in one indoor resting mosquito in Yauatong in the East Sepik Province (Bockarie *et al.,* 1996) is the highest so far recorded for *Anopheles* mosquitoes.

Bockarie *et al.* (Bockarie, Alexander et al. 1998), working in an area of intense perennial transmission of *W. bancrofti* by *An. punctulatus* in the Drekikier area attributed failure to detect infective mosquitoes for many months, following mass

treatment to the phenomenon of facilitation. In facilitation relationship, the lower point below which transmission will be interrupted can be achieved either by reducing density of parasites through mass treatment or density of mosquitoes by vector control.

This concept may play a role in lowering transmission in especially treated endemic areas (Bockarie, Ibam et al. 2000). In other endemic countries, Culex species are the principle vectors of *W. bancrofti* (Burkot, Taleo et al. 2002; Boyed, Waller et al. 2004). Although *Culex quinquefasciatus* and *C. annulorostris* are predominant species in the Drekikier area, they do not play a major role in the transmission of filariasis in the area (Bockarie, Tisch et al. 2002).

2.3.3 Historical background on MDA and vector control interventions in PNG

Studies of the efficacy of anti-filarial drugs have been conducted in PNG since the early 1980's (Kazura, Greenberg et al. 1993). The main findings from this work, conducted in collaboration with the WHO, established that single dose DEC (6 mg per kg body weight), ivermectin (400 μg per kg body weight), or a combination of the two drugs reduced Mf intensity by 50-90% for one year and that the efficacy of these regimens was similar to that of previously recommended 10 to 14 day courses of anti-filarial drugs. These studies have been important in the development of the formal declaration by the WHA that bancroftian filariasis be considered a target of elimination as a public health problem and ultimately eliminated, i.e., sustained interruption of transmission, by the year 2020.

Following these initial findings, a prospective study of 2500 persons living in a rural area of East Sepik Province showed that a single dose of DEC or DEC plus ivermectin reduced MF rate in people by 57.5 and 30.6%, respectively, and the annual transmission potential by 75.7-79.4% and 75.6-98.9%, respectively, after one year. The combination of the two drugs was more effective than DEC alone (Bockarie, Alexander et al., 1998). More recently, annual MDA continued for four years was reported to nearly eliminate Mf infections with no new infections in children reported and an overall decrease in disease by 25%, and the reverse of pre-existing

disease of the legs and male genitalia by 69 and 87%, respectively (Bockarie, Tisch et al., 2002). These findings support the notion that annual single dose mass treatment will be valuable in the control of lymphatic filariasis even in high endemicity areas such as PNG.

Several other studies have addressed issues regarding the impact of bednets not treated with insecticides on the prevalence of *W. bancrofti* infection, one such study was carried out on Bagabag island in Madang Province where both malaria and filariasis were endemic (Bockarie, Tavul et al. 2002). Bednet usage among residents was 60.6%, and the mean age of users (25.6 years) was similar to non-users (22.5 years). The overall *W. bancrofti* MF and Ag rates on the island were 28.5% and 53.1%, respectively. Bednet users had lower prevalence of *W. bancrofti* microfilaraemia , antigenaemia and hydroceles than non-users. An integrated community-based invertension involving mass drug administration and insecticide-treated bednets in the Mount Bosavi region of the Southern Highlands reduced rates of microfilaraemia in one village from 92% to 6% (Prybylski, Alto et al., 1994). Integrated control efforts involving mass treatment and vector control have also reduced microfilaria-positive rates in Ok Tedi area (Schuurkamp, Kereu et al. 1994) (Schuurkamp et al., 1994), Lihir island and Misima island (Selve, Bwadua et al. 2000).

Chapter Three

Review on lymphatic filariasis research in Papua New Guinea, with specific focus on entomology and Madang Province

3.1 Introduction

The type of research conducted on LF transmission in PNG is important to understand as it helps to understand the epidemiology of disease and what interventions may work for elimination purposes. There are several historical research articles and reports which show that LF is widely endemic, and more recently human prevalence mapping by the LF programme or part of research studies have help to determine different levels of risk across the country. However, it is important to collate all this information into one resource for the LF programme to assess all information and make key decisions. It may also help to guide where to conduct research studies.

Some studies have also assessed MDA intervention on transmission and this may provide some insight into how successful elimination may be. MDA studies on LF prevalence have been conducted in Southern Highlands, Western, East Sepik, Madang and New Ireland – understanding impact across different areas of the country with different prevalence levels is important. It may highlight areas that may need more help.

Despite some programmatic achievements, generally the challenges of delivering MDA and monitoring transmission in PNG have been big and resources quite limited due to other national priorities and few international stakeholders investing in the LF programme. It is important to consider other interventions that may impact on transmission such as vector control including ITNs or IRS. This is important as in PNG, LF is transmitted by *Anopheles* mosquitoes similar to those of malaria, and any intervention scaled up for malaria may also help the LF programme. Recently in

PNG (in 2012) there has been a large-scale distribution of LLINs through The Global Fund to Fight AIDS, Tuberculosis and Malaria. To understand the potential of vector control for malaria on LF it is important to understand the vectors driving transmission and their distributions across the country.

A scoping review of the research conducted to date on LF vectors therefore may help highlight the main vectors and also show the areas in need of further investigation. It may also help to determine if vector control is likely to help impact on LF transmission and consequently help the programme. This is important when the programme is slow to expand MDA activities.

3.1.1 Aim

The aim of this chapter was to provide a broad prospective on the research conducted on LF in PNG to-date, to better understand the epidemiology, help to identify gaps in knowledge and identify a research study area and direction that may help the LF Programme eliminate the disease.

Specifically, the work included

i) summarizing human prevalence distribution and data showing the impact of MDA
ii) collating and summarizing LF-specific entomological studies, highlighting publication profile, study features, field and lab procedures, species characteristics and methods/impact of vector control interventions
iii) describing the broad distribution and characteristics of the main *Anopheles* spp. associated with LF transmission in PNG and within Madang Province in relation to a proposed study site in Usino Bundi for field work

3.2. Methods

3.2.1 Human prevalence distribution and impact of mass drug administration

This section of work relates to work conducted as part of a collaborative study that I was involved with on human prevalence and the impact of MDA, and which I contributed to as a PhD student and the National LF Programme Coordinator for PNG at the time. My work involved organizing and conducting field sero-prevalence surveys with provincial teams, and compiling related programmatic data for the database and related publication by Graves et al. 2013.

To better understand the LF distribution in PNG, a systematic literature review on all LF human prevalence and MDA impact studies was conducted. The details are published in Graves et al., 2013 and are briefly described here.

A literature search using terms like Papua New Guinea, New Guinea, and Lymphatic Filariasis or *Wuchereria bancrofti* or *W. bancrofti* or filariasis or elephantiasis. Additional references were identified from published documents, WHO meeting reports, records, and MDA reports. Data on LF surveys in PNG since 1980 were extracted with locations, number of people tested, number of positives, sampling method used, age groups, and method of Mf examination were collected where available. Research studies testing interventions (mostly MDA, but some mosquito net projects) were extracted separately by village and time period where possible. Occurrence of any MDA or number of MDA rounds in locations of all surveys was noted, if given or available from other sources. The number of districts, how many publications, years when studies were conducted were also noted.

GPS coordinates of unknown locations were obtained from Geographic Names Server earthinfo.nga.mil/gns/html and/or Global Gazetteer www.fallingrain.com/world. Locations were assigned to districts using the 2010 district and provincial profiles from the National Research Institute of Papua New Guinea. For this chapter endemic districts were remapped using QGIS (http://www.qgis.org) based on three classification criteria.

Diagnostic tests included i) Blood slides/ thick films taken at night to maximize the number of Mf present in peripheral blood ii) ICT card test to detect antigen from the adult worm that is circulating in the peripheral blood iii) Og4C3 antigen ELISA a laboratory-based test also detecting antigen from the adult worm.

3.2.2. LF-specific entomological studies with specific cases studies

This section of the chapter focuses on a review of LF entomological studies, and was conducted to complement the work mentioned above in 3.2.1, to provide a source document for filariasis entomology in PNG and a background for my research, and also to highlight areas which would require further investigation in order to stimulate others to carry out further research on the entomological aspect of filariasis in PNG.

- Literature search terms included Papua New Guinea, PNG, New Guinea, lymphatic filariasis, LF vectors, mosquitoes, *Anopheles punctulatus* complex, *Anopheles punctulatus, Anopheles koliensis, Anopheles farauti* and combinations thereof. Malaria was also searched as similar vectors are responsible for transmission and may have related information on LF.
- Data were obtained from both published research (journal articles, thesis/dissertations as well as book chapters) and unpublished reports (district/provincial/national technical reports). These were collected from district/provincial health office, national health malaria surveillance and control office, PNG medical research institute, universities online, the internet or through PubMed search.
- Each document was assigned a reference number and its information recorded into a specific data collection form created on Excel spreadsheet. For each article the following information was summarised;
    - Publication profile: title of document, publication time (year/decade), type of document (research article, review article, report, thesis), journal/publisher and first author's affiliation (institution, organization)
    - Study features: locality (province, district, place if available), type of study (field, laboratory, or combination of both), time period of study if stated

- Field and laboratory procedures: method (landing catch, indoor/outdoor resting collections, light trap), stage of collection (adult, larval), vector identification method (morphological or molecular lab techniques), infection identification method (mosquito dissection, molecular lab tools),
- Species characteristics: main species incriminated, ecological habitats of adult and larvae, host seeking patterns/preference, flight range, spatial and temporal/seasonal abundance patterns, associated with LF mosquitoes in studies,
- Impact/methods of control/interventions: interventions associated with a reduction in abundance and/or infection and infective rates (MDA, ITNs, IRS)

Only articles containing information related to LF were included in the final database and descriptive analysis. The distribution of study locations were mapped to district level using QGIS software (http://www.qgis.org) to highlight where studies took place.

3.2.3. Broad distributions of *Anopheles* vectors incriminated for LF in PNG and specifically in Madang Province study site

This section of the chapter focuses on presenting a broad overview of the distribution of the main *Anopheles* vectors incriminated or confirmed from papers reviewed in the LF entomological review section 3.2.2. A number of historical reviews on *Anopheles* in PNG have already been published and the distribution of the different species and their ecological habitats summarised.

To highlight the distributions of the main *Anopheles* species in Madang Province, maps were created from the data points in relation and digitised to the province's elevation. A close up map of the different vectors species around the proposed study site in Usino Bundi was created to better assess the expected vectors and potential for LF transmission in the study area.

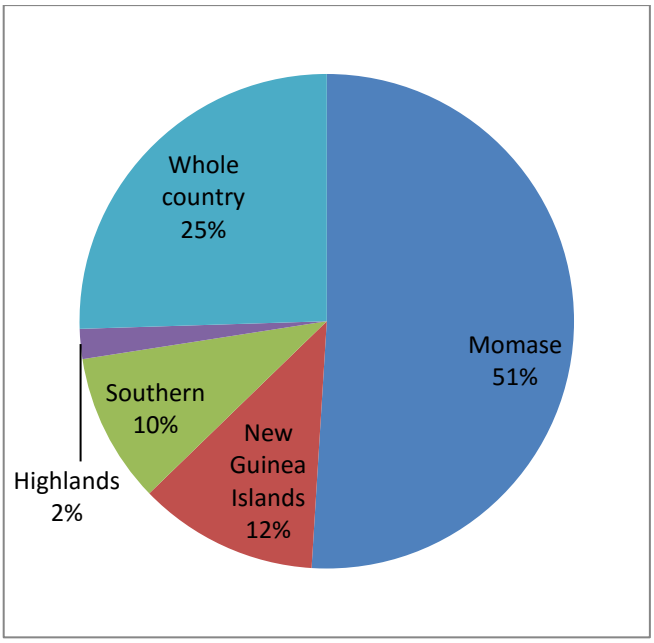
3.3. Results part 1

3.3.1. Human prevalence distribution and impact of mass drug administration

There were 312 LF prevalence survey sites between 1980-2011 using 3 main methods to determine LF prevalence, these are the Mf, ICT and OgC4 diagnostic methods. There were 155 Mf surveys conducted, testing between 6-1666 individuals (mean 211)/site, 149 ICT surveys testing 1-3799 individuals (mean 290)/site and 79 OgC4 surveys testing between 9-1322 (mean 209)/site. Some surveys conducted per site, included 2 or all of the diagnostic methods hence the total number of diagnostic methods is more than the actual number of surveyed sites.

By the initial GPELF endemicity criteria, from a total of 89 districts in PNG, 60 districts were found to be endemic, mostly lowland, coastal and island districts with an 'at risk' population of 4.81 million (70.4% of total population) whilst 0.73 million people (10.7%) live in nine unknown, yet to be surveyed districts.

Figure 3.1. Regional summary of human prevalence distribution publications in PNG between 1980 and 2011



3.3.2 Prevalence and GPELF criteria / classification

When all the surveys were combined, the estimated prevalence by diagnostic methods MF, ICT and Og4C3 were 27.5%, 12.9%, 48.8% respectively. These estimates are deemed biased due to different sampling sizes used and most LF research activities targeted high LF endemic areas. Alternatively, crude average estimates of each district were calculated for MF-18.5%, ICT-10.1% and Og4C3-45.4%. Although, these estimates don't address bias of surveys conducted in known LF endemic areas, they may be more appropriate representative of the general prevalence of LF in PNG.

The surveys were observed at three equal time points to see the changes over time, from 1983–1992 (10 years), 1993–2002 (10 years) and 2003–2011 (9 years). A decrease in MF and Og4C3 was observed over the 3 time periods while no big changes in ICT was seen in the latter 2 time periods. These are shown in Table 3.1 which was taken from Graves et al. (2013)

Table 3.1. Table showing the time periods and prevalence; the three GPELF endemicity criteria

Table 2 Summary of survey results by three time periods

	Mf			ICT			Og4C3		
	No sites	No persons	% pos	No sites	No persons	% pos	No sites	No persons	% pos
1983–1992	50	6539	30.4	0	0		2	976	64.7
1993–2002	76	19540	30.1	35	8502	13.4	47	9755	56.9
2003–2011	29	6608	7.8	115	34762	12.8	27	5169	28.9

Table 3 Classification of districts by three criteria schemes

	1 GPELF criteria	2 GPELF modified criteria	3 Alternative criteria
High endemic	60	36	34
Low endemic		25	15
Non endemic	20	20	31
Unknown	9	8	9
Total districts	89	89	89

Source: Graves et al., 2013

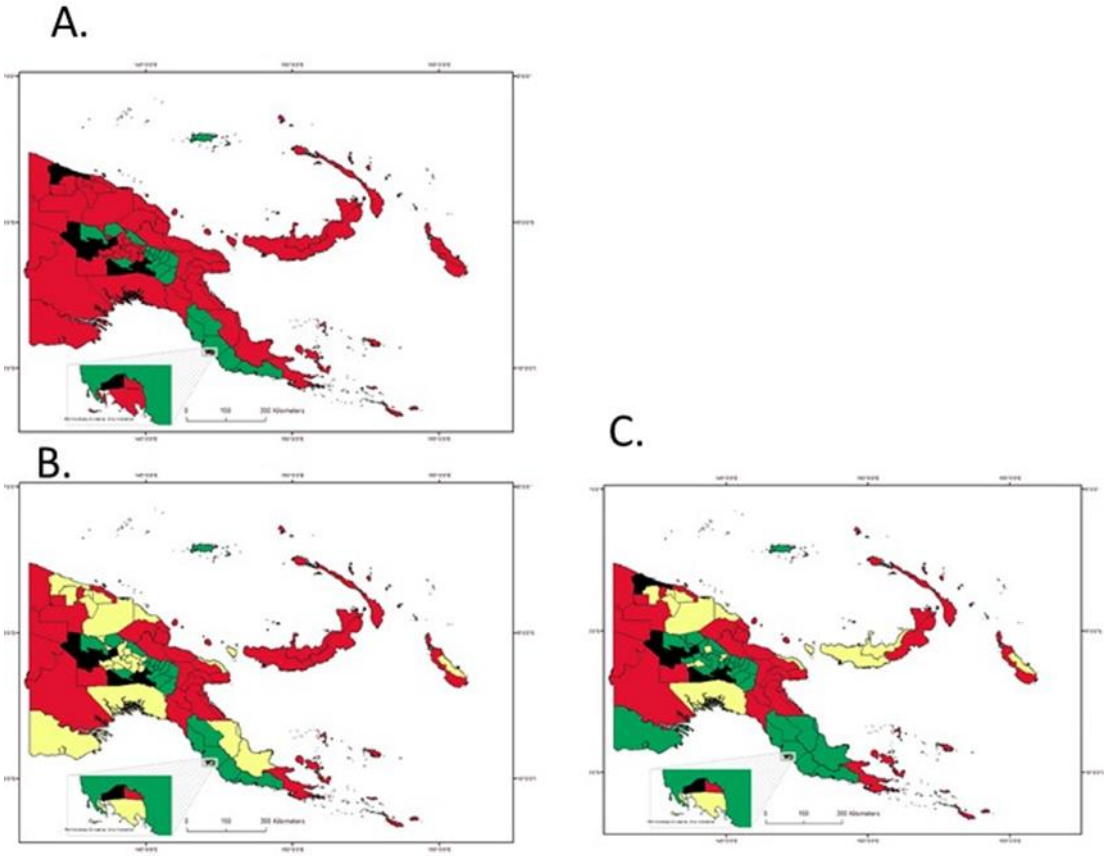
The first GPELF criteria for endemic and non-endemic districts was, any positive result (≥1 positive) by any of the three diagnostic methods in a district was classified as an endemic district, and negative results are non-endemic districts, shown in Figure 3.2_A. While this was previously used by the PNG LF Elimination Programme (PNGELF), Graves et al. (2013) highlighted another two modified GPELF criteria options, which reclassify the endemic districts into low and high endemic districts.

The two modified criteria have three categories of endemicity, the first modified criteria classified districts as follows; 0 positives = non-endemic, >0 - <5% = low endemic and ≥5% = high endemic (Figure 3.2_B) and if any unknown/untested district is surrounded with endemic districts, the lowest endemic category is assigned to that unknown.

The second modified criteria classified <1% = non-endemic, ≥1% - <5% = low endemic and ≥5 = high endemic (Figure 3.2_ C). The main difference is the first GPELF criteria had only 2 criteria while the two modified criteria had 3 categories, which further divided the endemic districts into low and high endemic districts.

The first GPELF criteria, identifies 60 endemic districts, while the second criteria identifies 36 of the 60 to be highly endemic districts (≥5% prevalence) while 25 are of low endemicity (>0 - <5% prevalence) and one of the unknown districts surrounded by 4 high endemic districts and 2 low endemic sharing boundaries with at least a low endemic district is classified as a low endemic district (Table 3.1 and Figure 3.2. A and B)

Figure 3.2. Districts by endemicity according to the 3 GPELF criteria



Maps showing classification of districts by endemicity, according to three criteria schemes. A. Map with districts classified using GPELF criteria scheme 1. Red: endemic, >0% pos; Green: non-endemic, 0% pos; Black: unknown; results from all types of test. B. Map with districts classified using modified GPELF criteria scheme 2. Red: High endemic, ≥5% pos; Yellow: Low endemic, >0% and <5% pos (or unknown but all adjacent districts >0%); Green: non-endemic, 0% pos; Black: unknown; results from all types of test. C. Map with districts classified using alternative criteria scheme 3. Red: high endemic; ≥5% pos; Yellow: low endemic, ≥1% and <5% pos; Green: non-endemic, <1% pos; Black: unknown; Mf results used if available, otherwise ICT.

Source: Graves et al., 2013

3.3.3 MDA impact

Most of the surveys took place before any MDAs were conducted, 2 research activities on annual MDA trials in Ambunti-Drekikir using DEC alone or DEC+ Ivermectin and Usino-Bundi using DEC+Albendazole had more than 3-4 MDAs carried out consecutively. Number of MDAs for each site was recorded if available. To see if MDA had an impact on prevalence, surveyed sites were categorized according to number of MDAs; 0, 1, 2, 3, 4 or > =5 MDAs prior to the survey. The

impact on prevalence of annual or twice a year MDA is shown graphically for Mf assays in Figure 3.3_A and for ICT and Og4C3 assays in Figure 3.3_B. Pre-MDA Mf rates were between 18.6% and 76.9%, after 3 and 4 rounds of MDA respectively, MF rates dropped down to 1.3% and 5.3% respectively. The impact on Mf prevalence was found to be very rapid and large (Figure 3.3_A) 5 rounds of MDA with DEC + Ivermectin, the MF rate was brought down to 5.3%, whereas the decline in antigen prevalence appeared to be slower as seen in Figure 3.3_B.

Figure 3.3. MDA impact on prevalence rates

A. **MDA impact on MF rates**

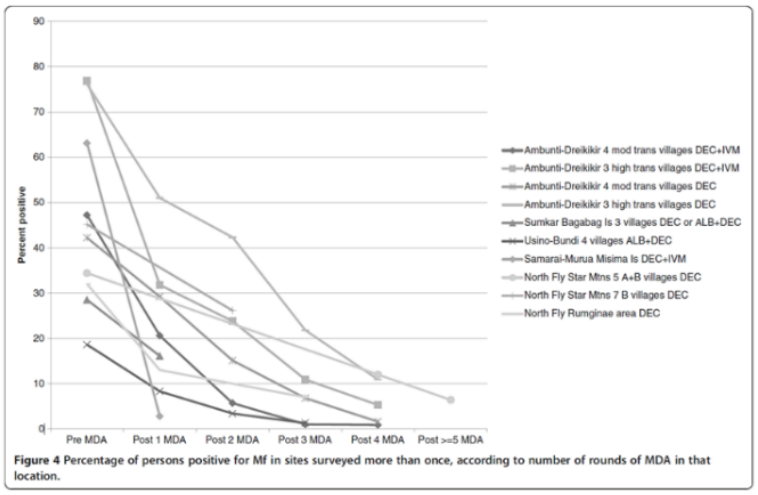


Figure 4 Percentage of persons positive for Mf in sites surveyed more than once, according to number of rounds of MDA in that location.

B. **MDA impact on Ag rates**

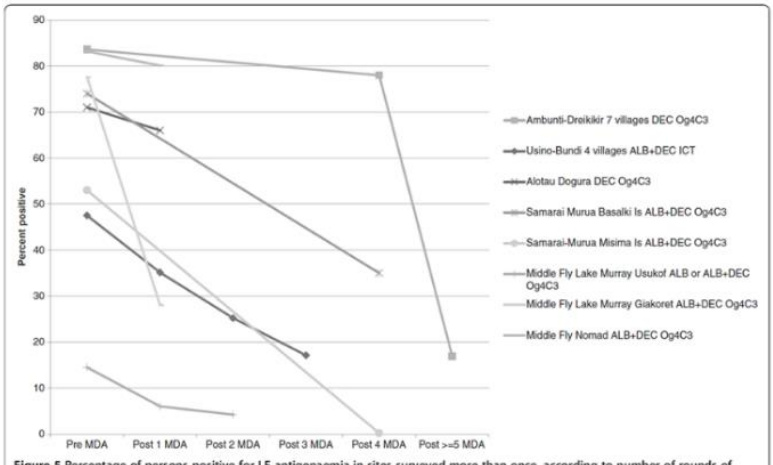


Figure 5 Percentage of persons positive for LF antigenaemia in sites surveyed more than once, according to number of rounds of MDA in that location.

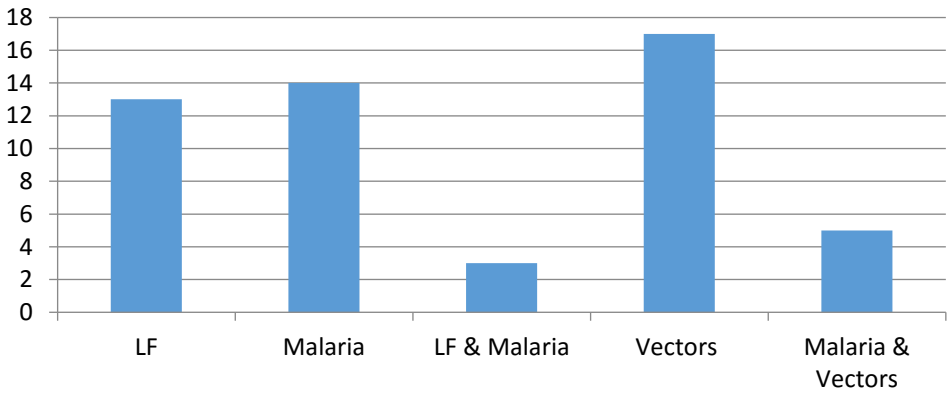
3.3. Results part 2

3.3.4 Review of LF entomological studies

Summary of literature search

The literature search including all terms produced 52 documents from 1934 to 2016, which included scientific research papers, reviews, local technical malaria reports and theses. In total, there were 15 documents on LF vector studies and 2 on LF / malaria vectors, and the remaining documents on *Anopheles* vectors and malaria as summarised in Figure 3.4 below. For the purpose of this review, only the 17 documents with information relating to the LF vectors in PNG were examined further and are listed in Table 3.2.

Figure 3.4. Overall summary of literature search



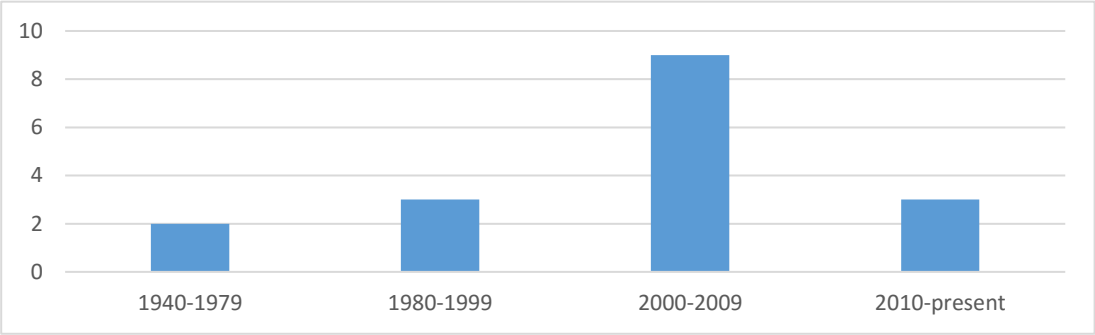
Publication profile of LF entomological studies

The LF entomology articles were published between 1946 and 2013, most of which were published in the last 20 years (n=14, 82%) and only 2 were published between 1946 and 1985. Prior to 2000, only 1 LF entomology article per year was published, the year 2000 saw the most LF entomological papers (n=4, 23.5%) published and most recently in 2013 (n=3, 17.6%) as well, shown in Figure 3.5. Table 3.2 summarises aspects of the entomological studies.

Table 3.2. Summary of LF entomological studies

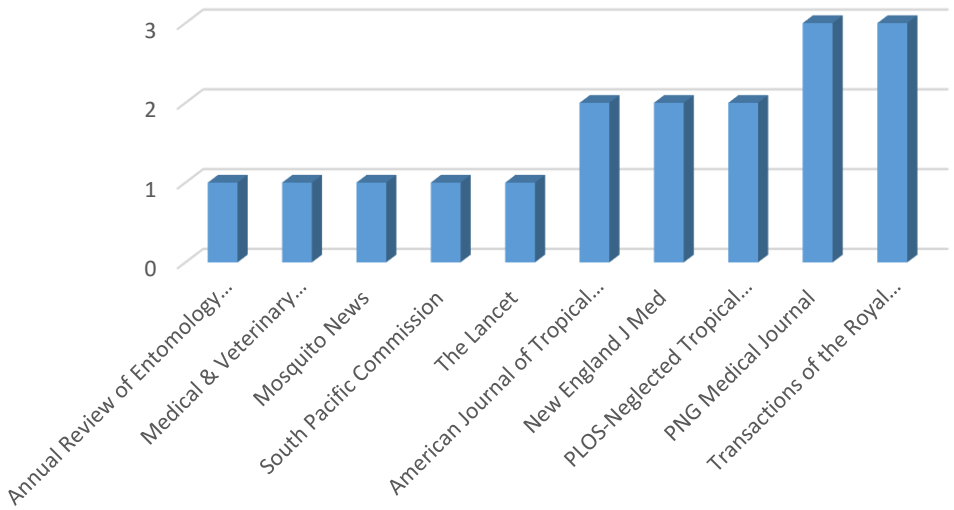
Ref	Reference title	Article type 1=Research 2=Report 3=Review 4=Book 5=thesis 6=other	First author's Institution	Journal / Publisher name	PNG Institution name	PNG Institution location	Publication year	Study type Field=1, Lab=2, Combined =3	Study Province (and District)
1	Studies on filariasis in Papua, New Guinea	1	US Navy	Mosquito News			1946	3	Milne Bay
2	Distribution of filariasis in the South Pacific Region	Technical Report	South Pacific Commission	South Pacific Commission			1954		
3	Vectors of Wuchereria bancrofti in Sepik Provinces of Papua	1	University of Sydney, Australia	Transactions of the Royal Society of Tropical Medicine and	PNGIMR	Madang Province	1986	3	East Sepik
4	W. Bancrofti Transmission in Papua New Guinea	1	PNGIMR, Madang, PNG	American Journal of Tropical Med & Hyg	PNGIMR	Madang Province	1996	3	East Sepik, Drekikir
5	Altitude and the risk of bites from mosquitoes infected with malaria and	1	Australia National University, Australia	Transactions of the Royal Society of Tropical Medicine and	PNGIMR	Madang Province	1997	3	Sandaun, Telefomin-august river
6	Randomised community-based trial of annual single-dose	1	PNGIMR, Madang, PNG	The Lancet	PNGIMR	Madang Province	1998	3	East Sepik, Drekikir
7	Towards eliminating lymphatic filariasis in Papua New Guinea: impacy of	1	PNGIMR, Madang, PNG	PNG Medical Journal	PNGIMR	Madang Province	2000		East Sepik-Drekikir
8	Control of lymphatic filariasis in a hunter-gatherer group in Madang Province	1	PNGIMR, Madang, PNG	PNG Medical Journal	PNGIMR	Madang Province	2000	3	Madang, Usino Bundi
9	The epidemiology and control of lymphatic filariasis in Lihir Island, New	1	James Cook University, Australia	PNG Medical Journal	PNGIMR	Madang Province	2000	3	New Ireland - Lihir
10	Mass treatment to eliminate filariasis in Papua New Guinea	1	PNGIMR, Madang, PNG	New England J Med	PNGIMR	Madang Province	2002	3	East Sepik, Drekikir
11	Impact of treated bednets on prevalence of Wuchereria	1	PNGIMR, Madang, PNG	Medical & Veterinary Entomology	PNGIMR	Madang Province	2002	3	Madang, Bagabag
12	A realtime PCR based assay for detection of Wuchereria bancrofti DNA in	1	Washington University, MO, USA	American Journal of Tropical Med & Hyg	PNGIMR	Madang Province	2006	2	Madang, Usino Bundi
13	The Impact of Repeated Rounds of Mass Drug Administration with	1	Washington University, MO, USA	PLOS-Neglected Tropical Diseases	PNGIMR	Madang Province	2008	3	Madang, Usino Bundi
14	A qPCR based multiplex assay for the detection of Wuchereria	1	Washington University, MO, USA	Transactions of the Royal Society of Tropical Medicine and	PNGIMR	Madang Province	2008	2	Madang-Usino Bundi
15	Role of vector control in the global program to eliminate lymphatic filariasis	3	Liverpool School of Tropical Medecine	Annual Review of Entomology (online journal)			2009	3	PNG
16	Insecticidal bednets and filariasis transmission in Papua New Guinea	1	PNGIMR, Madang, PNG	PNG Medical Journal	PNGIMR	Madang Province	2013	3	East Sepik, Drekikir
17	Mosquito-parasite interactions can shape filariasis transmission	1	University of Wisconsin, USA	PLOS-Neglected Tropical Diseases	PNGIMR	Madang Province	2013	3	Madang, East Sepik

Figure 3.5. LF Entomological literature timeline



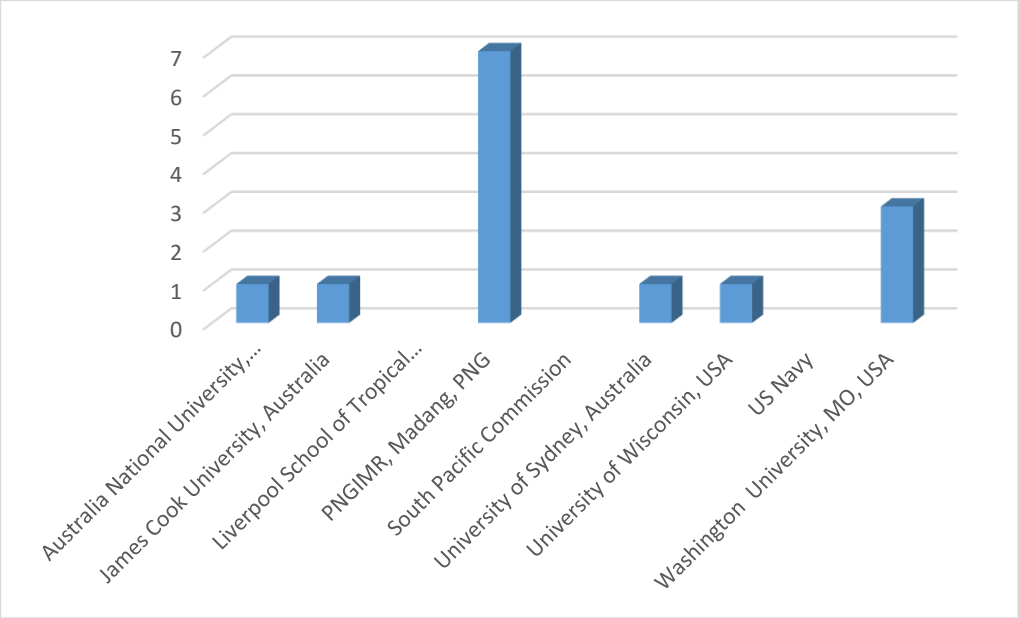
Most of the articles were research based (n=15, 88%) and were published in a variety of journals, with the highest number published in the Papua New Guinea Medical Journal and the Transactions of the Royal Society of Tropical Medicine and Hygiene (n=3, 17%) followed by PLOS-Neglected Tropical Diseases, American Journal of Tropical Med & Hyg and the New England Journal of Medicine (n=2, 11%) and the rest of the journals published an article each as shown in Figure 3.6. Apart from the two earliest publications, all publications after 1954 were conducted in collaboration with the PNGIMR, which is the National Medical Research Institute with its vector borne disease unit situated in Madang, the provincial capital of Madang Province.

Figure 3.6. Number of articles published per journal



Majority of first authors (n=7, 41.15) were senior research fellows with PNGIMR at the time of publication or were research fellows from University institutions from mainly the US and Australia institutes who collaborated with PNGIMR on the different entomological studies, this can be seen in Figure 3.7.

Figure 3.7. First author's institutions and affiliations



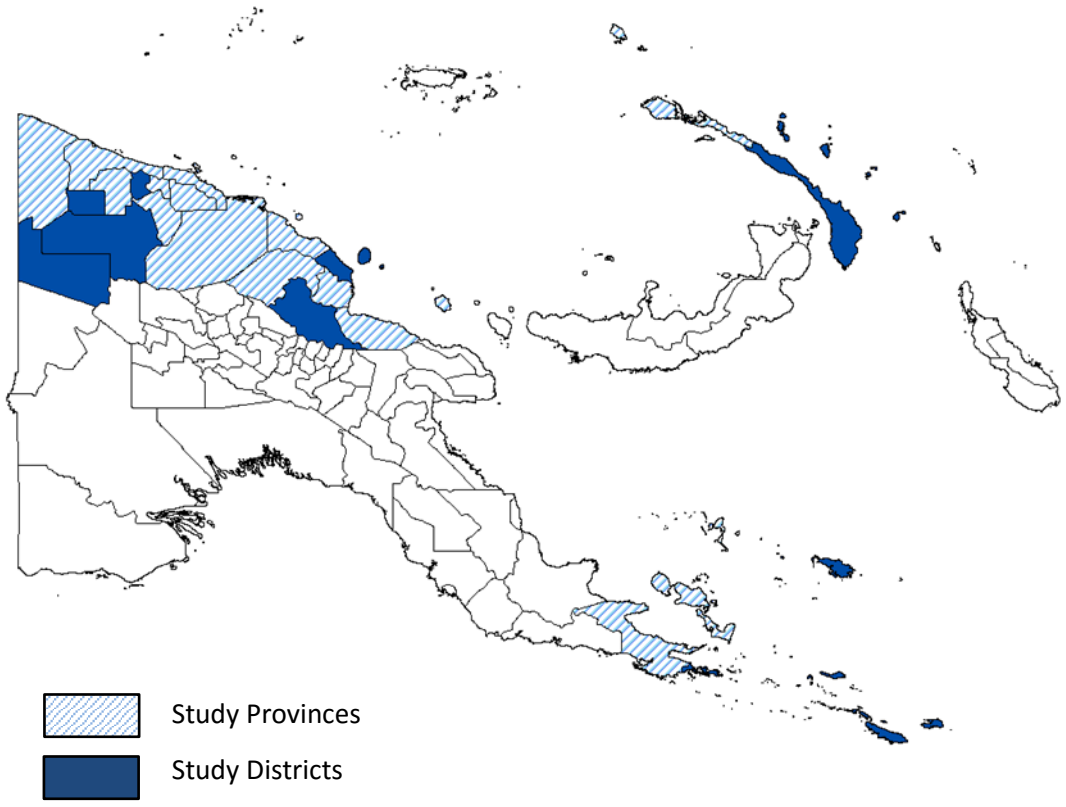
Study features

Most of the entomology research were locality specific and were conducted mainly in 2 provinces and in certain districts, East Sepik (Drekikir District) (n=5) and Madang (Usino Bundi district) (n=3) provinces as shown in Table 3.3. PNGIMR branches are located in these two provinces and most of the malaria, LF and other vector borne disease research are usually conducted in these provinces as evident by the data presented here. The research work in East Sepik were done and published between 1996 and 2000 while the research in Madang occurred from 2000 onwards. Both provinces are in the Momase region of PNG and contributed to this region having more LF entomology done compared to the other region as shown in Figure 3.8.

Table 3.3. Province and district where LF entomology research were conducted

Province, District (Locality)	No. of entomology publications
East Sepik	1
East Sepik, Drekikir	5
Madang and East Sepik	1
Madang, Sumkar (Bagabag)	1
Madang, Usino Bundi (Hagahai)	1
Madang, Usino Bundi (Naru)	3
Milne Bay (Sagarai)	1
New Ireland, Namatanai (Lihir)	1
Sandaun, Telefomin (August river)	1
Nation-wide	2

Figure 3.8. Map showing the provinces and districts where studies were conducted

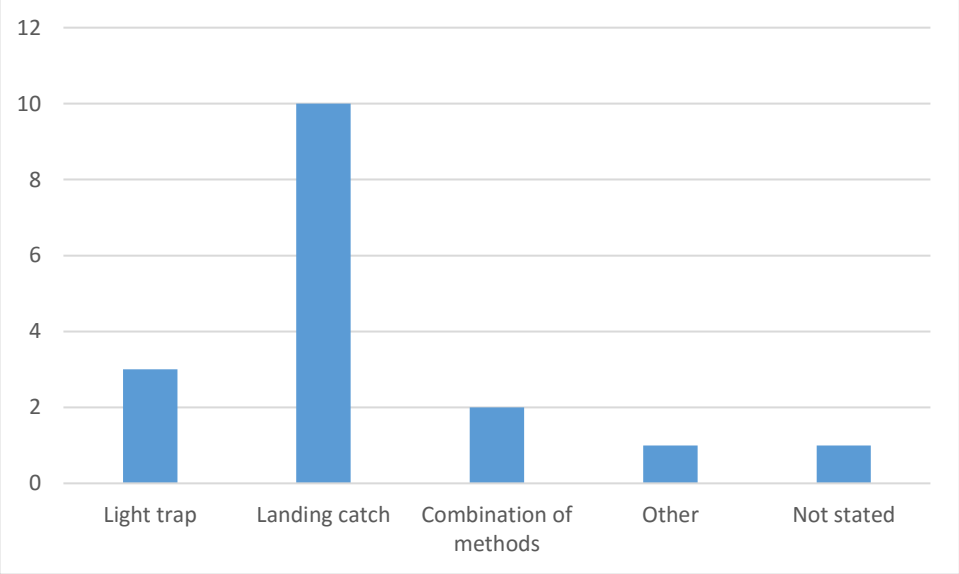


Majority of the articles were a combination of field and laboratory work (n=14, 93%) while only 2 articles were laboratory based researches and one was a technical report summarizing the Asia Pacific LF vectors. The laboratory based studies looked at testing new developed molecular techniques, the specificity of the conventional PCR and qPCR assays for detecting *W. bancrofti* DNA in vectors and both had the same first author.

Field and laboratory procedures

Field method (landing catch, indoor/outdoor resting collections, light trap), landing catch was the sampling technique mostly used (n=10, 58.8%) followed by light traps and a combination of these two methods. The review by Bockarie and others (2009) does not state what type of vector sampling methods were used and Erickson et al., 2013, used lab techniques to infect laboratory reared vectors to analyse vector-parasite interactions.

Figure 3.9. Mosquito sampling methods

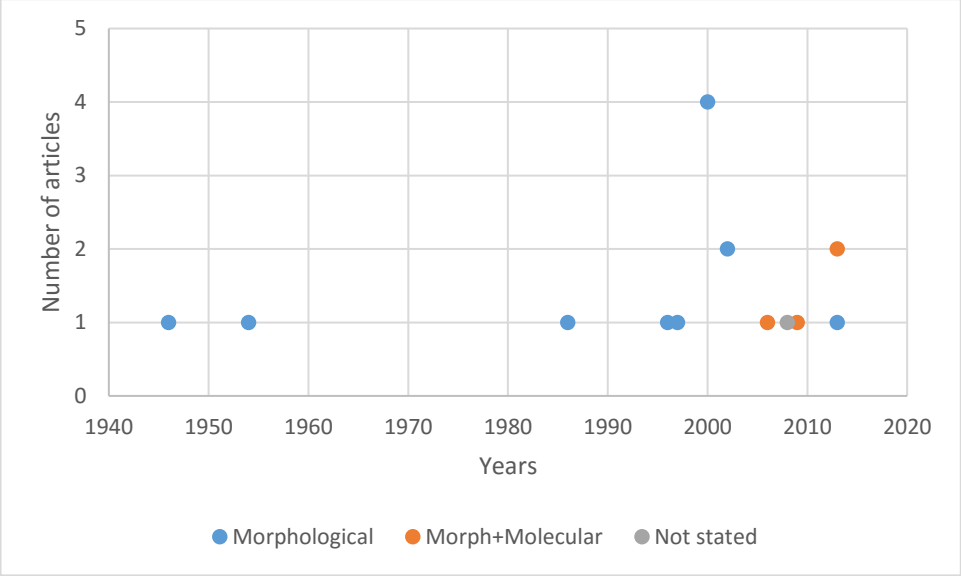


Adult vector samples made up 88% of collection while only one used laboratory reared larvae and one did not mention stage of vector. Species identification of vectors in the early years up to 2005 was principally done morphologically (Belkin, 1962), where the coloration of proboscis and sector spot on the wings were used to differentiate between the vector species of the *Anopheles punctulatus* complex.

Morphological identification was the main source of species identification before the development of molecular tools (PCR) in early 2000 which saw both the morphological and molecular techniques used together from 2005 onwards as seen in Figure 3.10_A. *W. bancrofti* infected mosquitoes in the early years were identified by individual mosquito dissection and observing the dissected specimens under a light microscope to detect the developing parasite larval stages in the mosquitoes. This was both labour intensive and require well trained technicians to identify the parasite stages in the mosquitoes. The microscopic detection of larval stages in the mosquito is still considered gold standard for infection in vectors since the conventional and taqman PCR are not able to detect the infective stage (third stage larvae – L3) of the parasite in the vector.

Figure 3.10. Summary of species identification and infection detection methods

A: Number of articles per year and vector species identification methods used



B: Number of articles per year and LF infection identification methods used

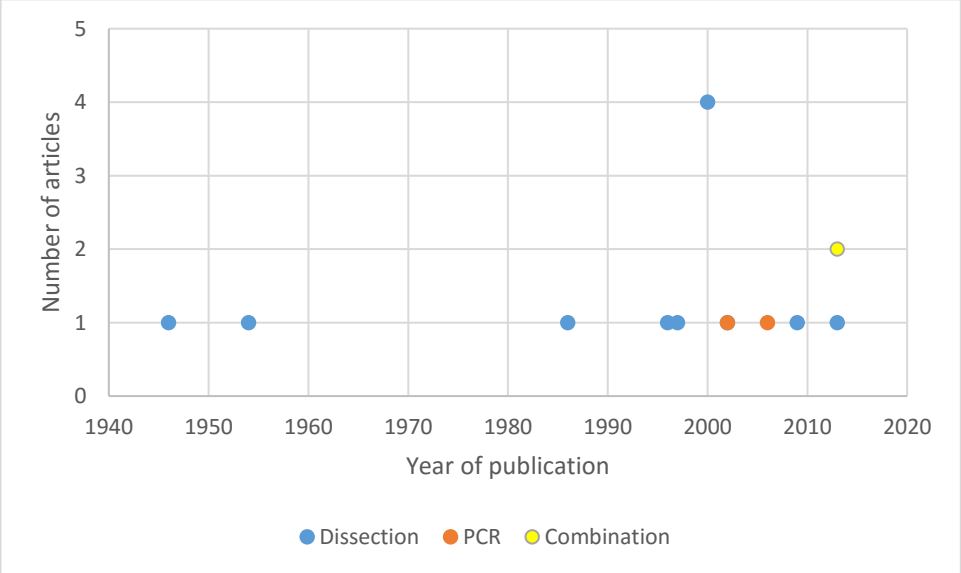
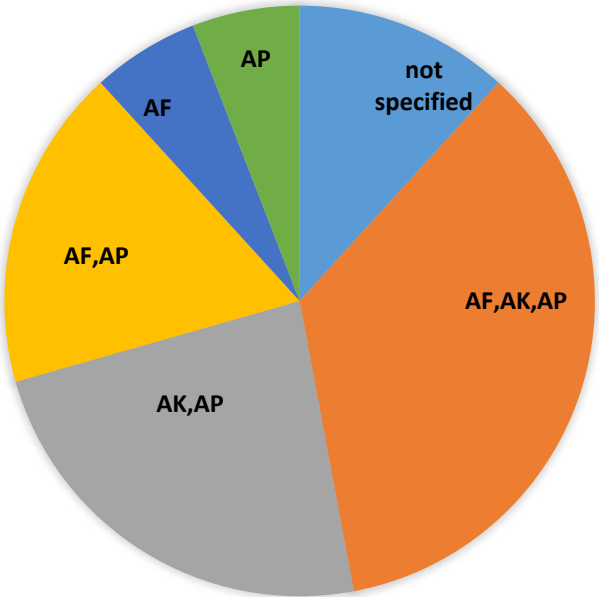


Figure 3.11. *W. bancrofti* main vectors as found in published papers



Species characteristics

Out of 17 articles, 6 of the papers state *An. farauti, An. koliensis* and *An. punctulatus* as the vector species, however in Reimer et al., 2013, where all 3 main vectors were present in Drekikir, an inland district of East Sepik province, *An. punctulatus* was the more dominant vector followed by *An. koliensis* while the smallest portion of *An. farauti* was not found to be infected nor infective. In Bockarie et al., 2002, research done in the same district, they only looked at the infection rate of *An. punctulatus* in that area, while Hii et al., 2000 found that on the island of Lihir, where all 3 vectors were found, *An. farauti* was the most dominant vector species present on the island. Iyengar (1954) in his technical report for SPC stated that *An. farauti* was a dominant vector in the New Guinea coastal provinces and islands. On Bagabag, an island in Madang province, Bockarie et al., 2000, found only *An. farauti* and *An. punctulatus* with *An. farauti* as the most dominant infective Anopheles while *An. koliensis* and *An. punctulatus* were stated as vectors in 4 of the articles and *An. farauti* and *An. punctulatus* identified as vectors in 3 articles.

In relation to ecological habitats of adult and larvae, host seeking patterns, flight range, there was no information specifically describing or examined in the publications reviewed on LF transmission.

With regard to spatial temporal patterns, Bockarie et al., (1998, 2000) examined abundance and infection rate monthly patterns over two years, which also included pre- and post- interventions. The highest rates of infection, both infected and infective stages in *An. punctulatus* were in August prior to MDA with DEC + ivermectin, after the intervention in September, the same months after 3 month lapse, saw a drastic drop in vector infection, with only the month of May showing infective mosquitoes.

Figure 3.12. Temporal patterns of LF infected mosquitoes over a two year period and in relation to the distribution of MDA

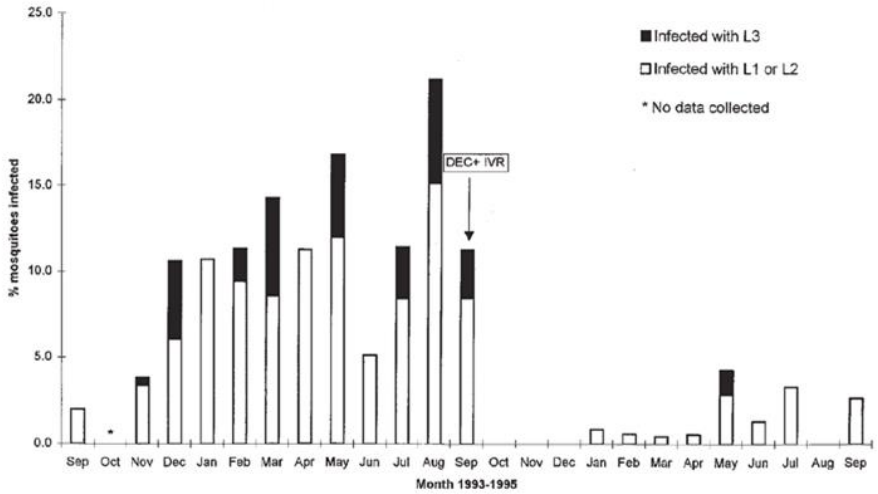


Figure 2. Effect of treatment-induced reduction in intensity of *Wuchereria bancrofti* microfilaraemia on uptake and development of microfilariae to infective larvae in wild-caught *Anopheles punctulatus* in Nanaha village.

Source: Bockarie et al., 2000

Methods of control

Over half the papers (n=10, 58%) did not examined or describe interventions / control measures, however 5 of the articles mentioned MDA trials in relation to the impact on LF transmission, 3 papers (17%) were on the MDA trials in the Drekikir district (n=3, 17%), testing DEC alone, DEC + Albendazole and DEC + Ivermectin which saw a combination of DEC and another drug more effective than DEC alone, although all treatment regimen had an effective impact on reducing LF infection in both humans and vector population. These studies in Drekikir also showed that 3- 4 rounds of MDA were most effective against low to moderate LF transmission villages compared to high transmission villages which may need 4-6 rounds of MDA.

Bed nets was considered the oldest method of control used against human-mosquito contact and was cited as a possible control measure for LF transmission where expatriates were sleeping under mosquito nets and were found to be free from LF whilst living among heavily infected individuals in Sagarai plantation, Milne Bay Province (Hopla, 1946).

Bockarie et al., in 2002 on Bagabag island in Madang suggests that untreated bed-nets prevents LF transmission while Reimer et al., 2013, showed a reduction from 1.8% infection rate in vectors prior to LLIN to 0.4% after LLIN distribution. Figure 3.13 is a figure from Reimer et al., which highlight the reduction in transmission with the distribution of bed-nets. There were no infective mosquitoes found after bed-net distribution and only two villages had infected mosquitoes with significantly low infection rates.

Figure 3.13. Temporal patterns of LF infected mosquitoes over a three year period and in relation to the distribution of bed-nets

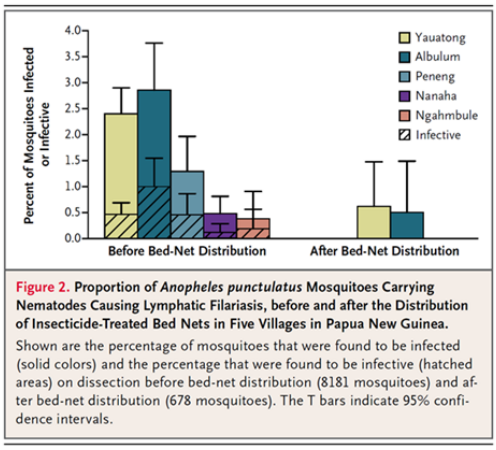


Figure 2. **Proportion of *Anopheles punctulatus* Mosquitoes Carrying Nematodes Causing Lymphatic Filariasis, before and after the Distribution of Insecticide-Treated Bed Nets in Five Villages in Papua New Guinea.**

Shown are the percentage of mosquitoes that were found to be infected (solid colors) and the percentage that were found to be infective (hatched areas) on dissection before bed-net distribution (8181 mosquitoes) and after bed-net distribution (678 mosquitoes). The T bars indicate 95% confidence intervals.

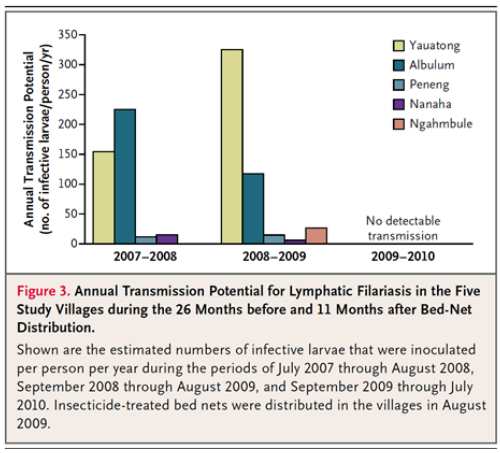


Figure 3. **Annual Transmission Potential for Lymphatic Filariasis in the Five Study Villages during the 26 Months before and 11 Months after Bed-Net Distribution.**

Shown are the estimated numbers of infective larvae that were inoculated per person per year during the periods of July 2007 through August 2008, September 2008 through August 2009, and September 2009 through July 2010. Insecticide-treated bed nets were distributed in the villages in August 2009.

Source: Reimer et al., 2013

Anopheles in PNG

The *Anopheles punctulatus* group of mosquitoes is the principle vectors of nocturnal periodic *W. bancrofti* parasite that occurs in PNG. This group of mosquitoes was first described by Donitz and others in the early 1900s (Donitz, 1901, Laveran, 1902 and Rozeboom and Knight, 1946). The *Anopheles punctulatus* complex was first suspected of filarial transmission in PNG when the disease was recognized as an endemic disease in the early 1900s (de Rook, 1938, Hopla, 1946). These suspicions were pursued in later years to establish 3 sibling species in the complex as principle vectors of *W. bancrofti*. Since then, a range of literature have concluded that certain species within the complex are responsible for the transmission of LF in PNG.

Mosquitoes of various genera are found in PNG, species from *Mansonia, Aedes, Culicine* and *Anopheles*. The *Culex quinquifasciatus* is a vector for *W. bancrofti* in many endemic areas of the world, but was found to be an inefficient vector in PNG while *An. punctulatus, An. koliensis* and *An. farauti* were found to be most common vectors in PNG (Bockarie et al., 1996). These three vectors are members of the *Anopheles punctulatus* group of mosquitoes and are also principle vectors of malaria parasites in PNG.

The three species are predominant throughout PNG depending on the geographical habitations, with *An. farauti* confined to coastal areas and can breed in fresh or brackish, permanent or temporary pools of water. While *An. koliensis* are mostly found in lowland inland areas and preferably breed in temporary pools, while *An. punctulatus* are dominant vectors in hilly areas and breed in sun-lit waters. The three species are known anthropophilic and anthropophagic vectors (Bockarie et al., 1996, Bryan, 1986).

Charlwood et al., (1986) have reviewed in detail the ecology and behavior of the *An. punctulatus* group of mosquitoes. Beebe & Cooper (2002) described the ecology in more detail and is seen in Table 3.4 summarizing the breeding site characteristics.

Table 3.4. Summary of ecological characteristics of main *Anopheles* vectors

Table 1
Geographic distribution summary for members of the *Anopheles punctulatus* group[a]

Species	(1) Northern Australia, monsoonal		(2) South-western PNG, monsoonal		(3) Southern PNG, southern plains, hot/wet		(4) Highland regions PNG (>1500 m), mild/wet	(5) Northern PNG Sepik/ Ramu plains, hot/ wet		(6) Guadalcanal Island, hot/wet	
	Coastal	Inland	Coastal	Inland	Coastal	Inland		Coastal	Inland	Coastal	Inland
A. farauti s.s.	+++		+++		+++	+	+	+++		+++	
A. farauti 7										+++	+++
A. farauti 2	++	++	++	+++	+++	+++	++	+	++	+++	+++
A. farauti 6							++				
A. farauti 5							+				
A. farauti 3	++	+++		++							
A. koliensis					++	++	+	+++	++	+ ?	+ ?
A. farauti 4								++	++		
A. punctulatus						+++	+	++	+++	+	++
A. sp. nr *punctulatus*						++			++		
A. clowi.									+		

[a] PNG, Papua New Guinea; +++, Extensive distribution; ++, limited distribution; +, sparse distribution; +?, doubtful distribution.

Source: Beebe and Cooper, 2002.

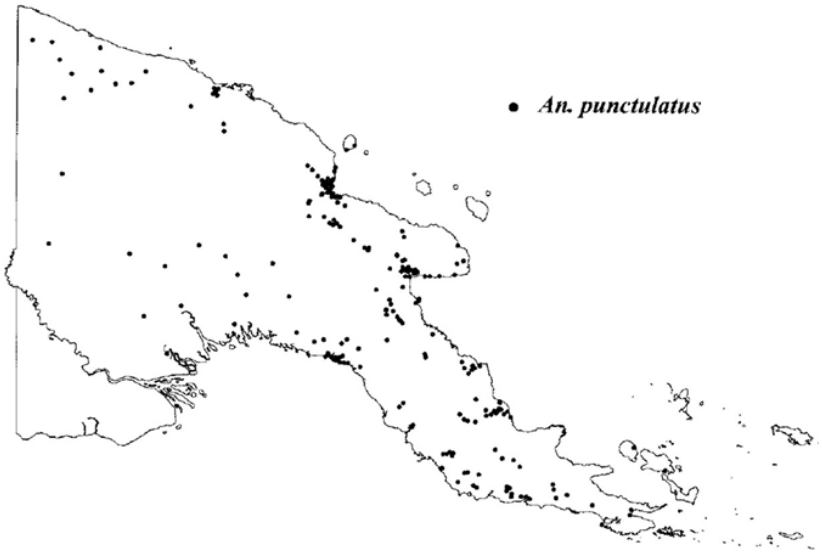
Photographs showing key differences in morphological characters of the vectors are shown in Figure 3.14. While Figure 3.15_A shows *An. punctulatus* as a sparsely distributed vector compared to *An. koliensis* distribution (Figure 3.15_B), which is more dominant in the upper North plains of the Sepik, Ramu and Markham plains and lower Southwest part (Gulf/Central) of mainland PNG. For *An. farauti*, Figure 3.16 A, shows *An. farauti s.s* to be a vector more popular along the coastlines, while *An. farauti 2* is a dominant vector in the inland lowland areas (Figure 3.16_B) while *An. farauti 4* is restricted to the upper North part of the country which makes up the Momase region of PNG. This species is found mostly away from the coastline and in the inner lowland areas as depicted in Figure 3.16_C.

Figure 3.14. Photos of the three main Anopheles vectors in PNG, showing differences in proboscis and wing sector spot, which are the key characteristics for morphological identification

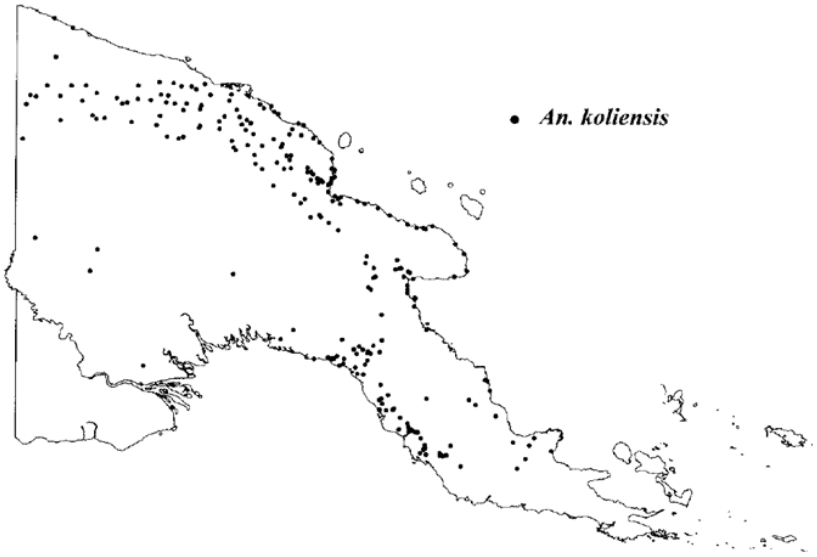
Source: Walter Reed Biosystematics Unit

Figure 3.15. Maps of *Anopheles punctulatus* (A) and *An. koliensis* (B) distributions

A.

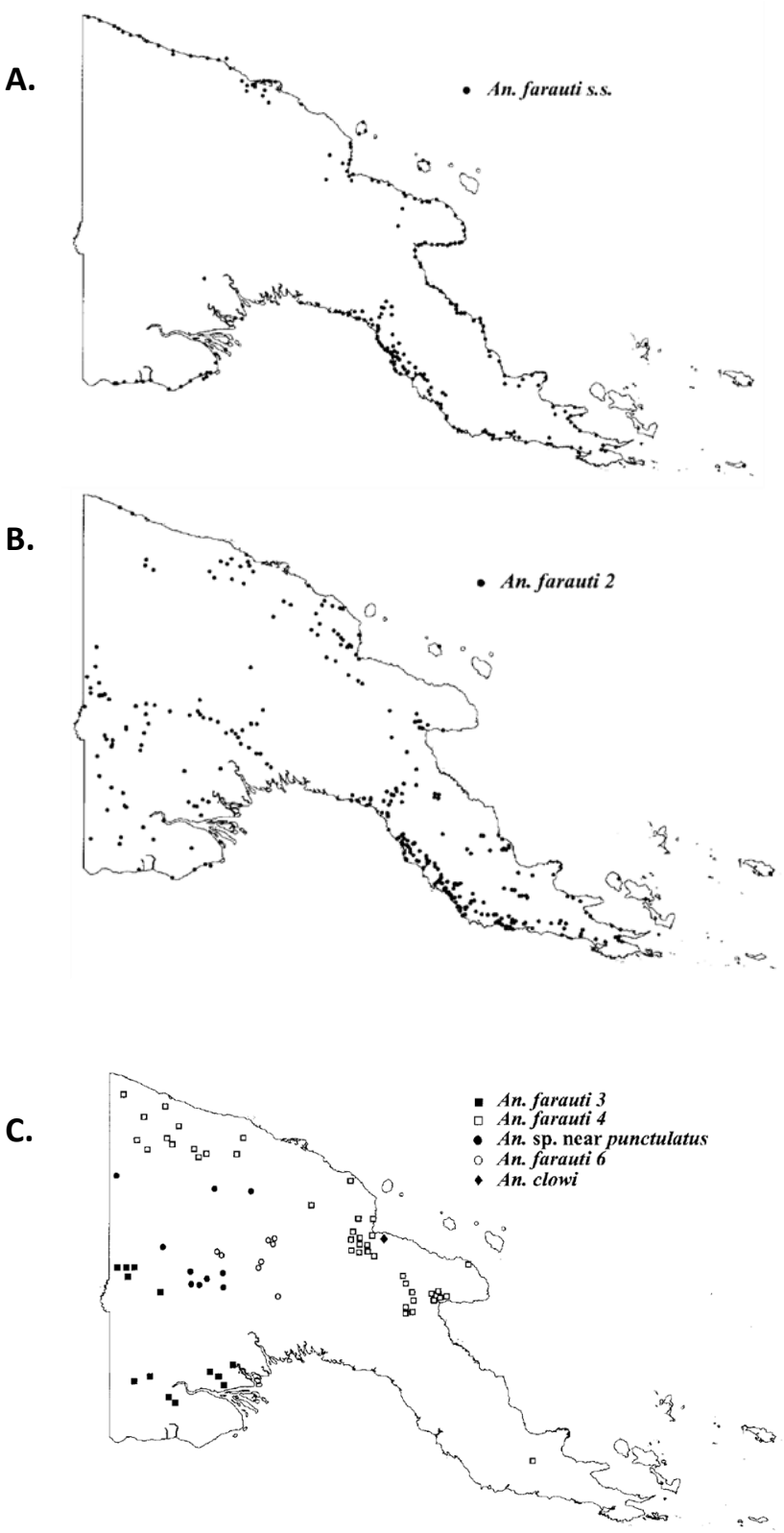


B.



Source: Cooper et al., 2002

Figure 3.16. Maps of *Anopheles farauti* sibling species distributions



Source: Cooper et al., 2002

3.3. Results part 3

3.3.5 Anopheles species in Madang Province and Usino Bundi study area

From the publication by Beebe et al., 2002, the distribution of known LF Anopheles vectors were digitized and presented in Figure 3.17 below. The *An. punctulatus* vector is more clustered around the western edge of mainland Madang, mainly in the coastal to lower inland areas as seen in Figure 3.17_B, while *An. koliensis* has a wider, less concentrated distribution throughout the province. The *An. farauti 4* is more common in the inner lowland plains on the southern part of Madang.

Figure 3.18 shows the study site where my project was carried out. And from the digitized map in that figure, it shows mainly *An. farauti 4* and *An. punctulatus*, hence they may play a role in the transmission of LF in the area.

Figure 3.17. Digitized maps of Madang *Anopheles* vectors

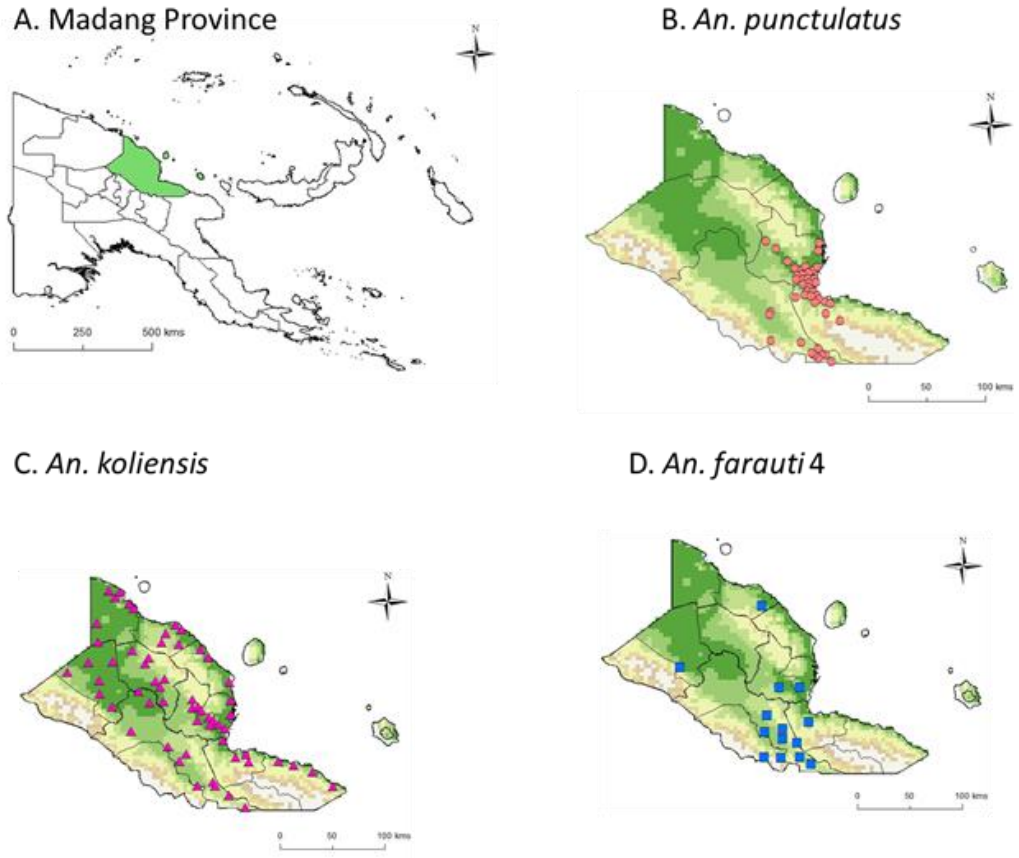
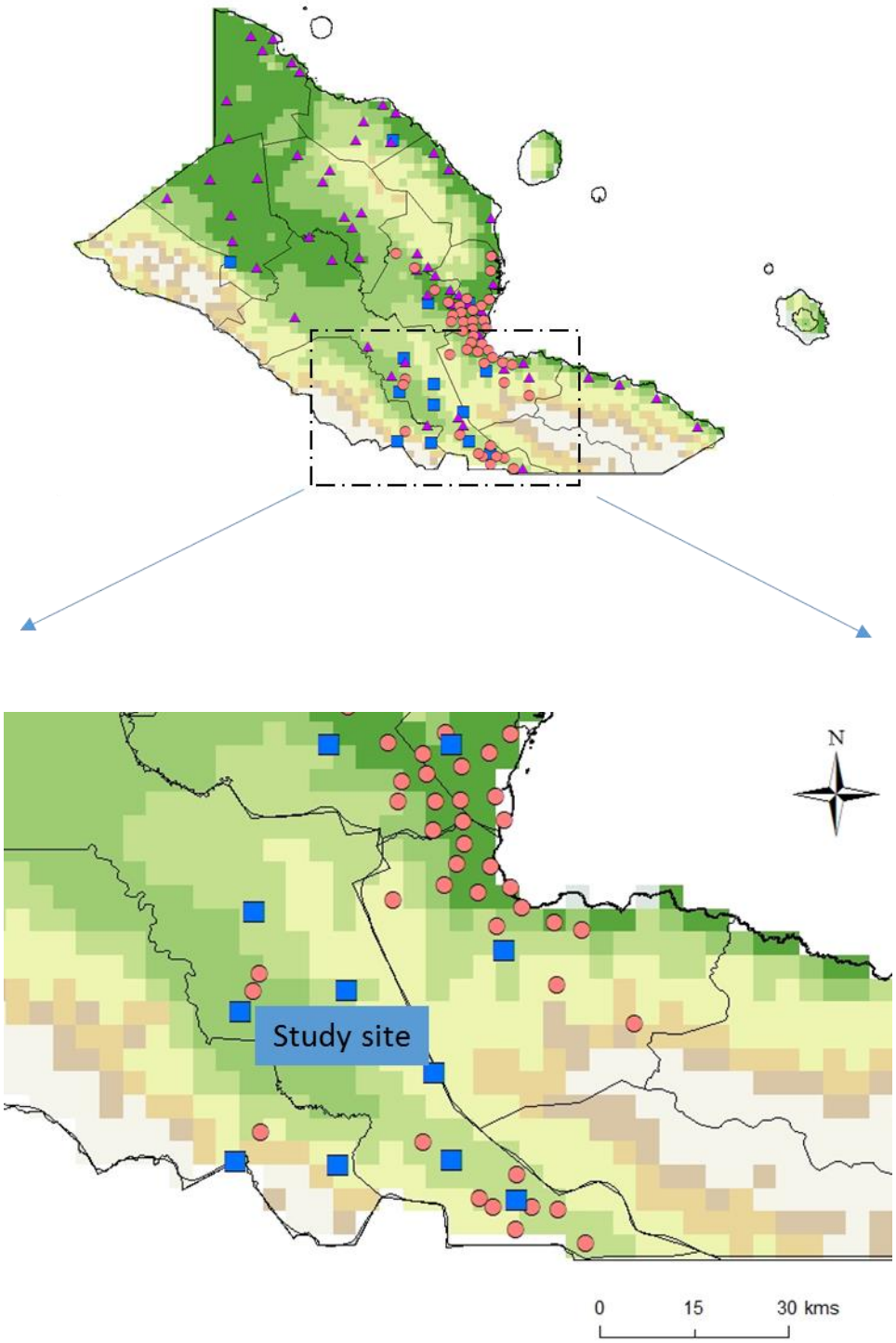


Figure 3.18. Madang Province and study area - highlighting main *Anopheles* vectors



3.4 Discussion

Overall this review of LF research in PNG shows the selective areas where studies have been conducted, highlighting high risk prevalence areas and main mosquito vectors as well as the wide range of gaps in our knowledge. The collation of this information will be helpful for programmatic purposes, facilitate the development of a national database which may be important for the WHO elimination dossier requirements, as well as information for future researchers so that they know where and what to investigate further.

The work summarized on the human prevalence and MDA impact section highlight that overall LF prevalence was found widely across PNG, however the levels of endemicity varied significantly with lower prevalence rates found in more recent years compared with historical studies (Graves et al., 2013). The reasons for this are not completely clear, but could be related to interventions or environmental changes such as bush clearing, rural to urban migration, however the latter points have not been investigated.

The examination of three different criteria to map endemic areas was useful and showed that the classification of high and low areas may help the LF programme to target the areas needing intervention the most. This is important as the resources for the LF programme are minimal and currently only a few international stakeholders are supporting the programme. It will therefore be critical to target very high risk areas first.

The analysis of the two main interventions of MDA and ITNs/LLINs showed that they are effective and LF transmission can be significantly reduced, with a clear decline in MF and Ag after MDA. However, in the absence of a large scale MDA programme at this current time in PNG, it may be better if the LF programme links with the malaria control programme and tries to target high risk areas with ITNs/LLINs, especially as this intervention has shown to be highly effective as shown in the historical work (section 2.3) and more recently by Reimer et al., 2013.

The entomology review highlighted the main vectors associated with LF transmission in PNG. However, the number of studies and locations where studies had been conducted was quite limited. This may be because there is only one main institute, PNGIMR driving the research in the areas and stay within well-established study zones. This has resulted in large gaps in knowledge in many areas of the country. Future studies should look to expand to new neglected areas of the country if local capacity is capable of doing so. It is not known if the *Anopheles farauti*, *An. punctulatus* and *An. koliensis* in the other regions will also have the same transmission patterns and also if the interventions of MDA and ITNs will also impact the same way.

It is likely that the most recent scale up of LLIN/ITNs across the country will impact on LF transmission, but it is also possible that they could change the behavior of mosquitos and the biting patterns. Bockarie et al., (2000) showed that even after MDA intervention, that the peak transmission month (measured by the number of infected mosquitoes) changed from August to May. In PNG, men and adolescent boys tend to stay up late at night, and it may be that they are less likely to use LLINs as it can be very hot and many people sleep outside away from nets too. This puts this sub-group of the population particularly at risk.

The proposed study site in Madang province shows that all main LF vectors are present – but mainly *An. farauti 4* - the work conducted in chapter 6, the entomology component of this study should be able to highlight which vectors are the main ones involved in transmission in this area.

Chapter Four

Mapping Antigen Prevalence and Risk Factors of Lymphatic Filariasis in Usino Bundi District, Madang Province

4.1 Introduction

Papua New Guinea has a population of 7.2 million inhabitants; of which 2.7 million people are estimated to be at risk of infection, predominately in the lowlands of the country (Graves et al., 2013). In PNG, LF is caused by *W. bancrofti,* which is transmitted by *Anopheles* mosquitoes similar to malaria and the main species include *An. farauti*, *An. punctulatus*, *An. koliensis* (as highlighted in the review in Chapter 3). A recent review on human prevalence by Graves et al., 2013, highlighted the main endemic areas of the country, however there are still many unmapped areas with well define prevalence information. Similarly, the burden of clinical disease and associated risk factors are also not well defined, and no specific risk factor survey has been conducted and published on LF from PNG.

The National LF Elimination Programme in PNG is yet to scale up its national-wide intervention programme using MDA, or address issues of morbidity management adequately to alleviate suffering caused by the disease. It is behind GPELF targets. However, In the absence of any scale up of MDA, it could benefit from the recent 2012 distribution by the Global Funded Malaria Programme of long lasting insecticide treated nets (LLINs) as they have shown to impact transmission and reduce prevalence in PNG and elsewhere (van den Berg et al., 2013, Reimer et al., 2016).

Some of the highest rates of LF in PNG are in the Madang Province where previous studies have been conducted and shown high prevalence, however they have mainly been in one region of the province and there is little information on the risk elsewhere and/or in close proximity to the Central highlands. Standard

programmatic mapping by WHO guidelines (WHO 2013) have not been conducted in other areas of this province. The standard recommendation includes testing up to 100 people in a village within a defined area to determine the presence of infection and the need for MDA. There is little information in the Usino Bundi district of Madang, near the base of the highlands therefore it provides an opportunity to conduct a mapping and risk factor survey.

4.1.1 Aim

The aim of this study was to determine the LF prevalence in an unmapped area of Madang Province, identify key demographic and environmental factors associated with transmission, and assess local knowledge of the disease and the National LF Programme. This information will help to identify high risk groups, risk factors so that appropriate public health awareness campaigns can be appropriately targeted.

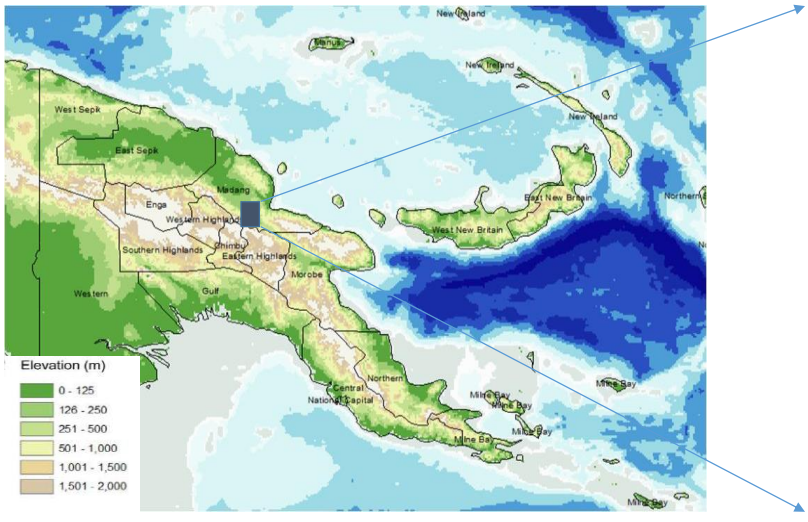
4.2 Methods

4.2.1 Study area

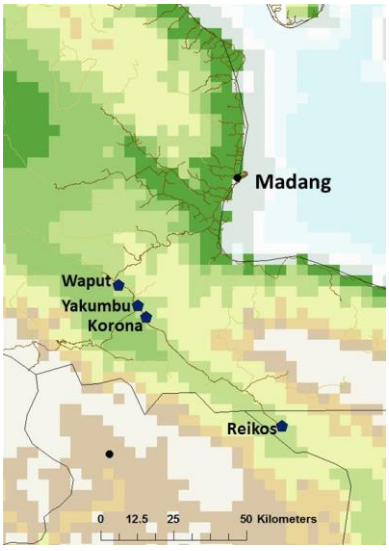
The study was conducted in Usino Bundi District in the eastern area of Madang Province (Figure 4.1_A), which is within the Momase region of PNG, and known for high malaria and LF transmission (Weil et al., 2008, Alexander et al., 2000, and Benet et al., 2004). All 6 districts in Madang have been shown to have active filarial transmission in the last decade (Bockarie et al., 2002 and Graves et al., 2013). Usino Bundi area was selected as LF transmission had only been assessed in one area on the North West perimeter of the district (Weil et al., 2008). The district has a population of approximately 60 thousand people with an average of 5.9 people per household (PNG National Census, 2013). The majority of people are subsistence farmers living in houses made from bush materials with elevated floors and sago palm leaf roofs. Walls are either made of woven split bamboo, timber or sago palm stalk with large unscreened windows. The topography is diverse with tropical mountain rainforest leading into the vast grassland plains. It rains throughout the year however over 70% of the precipitation occurs between November and May. The rest of the year constitute the dry season.

Figure 4.1. Map of Papua New Guinea and Usino Bundi District study sites

A. PNG and site of study area



B. Four study sites



4.2.2 Study design and prevalence sampling

A cross-sectional survey was conducted in April, 2013 and included a combined LF antigenemia (Ag) prevalence survey and household risk factor questionnaire conducted in four randomly selected villages in Usino Bundi. The sample size was based on the WHO guidelines for rapid mapping of bancroftian filariasis in endemic areas, which requires up to 100 people to be tested in each village [WHO, 2012]. This survey selected individuals $\geq$ 6 years of age through a multi-stage random process. First, from a central point of the village a random direction was selected by spinning a bottle, and every second household selected until the end of the village. The process was repeated until 100 households had been selected except for Korona village which only had 90 houses at the time of survey and Raikos community which had only 99 houses. Second, an eligible individual within the household was randomly selected and invited to participate in the survey.

A team of two technicians (one for interviewing and recording answers for the household survey and the other to perform the Ag prevalence test) and a local volunteer (a person of good standing in the community) who assisted with identifying the location of houses within village boundaries. Consenting individuals were tested for filarial antigen using the rapid diagnostic BinaxNOW® Filariasis ICT test shown in Figure 4.2_A (Alere Inc., Scarborough, ME) (Weil et al., 1997). From each individual, a 60µl of finger-pricked blood was obtained for the card test (Figure 4.2_B) with strict adherence to the manufacturer's instructions. Any individuals testing positive were provided with information on prevention of LF, referred to the local health clinic for treatment and reported to the National LF programme, Department of Health.

Figure 4.2. Immunochromatography rapid diagnostic test kit that was used for testing for the *W. bancrofti* antigenemia

A. ICT kit

B. Card test used to determine Ag presence

4.2.3 Risk factor questionnaire

The risk factor questionnaire was used to gather information on the following factors and the relationship with ICT positivity was examined;

- demographic characteristics **(**village, sex, age, education level, length of residency, number of household members),
- housing/infrastructure information (house type, number of rooms, source of water, toilet type, refuse habits, drainage system availability, proximity of household to village edge),
- knowledge of LF and the national LF elimination programme (cause of disease, transmission, prevention, treatment history from national programme) including
- use of interventions (MDA, LLINs, mosquito repellents),
- evidence of LF clinical conditions in their household (lymphoedema, hydrocoele, other).

Figure 4.3 shows one of the field team members collecting information about the village and households to be include in the survey, before the implementation of the questionnaire.

Figure 4.3. Field team members collecting information in Waput village

4.2.4 Data analysis and maps

Data in the field were recorded on paper forms and were later entered into Microsoft Excel database and later exported into PASW Statistics version 22 (SPSS Inc., Chicago, IL, USA) for statistical analysis. Antigenemia prevalence was expressed as the percentage of infected individuals over the total number of individuals examined by the different factors. The intensity of infection was computed when the count was available as arithmetic means, and the sampling fluctuations estimated using the 95% confidence interval (CI).

Descriptive and statistical analyses were conducted to compare Ag prevalence level and mean infection between the villages, sexes, age groups, level of education, length of residency, number of people in the house and the number of bedrooms. Cross-tab comparisons and Chi square test with p-values <0.05 as significant were used. Difference between infection rates by each of the demographic, housing/

infrastructure, and intervention variables were also highlighted using graphs of mean measures and standard errors of the means.

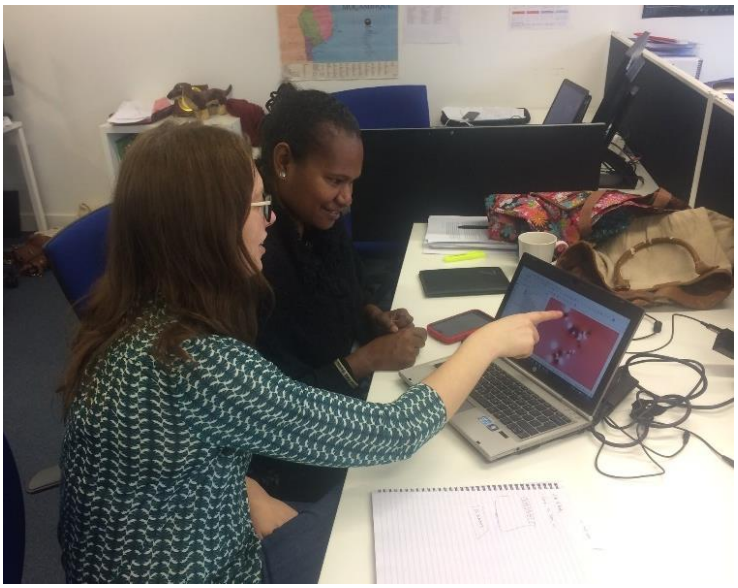
The geographical coordinates of each village and household were recorded using a high sensitivity global positioning system [GPS eTrex; Garmin (Europe) Ltd, Southampton, U.K.]. Maps to show LF distribution and prevalence of LF antigenemia were created using a freeware geographical information system (GIS) software QGIS http://www.qgis.org/

4.2.5 Consent and ethical consideration

Proper ethical procedures were followed and both LSTM ethics committee (Appendix 1) and the PNG Medical Research Advisory Council (Appendix 2) gave approval for the study to be conducted.

The provincial health advisor and respective district health manager were informed prior to the field activities and community leaders were also made aware of the study activities that would be taking place in their community once the villages were selected. The community leaders helped to let the villagers know about the upcoming field activities.

Figure 4.4. Learning QGIS software for mapping data and spatial analysis



Source: Centre for Neglected Tropical Diseases, LSTM

4.3 Results

4.3.1 Demographic characteristics and prevalence

The survey was conducted over a 3 week period in April, 2013 (Appendix 3). The four study villages randomly selected included Waput, Yakumbu, Korona and the Raikos community located in the Ramu Agro area of Madang Province (Figure 4.1B). Each study village comprised of three to four hamlets as shown in Table 4.2. A total of 389 houses and individuals were surveyed. While at least 100 individuals per village were targeted, Raikos only had 99 households and Korona only 90 households and therefore all households were included in the survey. Of the 389 individuals surveyed, 40% (n=157) were household heads (n=108,27.7% males; n=49, 12.6% females), all of those participated in the ICT testing were randomly selected to test for Ag prevalence at the household level. Table 4.1 shows the composition of people tested for Ag prevalence at household level.

Table 4.1. Composition of household individuals tested for antigenemia

Status	Female		Male		Total	
	n	%	n	%	n	%
Household owner	49	12.6	108	27.7	157	40.4
Household member	107	27.5	125	32.1	232	59.6
	156	40.1	233	59.9	389	100

Overall, the Ag prevalence was 8.7% with a wide range of positivity found in three villages; Korona (28.9%), Yakumbu (5%) and Waput (3%), this is shown in Figure 4.5_A. None were detected in Raikos community (Table 4.2). There was variation at hamlet level, where Ag was prevalent in all 3 hamlets of Korona village with the main hamlet Korona having the highest prevalence (n=49, 33%) followed by Tongona (n= 24, 33%) and Koinduna (n=17, 12%) as shown in Figure 4.5_B. Only one hamlet, Urigina in Yakumbu had 5 Ag positive individuals (n=43, 5%) and 2 hamlets in Waput had 1 and 2 Ag positive individuals in Koiye and Danaru respectively (5%).

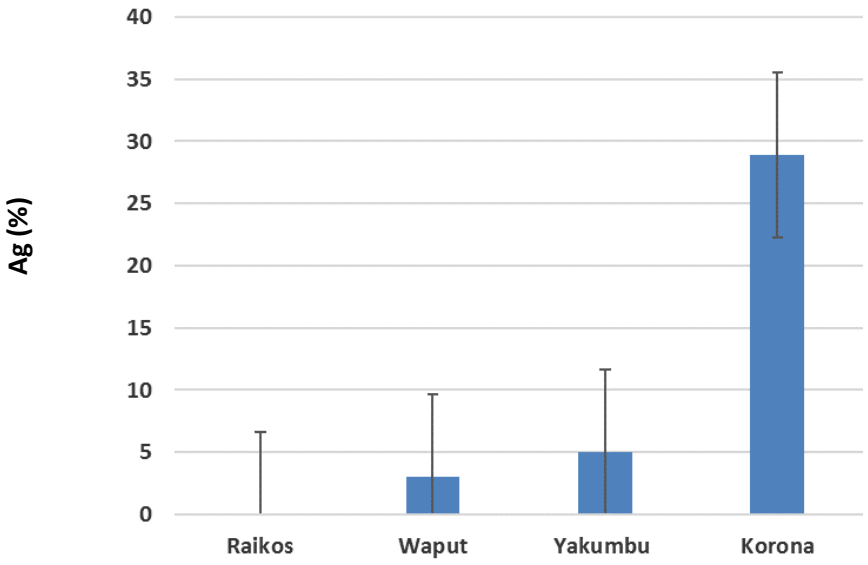
Table 4.2. Ag prevalence at hamlet level

Village	Hamlet	Negative	Positive	Total	%
Korona	Korona	33	16	49	33
	Tongona	16	8	24	33
	Koinduna	15	2	17	12
Yakumbu	Yakumbu	34	0	34	0
	Ass Mambu	6	0	6	0
	Urigina	38	5	43	12
	Waruna	17	0	17	0
Waput	Waput	35	0	35	0
	Danaru	19	2	21	10
	Koiye	43	1	44	2
Raikos	Bora	50	0	50	0
	Kapul	4	0	4	0
	New camp	45	0	45	0

The maps shown in Figure 4.6 shows the ICT positive households in the high prevalent Korona village highlighting the Ag prevalence is widespread and present in all three hamlets with Korona hamlet having the highest number of people positive. In Yakumbu, Ag prevalence was only present in one hamlet, Urigina. While Waput had dispersed Ag prevalence.

Figure 4.5. Ag prevalence by sex and age distribution of the study population

A. Ag prevalence by village



B. Ag prevalence by hamlet

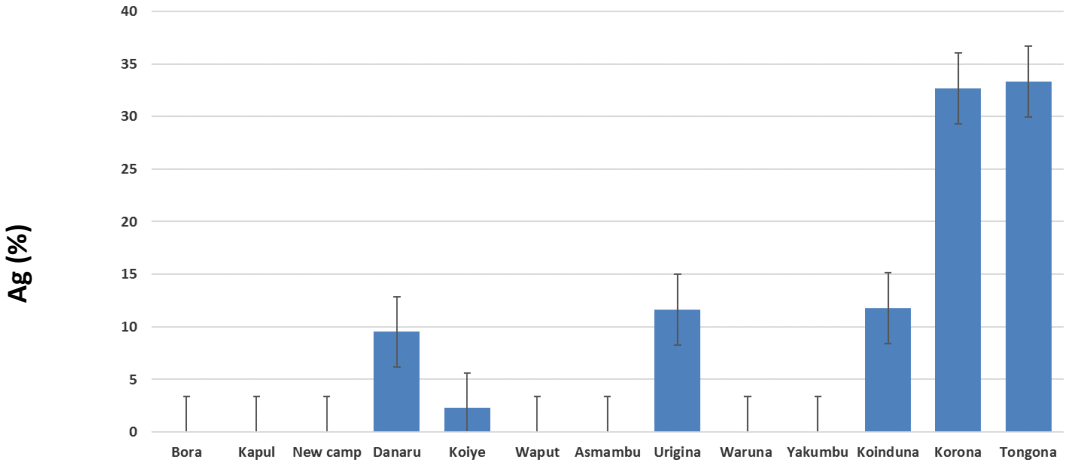
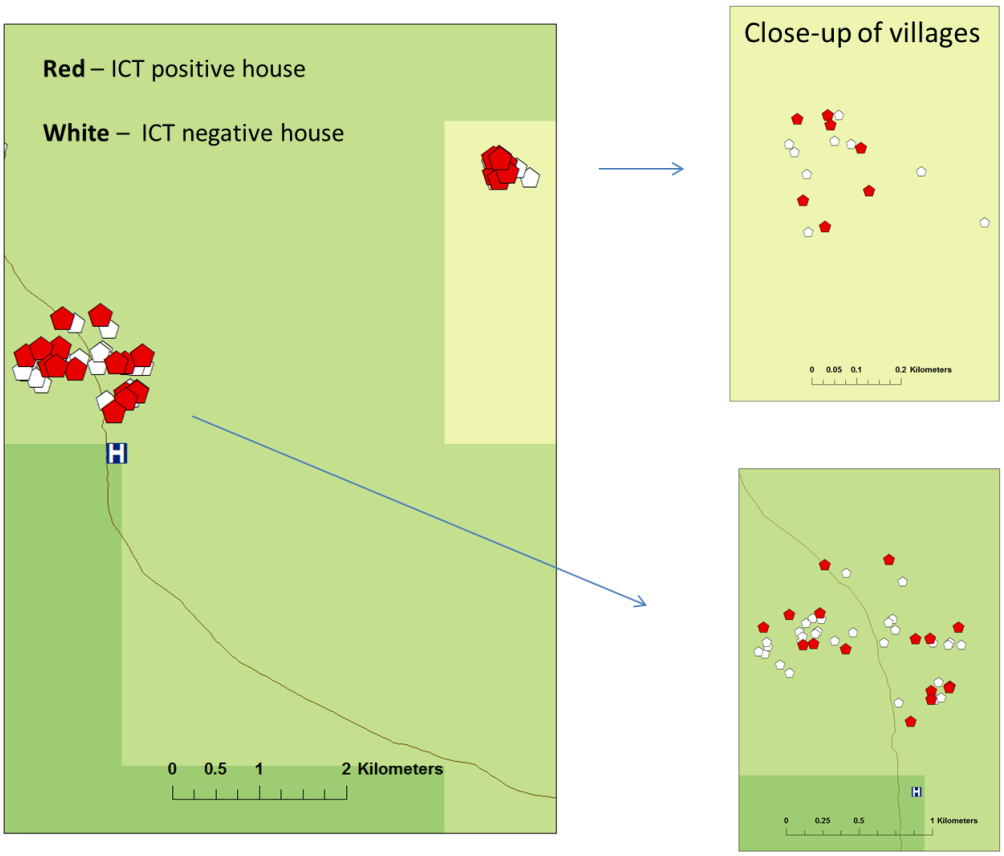


Figure 4.6 Maps of Ag positive distribution in Korona village and hamlets



4.3.1.1 Individual risks

Sex and age: Of the 389 randomly selected individuals, there were 233 males (59.89%) and 156 females (40.10%) tested using the ICT. There was no significant difference in the proportion of males and females in each village (chi square, p-value ≥ 0.05). The age of individuals tested ranged from 7 to 71 years (mean age=26.01) which was similar between males (range 7 to70; mean= 25.74) and females (range 7 to 68; mean=26.43). Males (10.7%) were found to have nearly double the prevalence of females (5.7%) but this is not significantly different (0.090 >0.05). However, there is significant difference in the age groups (p-value=0.000) and a stepwise increase in prevalence with age was found with the 7-19 age group recording the lowest prevalence (0.7%) and those aged over 49 years the highest (33.3%). This is shown in Figure 4.7.

4.3.1.2 Household risks

Education: The majority of surveyed household individuals had no formal education (n=111; 10.8%) or primary school education (n=203; 9.4%) only with similar prevalence rates, which were approximately twice as high as those who had secondary or higher education levels (n=74; 4%).

Household size (people and rooms): The average number of people living in households ranged from 5.5 in Korona to 6.6 in Yakumbu (overall average 6 people/house). There was no difference in ICT prevalence between households with <6 people and household with > 6 people living in them. The average number of bedrooms per house ranged from 2.2 at Raikos to 3.2 at Yakumbu (overall 2.7 bedrooms) with no significant difference in prevalence found between household with less than 3 bedrooms (n=180; 3.5%) and those with 3 or more bedrooms (n=209; 5.1%).

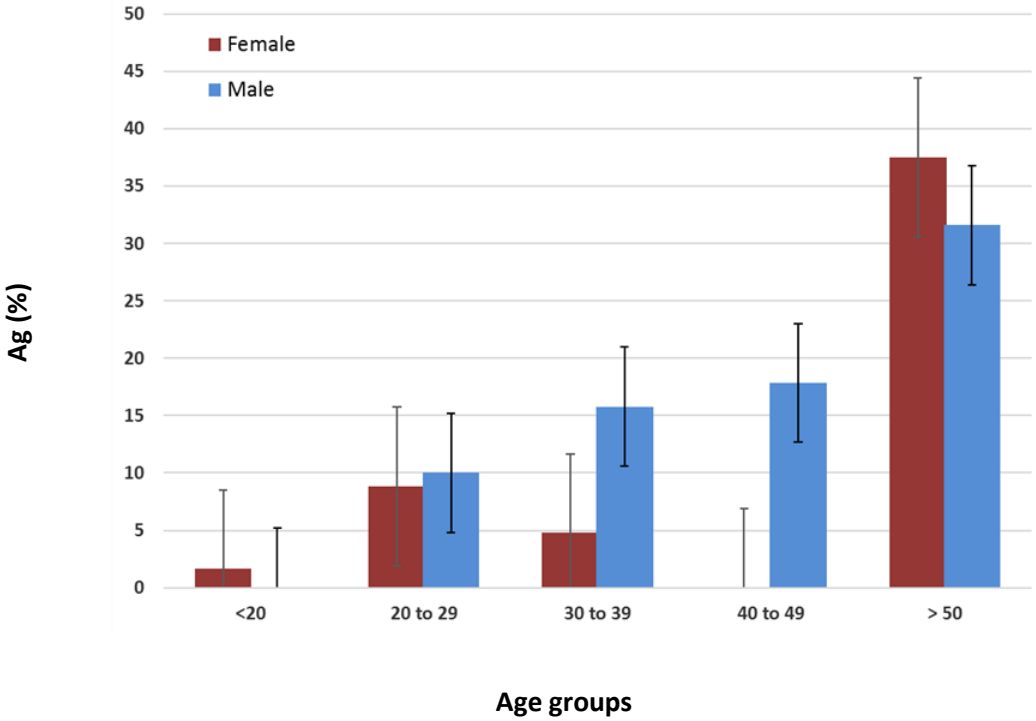
Length of residency and travel patterns: Approximately two thirds stated that they had lived in the village 10-15 years (n=128) or their entire lifetime (n=227) with prevalence rates of 0.8% and 14.5% respectively. A small proportion stated 'other'; (visitors/seasonal workers in the case of Raikos, n=34, 0%), and were found to have no Ag prevalence. A significant difference was seen with the length of residency, p-value=0.00. All those surveyed stated that they travel periodically between different locations within Madang Province for work, school or other traditional activities like bride price, attending funerals and visiting relatives.

Table 4.3. Summary of demographic characteristics and ICT prevalence

	Total	ICT positive (N)	ICT positive (%)	Stats
				Pearson Chi-Square (p-value)
Demographic Characteristics				
Individual risks				
Sex				
Female	156	9	5.7%	
Male	233	25	10.7%	
				0.090>0.05
Age Group				
7-19	131	1	0.7%	
20-29	94	9	9.5%	
30-39	79	10	12.6%	
40-49	36	5	13.8%	
> 49	27	9	33.3%	
				0.00 <0.05**
Household risks				
Village				
Korona	90	26	28.9%	
Waput	100	3	3.0%	
Yakumbu	100	5	5.0%	
Raikos	99	0	0%	
				0.00 <0.05**
Education				
No school	111	12	10.8%	
Primary	203	19	9.4%	
Secondary + above	74	3	4.0%	
				0.246 >0.05
Length of Residency				
<10 years	128	1	0.8%	
>10 years	227	33	14.5%	
Other**	34	0	0%	
				0.00 <0.05**
People in Household				
< 6 average	178	19	10.7%	
> 6 average	211	15	7.1%	
				0.215 >0.05
No. Bedrooms				
< 3 rooms	180	14	7.8%	
≥3 rooms	209	20	9.6%	
				0.533 > 0.05

**p value is significant

Figure 4.7. Ag prevalence by sex and age distribution of the study population



4.3.2 Housing/Infrastructure characteristics and prevalence

House type and height: There were three main types of houses in the four villages surveyed. Photos showing examples of the different house structures are shown in Figure 4.8. The majority were semi-permanent (n=103; 26.5%) which are constructed from bush materials but had a tin roof instead of woven sago leaves, bush material houses (n=216; 55.5%) constructed from sticks and bamboo blinds for walls and flooring with woven sago leaves as roofs. A smaller proportion were permanent houses (n=57), which were constructed with timber, corrugated iron roofing and screened windows and were mostly located in Raikos. No individuals living in the permanent houses were found to be Ag positive, compared with 9.7% and 11.1% recorded for the semi-permanent and bush material houses respectively (Table 4.3). There was a highly significant difference between semi-permanent and bush material houses and the permanent which is see in Figure 4.9.

Water source and toilet type: Households that sourced water predominantly from streams/rivers (n=188) or from tap water (n=181) through channelled piped from nearby lake are similar, with the Ag prevalence of people using both these water sources as 9.57% and 8.28% respectively. The majority of houses had their own pit toilets which were located close to the main house, no more than 12m away (n=322), and the few that shared (n=3), were nuclear family members with houses side by side (2 brothers or a father and newly married son). There were no significant difference found in water use and toilet type.

Refuse habits and drainage system: Nearly two thirds of households used dugout pits in close proximity to their houses as the main source of refuse disposal (n=193; 13.5%), there is higher Ag prevalence associated with this group of houses and is found to be significantly different (p-value=0.014, Table 4.3) to households that used rivers/stream or bushes (n=102; 3.9%) or a common dugout pit (n=91; 4.4%) as illustrated in Figure 4.10. Two thirds of houses had a drainage system present (n=258; 9.9%) and the Ag prevalence was similar to those houses without a drainage system present (n=118; 8.1%).

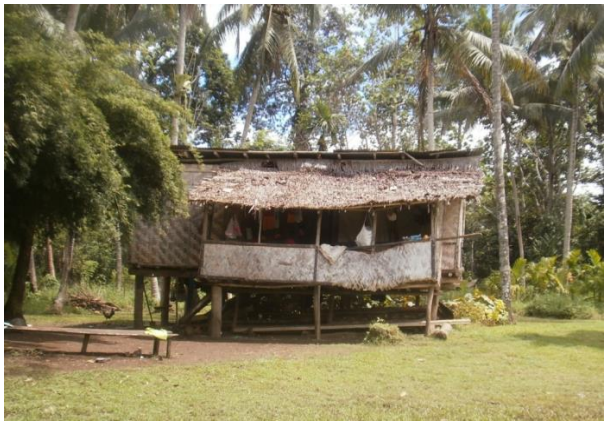
Table 4.4. Summary of housing and infrastructure characteristics and ICT prevalence

	Total	ICT positive (N)	ICT positive (%)	Pearson Chi-square P-value
Housing/ Infrastructure Characteristics				
House Type				
Permanent	70	0	0%	
Semi-permanent	93	10	9.7%	
Bush material	192	24	11.1%	
				0.015 <0.05**
House raised				
Yes	338	34	9.1%	
No	17	0	0.0%	
				0.192 >0.05
Water source				
Streams/river	188	18	9.6%	
Bore hole/rainwater	4	1	25.0%	
Tap water (from lake)	181	15	8.3%	
Combination	15	0	0%	
				0.678 >0.05
Toilet type				
Shared toilet	57	3	5.3%	
Own toilet next to house	322	30	9.3%	
Bush/river	10	1	10%	
				0.601 >0.05
Refuse habits				
River/stream/bush	102	4	3.9%	
Common dug out pit	91	4	4.4%	
Dugout pits next to house	193	26	13.5%	
				0.014 <0.05**
Drainage system				
Yes	258	21	8.1%	
No	131	13	9.9%	
				o.556 >0.05
Proximity to village edge				
<12m	232	15	6.5%	
>12 m	157	19	12.10%	0.053 >0.05*

Figure 4.8. Typical houses in the study areas and Ag prevalence

I) Permanent structure

ii) Semi-permanent structure



iii) Bush material structure

Figure 4.9 Differences in Ag prevalence by house type

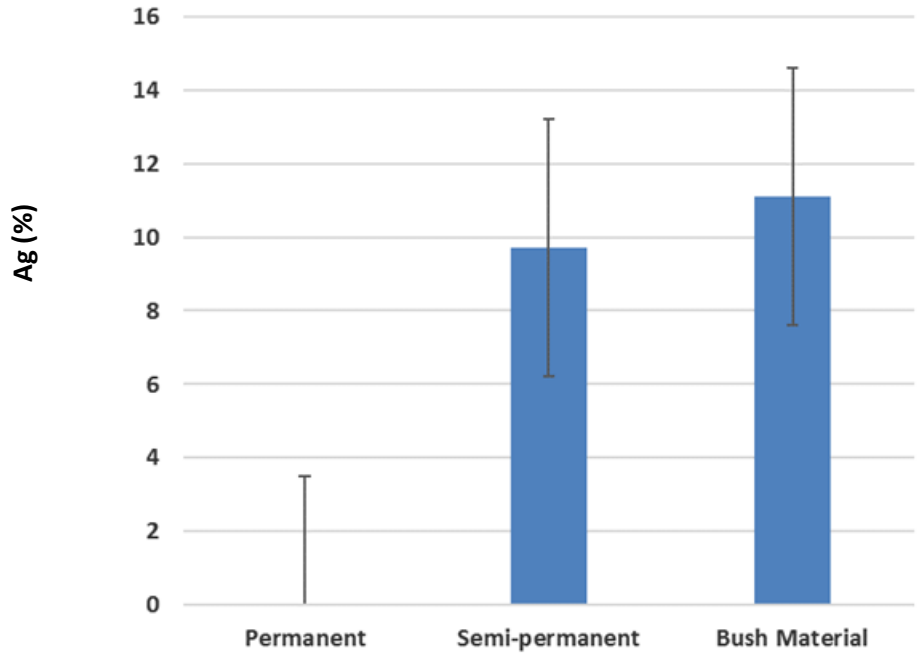
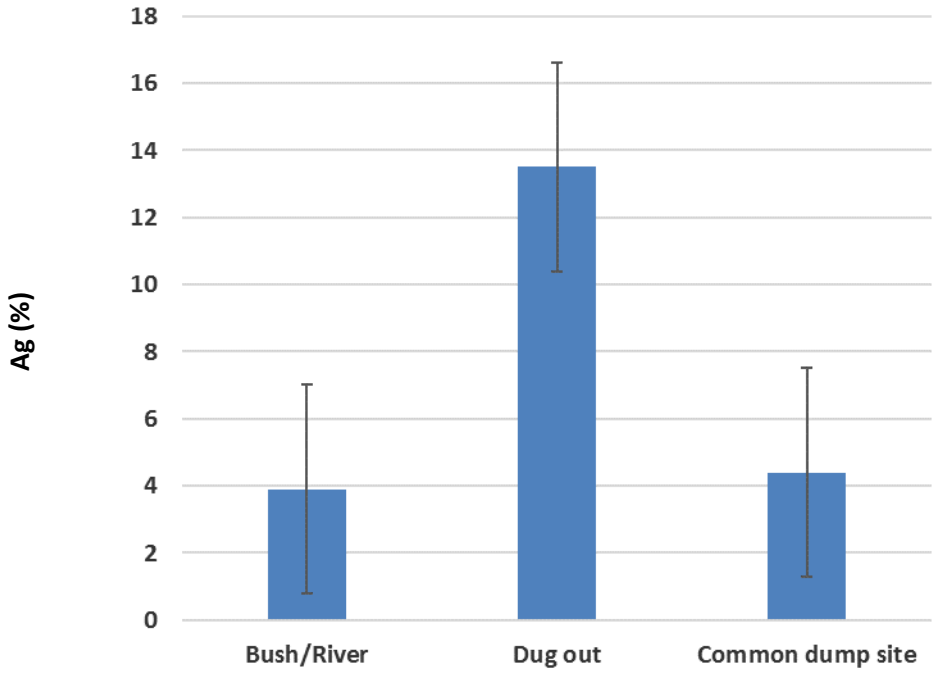


Figure 4.10 Prevalence of Ag by refuse habits



4.3.3 Knowledge of LF and the National LF Programme and disease prevalence

Knowledge of LF: We found that 90.2% (n=351) of the people interviewed did not know about the disease and its symptoms, (Table 4.5). From the 9.8% that had some knowledge of LF, 5% of them were LF positive compared to 10% of those who did not know about the disease (Figure 4.11).

LF Programme: About 98% (n=384) of them have never heard of the national LF program responsible for mass awareness and providing chemotherapy to help control and eliminate LF in the country. All of the individuals that took part in the survey and ICT testing said they want to be treated if they are at risk of infection. Although no one had heard of the programme, three people stated that they had seen and/or heard of MDA activities in other provinces.

Figure 4.11. Composition of Ag infected individuals and their knowledge of LF

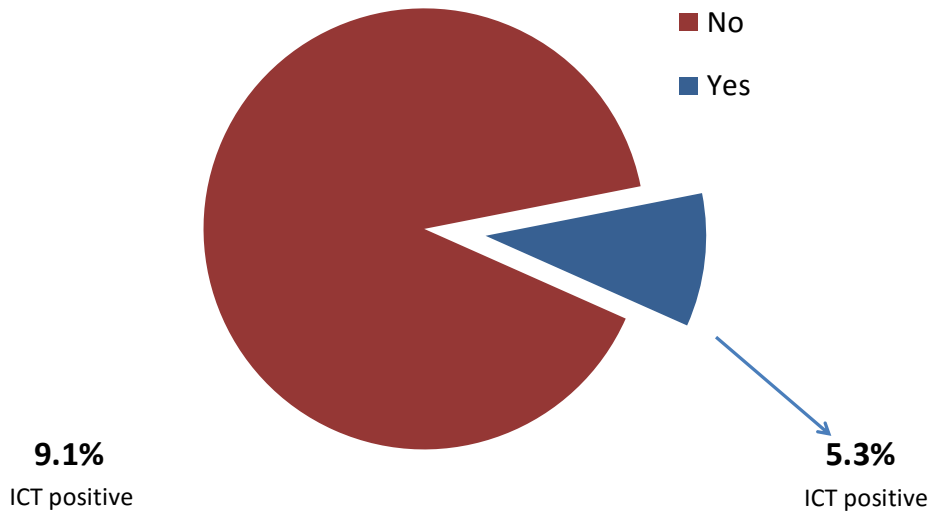


Table 4.5. Summary of knowledge of disease, LF programme and use of intervention and ICT prevalence

	Total	ICT positive (N)	ICT positive (%)	Pearson Chi-Square P-value
Knowledge of LF and National Programme				
Heard of LF				
Yes	38	2	5.3%	
No	351	32	9.1%	
				0.424 > 0.05
Know of National LF Programme				
Yes	5	0	0.0%	
No	384	34	8.9%	
				0.486 >0.05

4.3.4 Use of different types of interventions (LLINs, mosquito repellents)

LLINs: All participants stated they received LLINs in 2012 as part of the national malaria control campaign. The number of LLINs per household ranged from 0 to 10, and the number of people sleeping under nets were on average. Based on the number of nets and number of people sleeping under LLINs in each household, the proportion of each household covered by LLINs was quantified and found to range from 57.2% in Raikos to 64.5% in Yakumbu (overall coverage rate 61.1%).

Mosquito coils/repellents: Overall very few households used local repellents or mosquito coils, although there were no significant difference, those who used some form of repellent regularly, were found to have lower Ag prevalence (n=43; 4.7%) compared with those who did not (n=346; 9.2%).

Table 4.6 Reported use of vector control interventions

				Pearson Chi-Square P-value
	Total	ICT positive	**ICT Positive (%)**	
Mosquito coils / repellents				
Yes	43	2	4.7%	
No	346	32	9.2%	
				0.314 >0.05
LLIN in house				
>3 LLIN	115	14	12.1%	
<3LLIN	266	20	7.5%	
				0.143 >0.05

4.3.5 Presence of clinical conditions

Individuals were visually checked for clinical symptoms of LF and were also asked if anyone in their household had symptoms of LF. Those who had symptoms were recorded and members of their household with symptoms were visually confirmed. A total of 12 cases were identified, the majority (n=10) of those were from Korona, the village that had high Ag prevalence. The elephantiasis of lower limb of an adult female is shown on Figure 4.12. Most of these clinical symptoms were of the lower limbs and 7 people stated they had their condition for more than 7 years. When asked if they needed assistance with day to day care, 4 individuals out of the 12 stated that they do, and these people suffered from swollen leg, hydrocele or both. None have received treatment for their condition.

The relationship between the number of clinical cases and the ICT prevalence was compared at hamlet level and it was found that the hamlets in the high risk Korona village collectively had the highest number of cases, while Waput and Yakumbu had only a few and Reikos had none (Figure 4.13).

Figure 4.12. Elephantiasis of left leg of an adult female from Yakumbu village

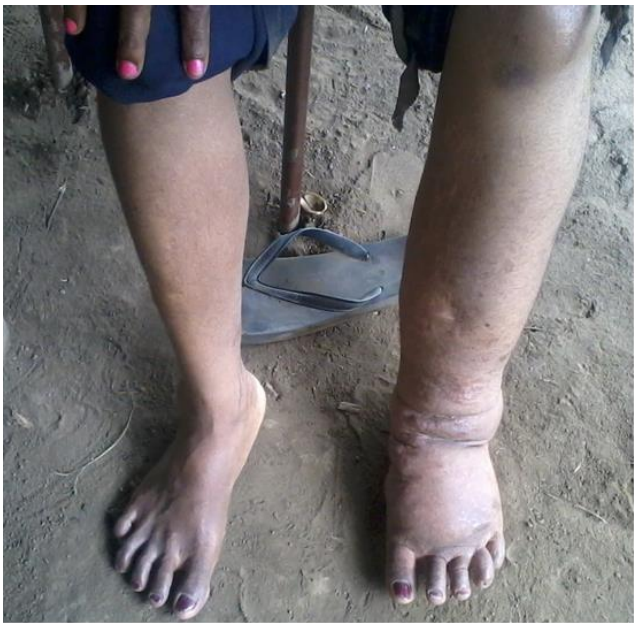
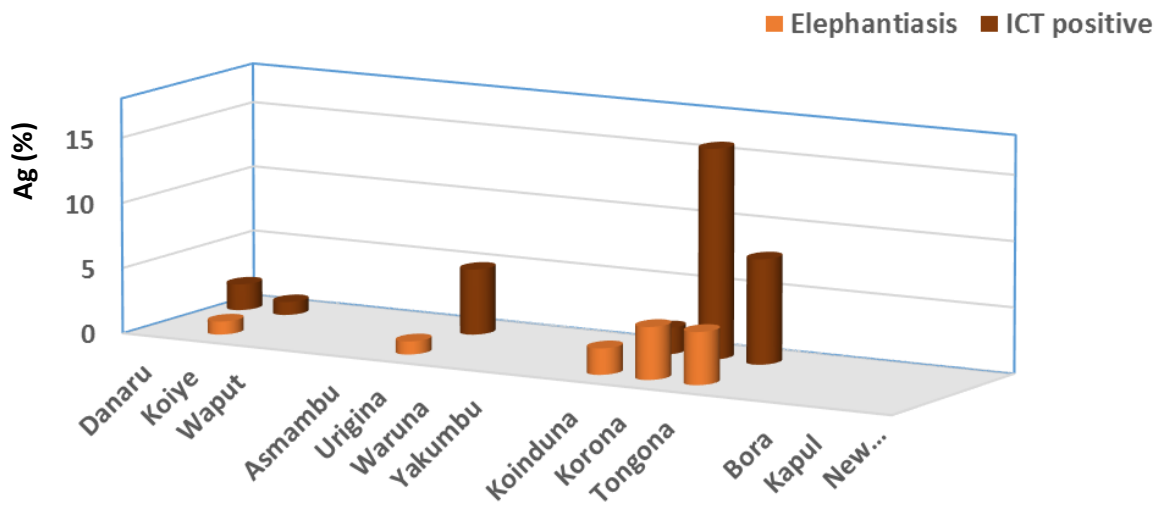


Figure 4.13 Relationship between clinical cases and ICT positive individuals at hamlet level



4.4 Discussion

This is the first study to examine LF Ag prevalence, associated risk factors and knowledge of programmatic activities in Usino Bundi District of PNG. The demographic and environmental factors that were found to be associated with the presence of LF Ag will be useful information for National LF programme to help with the planning of implementation activities. This chapter specifically looked at determining LF Ag presence and prevalence in four villages separated geographically from each other by 5 to 50km, the results show great variation between villages at a relatively small scale.

The LF was scattered throughout the three villages, with Korona village having the highest Ag prevalence, while no Ag was detected in Raikos community. The three villages with positive cases were found to be in close proximity to each other, but had quite variable prevalence rates to each other, indicating the focal nature of LF. This may be related to the flight span of known local *Anopheles* vector species, which is approximately <2km. The higher Ag rates in Korona could also be related to the river that runs through it. Such variation has also been shown in Drekikir in the East Sepik Province by Tisch and others (2001) where villages in close proximity were found to have different Ag prevalence.

In this study, Raikos village was found to be not at risk, which may be related to the fact that it is has more semi-permanent and permanent houses, which may be more protective than the bush material houses. There is little literature on the risk of LF and housing structure, however a recent review by Tusting et al., (2017) conducted a multi-country analysis of housing structure in Sub-Saharan Africa and found that housing quality is an important risk factor for malaria. Lweitoijera et al., (2013) also found that although there were significant reductions in vector density and malaria infection through vector control methods, malaria vectors still entered houses of poor design that allowed for mosquito entry. Raikos villages is also located the furthest from the other three villages and is at a higher altitude with reduced

natural vegetation around it due to plantation development which could have implications for vectors habitats and thus reducing risk.

The length of residency and migratory habits of residents appeared to be associated with LF risk, for example, the residents of the three positive villages were mostly life-time community members were more likely to be Ag positive as they are exposed to infection over time. Whereas Raikos composed of mixed residents of life time residents and seasonal workers for the Agro Industry that is located close to it, had no Ag prevalence. A review on the impact of migration by Ramaiah in 2014 indicates the impact of residency and migratory habits does influence LF transmissions.

Other important demographic factors were related to age and sex. While no significant difference between males and females was found overall, the prevalence in the older male age category of 40-49 year olds, was significantly higher than the younger age males and also the female age groups. This is a similar finding to Weil et al., (2008) who found no gender differences but higher antigen prevalence in older males in Madang Province. Terhell and others (2000), further showed that older age groups developed LF Ag much faster than younger age groups despite equal lengths of exposure in the same area. There was also correlation between Ag positives and morbidity prevalence which is important as any future Ag mapping projects may help to identify patients in need of clinical care. Tisch et al., (2008) also showed a correlation between Ag positive people and the presence of clinical morbidity in villages within a 20-30km of each other in East Sepik province, PNG.

Information on other potential risk associated factors were collected, however there was no indication of any association between these factors and Ag prevalence. However, refuse habits indicated a potential risk and could most likely implicate vector habitats where a higher Ag prevalence is associated with dug-out pits closer to residential areas encouraging closer breeding sites of vectors possibly. Cooper and others (2002) looked at habitats and distribution of the *An. punctulatus*

complex in PNG and noted that *An. punctulatus* can breed in temporary man-made or natural rain collected shallow pools of water with decaying plant matter.

Importantly, this study found that there was a large gap in general knowledge on LF and none of the people interviewed knew about the National LF programme. This could be partly due to the fact that the National LF program is slow to expand its activities and has only been conducting MDA in one island province for the last three years. This finding shows that a significant scale up of public health awareness on the disease needs to be done, specially targeting people living in high risk areas who have little knowledge about the risk of infection and disease. Specific help for those with clinical conditions also needs to be addressed. The LF programme needs to develop and distribute country specific IEC materials to high risk areas, and these could be adapted from existing WHO materials and help facilitate the elimination process.

Chapter Five

Micro-Mapping and Spatial Analysis of Microfilaria Prevalence in a Highly Endemic Village

5.1 Introduction

As part of the Global Programme to Eliminate LF, the WHO recommends that one site in an endemic implementation unit for MDA needs to be selected as a sentinel site so that the impact of the programme can be monitored over time, i.e. before MDA starts (known as baseline), at midpoint of MDA (after 3 rounds of MDA) and at the end of 5-6 MDA rounds with effective coverage rates which are considered to be >65% of the total population (WHO, 2013). In early part of the global programme MF (night blood when the nocturnal parasite is active) survey of 300-500 people were required for each sentinel site, as it provides more information of the level of problem within a community (WHO 2013).

Chapter 4 conducted a survey to identify the risk in a relatively small defined area of Madang Province. In the first survey the village 'Korona' was found to have the highest rates of LF infection and disease, and was therefore selected for further investigation so it could be used as a sentinel site. However as there is little information on LF in PNG an Mf survey of the approximate 300 people living in this population was considered an important activity to conduct and will help determine the level of current infection. It was also an opportunity to explore some risk factors in more detail as they have shown to vary at a micro level elsewhere. For example, in Tanzania the number of people per house, and vector control such as LLINs may vary and be associated with micro-risk (Russell et al., 2013) and in another study different vectors have different associations with human density or 'biomass' (Kaindoa et al., 2016). Similarly, in Yemen, a study found certain hamlets at more

risk with higher numbers of mosquitoes when many people gathered in the evening (Al-Eryani et al., 2016).

5.1.1 Aim

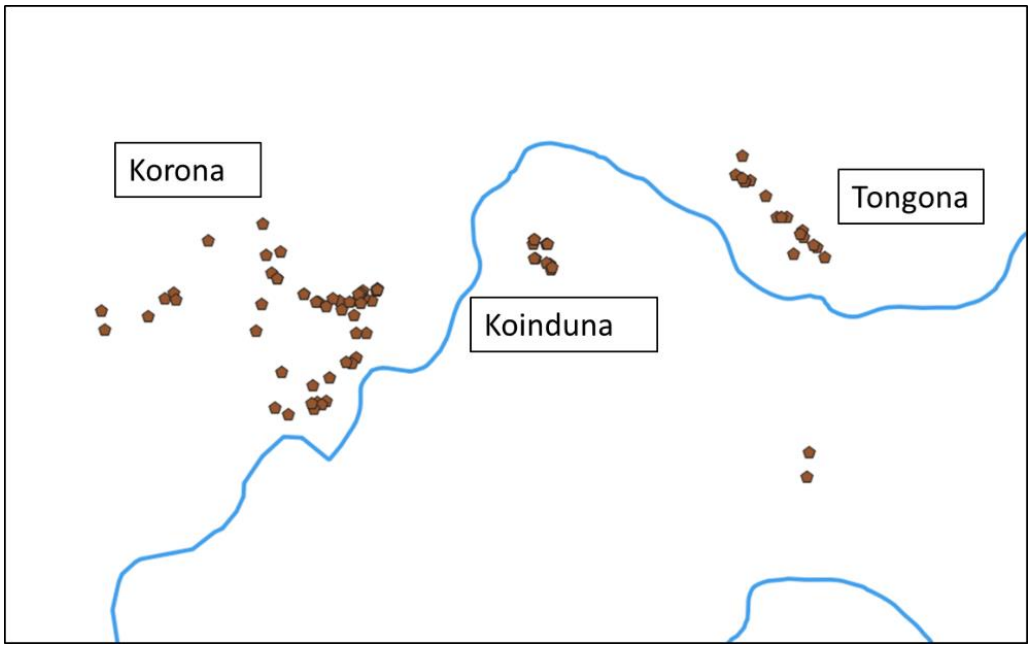
The aim of this study therefore is to build on the findings and data collected in Chapter 4 and to conduct a MF survey of approximately 300 people in the highly endemic village of Korona (to be used as a sentinel site) and examine demographic and housing infrastructure and intervention risk factors in more detail at micro geographical scale.

5.2 Methods

5.2.1 Study site and sampling

The part of the study focused on the village Korona with the highest number of ICT positive cases from Chapter 4, and included a microfilaria (Mf) and house risk exposure survey which was conducted in January of 2014. The three study hamlets of Korona village included Korona, Tongona and Koinduna (Figure 5.1). All individuals from the first survey and their family members living in the same house were asked to take part in the Mf and risk survey. The Mf survey was conducted to confirm active disease transmission in each household and better understand how it may vary across the village. The risk exposure survey was conducted to better understand the transmission dynamics at household level. Approximately 300 individuals $\geq$ 5 years were targeted. A team of 3 technicians and one local assistant conducted the survey.

Figure 5.1. Map of study sites and distribution of households



5.2.2 MF survey

Consenting individuals were tested for active transmission by a Mf night blood survey conducted between 10pm and 2am coinciding with nocturnal periodicity of *W. bancrofti* (Melrose, 2000). Venous blood of 5ml was collected per person using EDTA coated vacutainers due to difficulty of transportation and distance of travel between study site and place of slide preparation. The samples were stored in cool boxes containing ice packs and brought back to the laboratory the next day. Before preparing the slides, the vacutainers were spun to homogenize the sample before a slide was made from 3 strips of 20µl of thick blood smears. The slides were flooded with distilled water for at least 3 minutes and left to air dry (Figure 5.2_A). Stains were fixed with methanol then stained with Giemsa for 50 minutes and left to dry (WHO, 2013). The slides were then observed at a magnification of x100 by a 1st and 2nd reading for confirmation by two technicians (one of them myself) (Figure 5.2_B).

Figure 5.2. Preparation of MF slides

A) Prepared MF blood slides being air-dried prior to staining

B) Sorting MF slides to be checked under microscope

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5.2.3 Risk factor questionnaire

The questionnaire shown in Appendix 4 was used to gather information on the following factors in relationship to MF positivity. Some household level data was match from the initial ICT survey (variables linked from the ICT survey are denoted by *)

- demographic characteristics (village, sex, age, length of residency, number of household members, number of bedrooms, sleeping patterns – house boys),
- housing/infrastructure information (house type, roof type, source of water*, toilet type*, refuse habits*, drainage system*, proximity to village edge),
- use of interventions (LLINs, mosquito repellents*) and
- evidence of LF clinical conditions (lymphoedema, hydrocele, etc.,).

5.2.4 Data management, analysis and mapping

Data in the field were recorded on paper forms and were later entered into Microsoft Excel database and further exported into PASW Statistics version 22 (SPSS Inc., Chicago, IL, USA) for statistical analysis. Mf prevalence was expressed as the percentage of infected individuals over the total number of individuals examined by the different risk factors. The intensity of infection was computed when the Mf count was available as arithmetic means (MF/µl). The household prevalence was also examined to get crude estimates of infection densities by examining the number of people infected per number of household members.

Descriptive and statistical analyses were conducted, including cross-tab comparisons made by statistical test Chi square test with p-values <0.05 as significant. Difference between infection rates by each of the demographic, housing/infrastructure, and intervention variables were also highlighted using graphs of mean measures and standard errors of the means.

The geographical coordinates of each household were recorded the same as outlined in Chapter 4. Maps were created using QGIS software to show Mf prevalence and density distributions across the three hamlets. The interpolation tool in QGIS was used to create buffer zones around each household and its infection rate at approximately 3m intervals to show the high and risk zones around each house and how they vary within each hamlet. Selected significant variables were overlaid on the interpolated maps to determine if there was an obvious spatial relationship between infection and risk factor.

5.3 Results

5.3.1 Demographic characteristics and prevalence

The survey was conducted over a week period in January, 2014 with a team of 9 people, including myself and 2 local aids. In total 301 individuals from 84 houses were surveyed. Each study hamlet was visited and a total of 51 households from Korona (187 people), 19 households from Tongona (n=67 people), and 14 households from Koinduna (47 people) were included. While all individuals per household were targeted, due to work and other domestic commitments or travel arrangements, and also the late time of survey (Mf survey took place between 1000-0200hrs) approximately 20 -80% of household members were included.

Overall, the Mf prevalence was 29.9% (n=90/301). There was an association with ICT positive households (from survey in Chapter4), with a higher Mf prevalence in ICT positive households compared with non-ICT positive households as shown in Figure 5.3. More generally, across the three hamlets, a wide range of prevalence was found; Korona (24.6%; n=46/187), Koinduna (31.9%; n=15/47) and Tongona (43.3%; n=29/67), which is shown in Table 5.1. The Mf prevalence in Tongona hamlet was found to be significantly higher than Korona and Koinduna (Figure 5.4_A). When Mf rates were examined by village, Koinduna (21.6/µl) had a higher average count compared with Korona (16.6 /µl) and Tongona (17.3/µl) Figure 5.4_B.

Figure 5.3. Average Mf count by ICT positive and non-ICT positive house

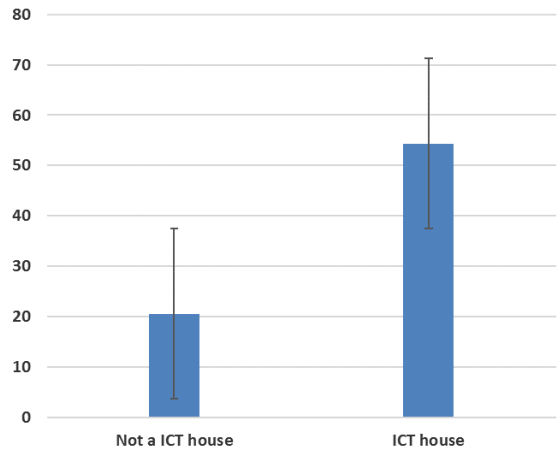
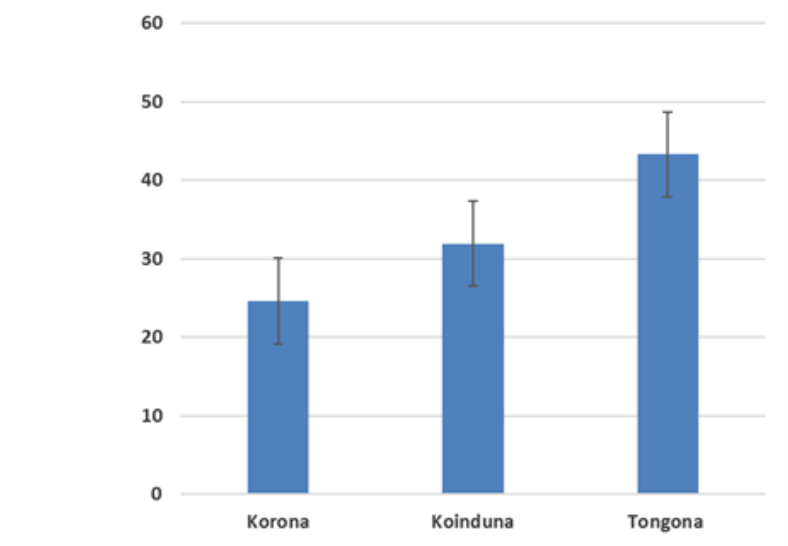


Figure 5.4. Mf prevalence and average Mf/µl by hamlets with standard error bars indicating significant differences

A. Mf prevalence by hamlet



B. Average Mf/µl count by hamlet

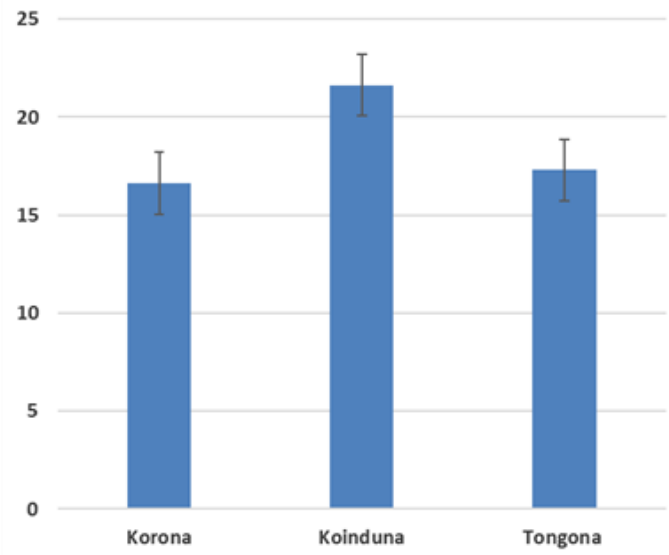


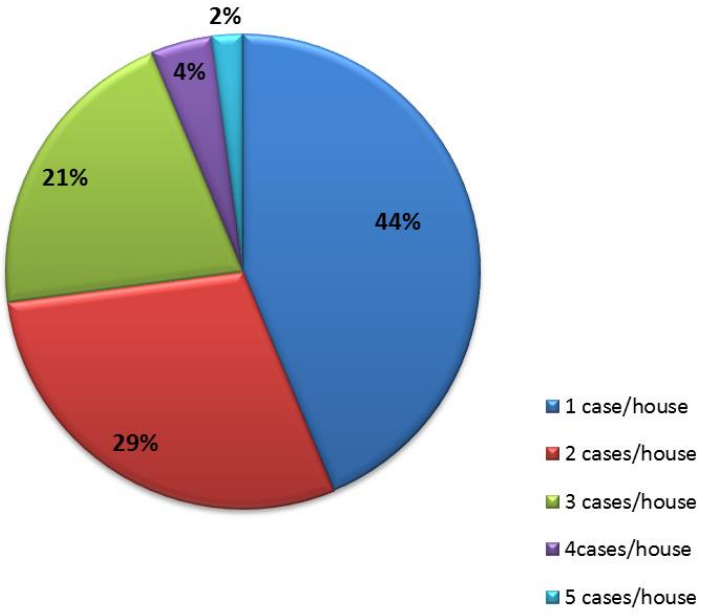
Table 5.1. Summary of demographic characteristics and Mf prevalence

	Total	Mf positive (N)	Mf positive (%)	Stats
				Pearson Chi-Square (p-value)
Demographic Characteristics				
Hamlet				
Korona	187	46	24.6	
Tongona	67	29	43.3	
Koinduna	47	15	31.9	
				P=0.016**
Sex				
Female	1284	29	23.4	
Male	177	61	34.5	
				P=0.039**
Age Group				
<10	37	3	8.1	
10-19	126	25	20.5	
20-29	45	17	37.0	
30-39	45	13	28.3	
40-49	32	22	71.0	
> 49	17	10	52.6	
				P=0.00**
Houseboy				
Yes	31	8	25.0	
No	270	81	30.5	P=0.522
Length of Residency				
<5yrs	6	0	0	
5-9yrs	1	0	0	
10-14yrs	3	0	0	
Lifetime	291	90	30.9	
				P=0.309
People in Household				
< 6 average	107	33	30.8	
≥ 6 average	194	57	29.4	
				P=0.712
No. Bedrooms				
< 3 rooms	63	23	36.5	
≥3 rooms	238	67	28.2	
				P=0.077
Sleeping pattern				
Other house	4	2	50.0	
Only this house	297	88	29.6	
				P=0.367

** P-value less than 0.05 indicating significant difference

In total there were 48 positive households out of the 84 surveyed. The proportion of households with number of Mf positive individual is shown in Figure 5.5, highlighting that nearly half (n=21, 43.8%) of the households had at least one Mf positive case, while nearly one third of households had two Mf positive cases (n=14; 29%) and nearly one third of households had three or more positive Mf cases (n=13; 27.4%). There was no pattern to the three houses reporting ≥4 MF cases per houses with one house from each hamlet.

Figure 5.5. Proportion of Mf positive houses showing number of Mf cases found in the house

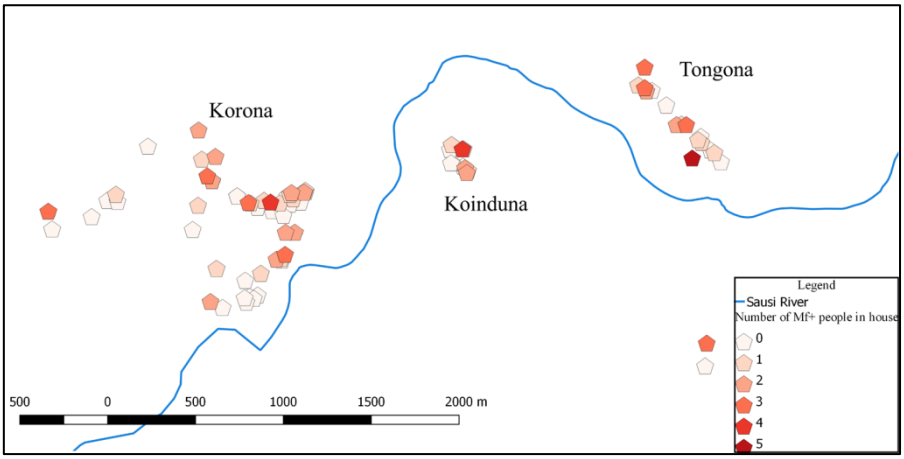


The number of Mf positive individuals per household and number of people per household are shown in Figures 5.6_A and B respectively, showing a similar distribution in population and a wide spread of Mf infection across all hamlets. The proportion of Mf positive people per number of people per household is shown in Figures 5.6_C.

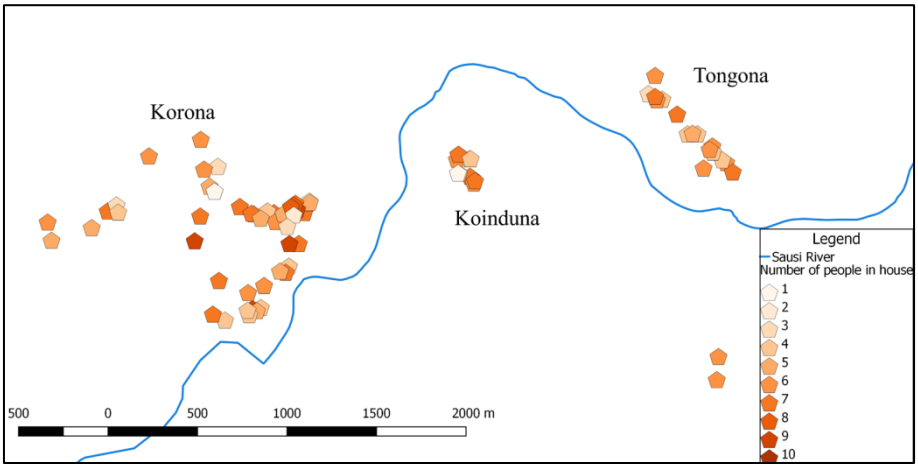
There is a wide distribution of Mf infection at household level which is evident throughout all hamlets, with higher Mf positive people per number of people per household in Tongona. The interpolation of the number of Mf positive people per households in each hamlet is shown in Figures 5.6._A-C. This helped to define higher and lower risk zones within hamlets. Korona had a lower Mf positive people per household which was distributed towards the outer edges of the hamlet, shown in Figure 5.7_A, compared to Koinduna and Tongona which showed higher Mf positive people per household with single point intensities towards the south west edge and northern parts of the hamlets respectively as seen in Figure 5.7_B and C.

Figure 5.6. Prevalence maps of Mf positivity and house population numbers

A. Mf positive people per house



B. Number of people per house



C. Number of Mf positive people per number of people in house (Mf house rate)

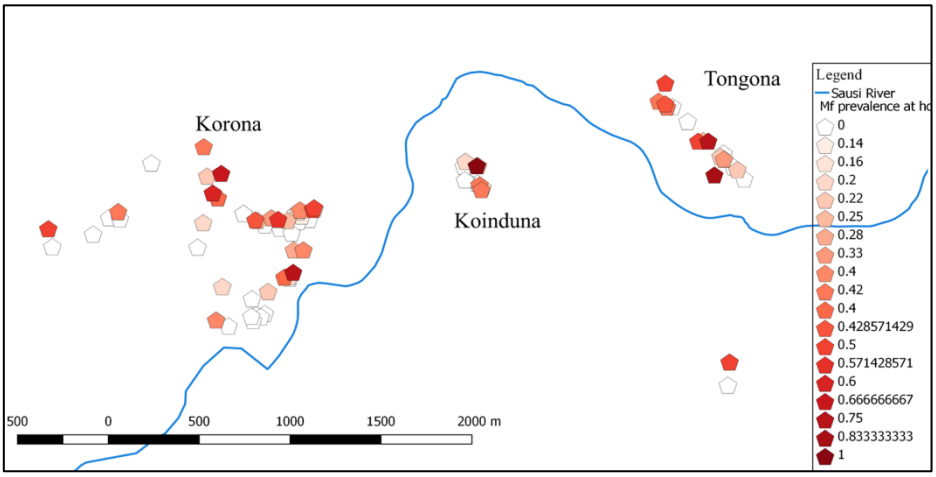
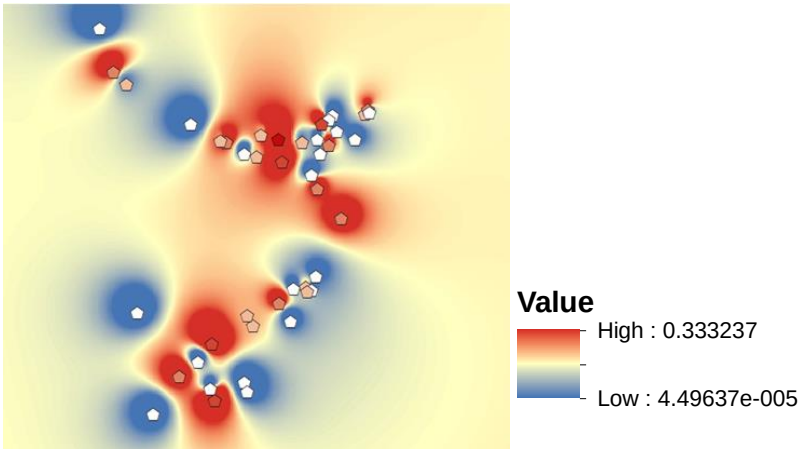
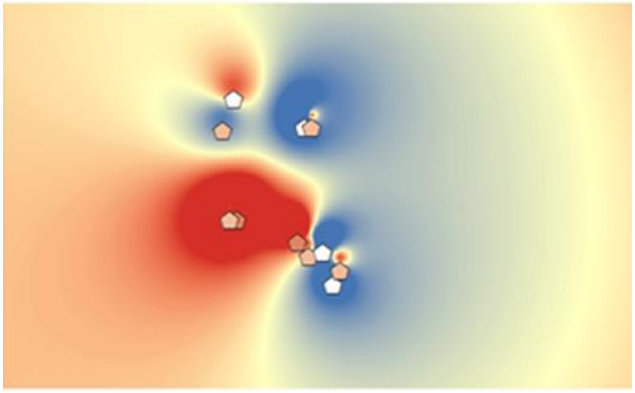


Figure 5.7. Interpolated maps of number of Mf positives per household in each hamlet

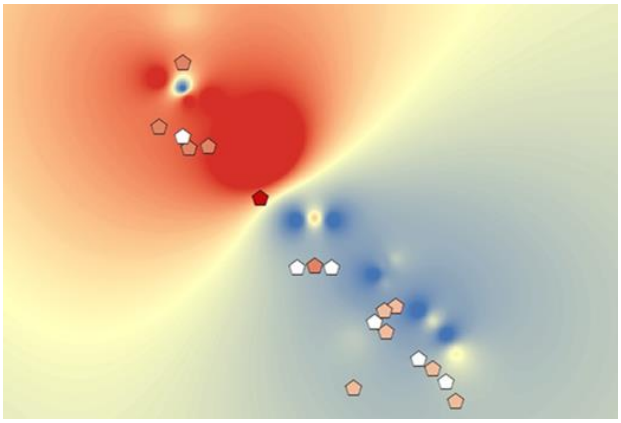
A. Korona hamlet



B. Koinduna hamlet



C. Tongona hamlet



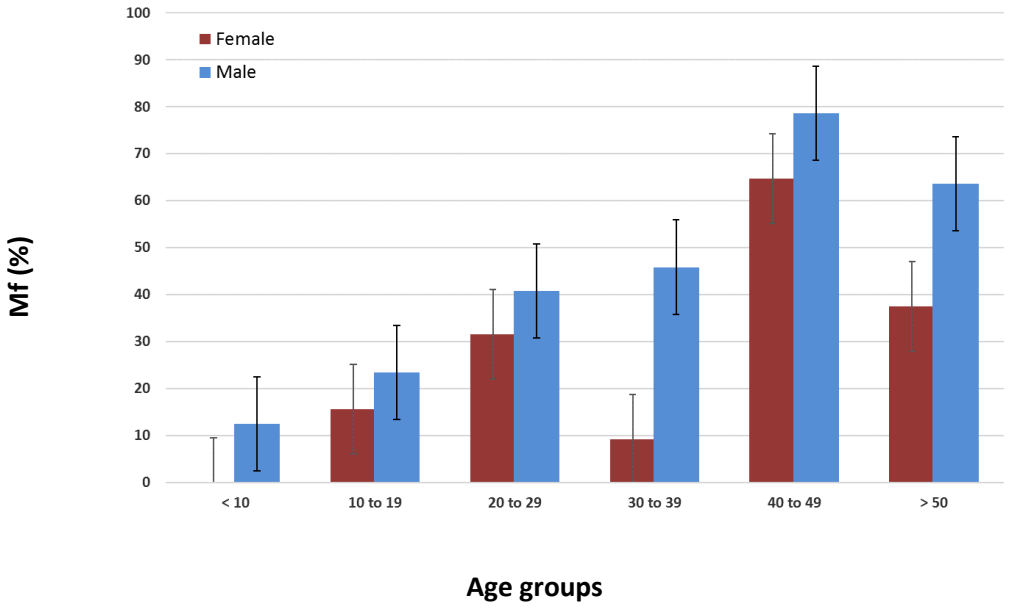
Sex and age: Of the 301 tested individuals, there were 177 males (58.8%) and 124 females (41.2%). There was no significant difference in the proportion of males and females in each village (chi square, p-value $\geq$ 0.05). The age of individuals tested ranged from 6 to 70 years (mean age=23.8) and was similar between males (range 6 to 70; mean= 22.7) and females (range 6 to 69; mean=25.2). Overall the age average of Mf positive people was 31.3 years, which was significantly higher than the average age of Mf negative individuals which was 20 years ($p < 0.05$). When comparing Mf positivity by sex, males (34.5%) were found to have a higher prevalence than females (23.4%) which was significant (p=0.039), shown in Table 5.1.

There was an overall increasing trend in prevalence with age and significant differences were found between certain age groups and by sex. The 40-49 year olds of both sexes were significantly higher compared to the other age groups, this is shown in Figure 5.8A (male blue; female red). Males in this age group had 78.6% Mf positivity and females 64.7% positivity. While the prevalence in individuals aged between 10 and 39 years was significantly lower than the 40-49 age group, there was a gradual stepwise increase in prevalence with males in the 20 to 29 year age group (40.7%) having a significantly higher prevalence to males <10 year old (12.5%). There was a decline in prevalence in the >50 year age group by sex.

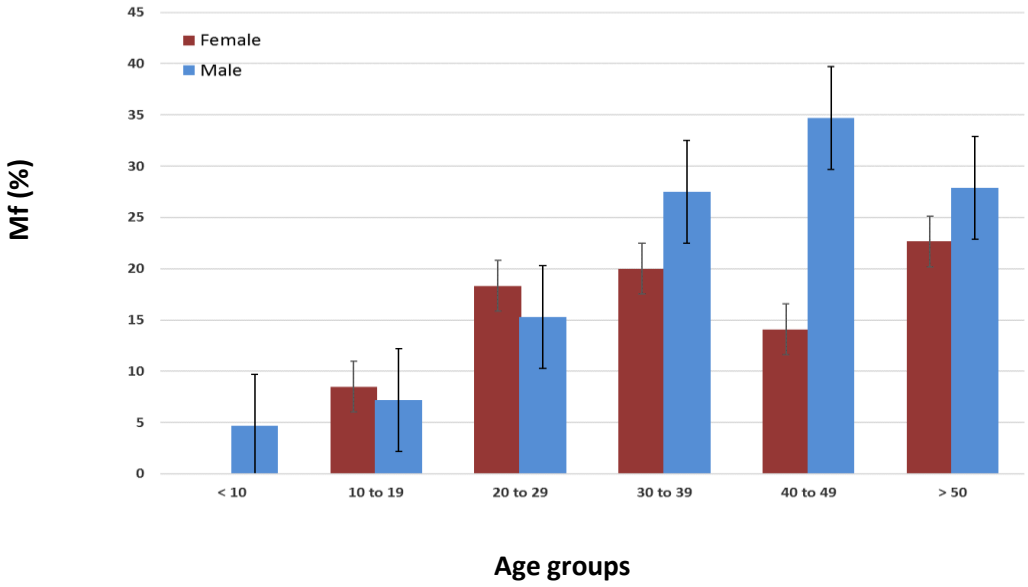
Similarly, there was an overall increasing trend in the average Mf count with age and significant differences were found between certain age groups and by sex. The average Mf counts for males was 18.9/µl and for females was 15.1/µl ($p<0.05$). For males the 40-49 year olds were found to be significantly higher compared to <30 year age groups, this is shown in Figure 5.8_B (male blue; female red). Males in this high risk 40-49 year age group had an average of 34.7/µl, the lowest was recorded in the <10 year age group. For females, the highest Mf counts were in the 30-39 year and >50 year age groups with counts of 18.3/µl and 22.7/µl respectively.

Figure 5.8. Mean Mf prevalence and Mf counts by sex and age distribution

A. Mf prevalence



B. Mf count



Length of residency: Almost all individuals (96.7%) reported they were permanent residents and all those were Mf positive (n=90) have lived in the area all their lives. Of those who were not permanent residents, a small number were either health workers or teachers stationed in the area and few were visiting relatives (n=9) with none found to be Mf positive (Table 5.1).

Household size (people and rooms): The average number of people living in households ranged from 6.2 in Korona and Tongona and 5.4 in Koinduna, with an overall average of 6.1 people per house. There was no difference in Mf prevalence between households with <6 people (n33; 30.8%) and households with ≥ 6 people (n=57; 29.4%) (Table 5.1). The average number of bedrooms per house ranged from 3 in Korona to 3.2 in Tongona and Koinduna (overall average 3.1 rooms) with no significant difference in prevalence between household with less than 3 bedrooms (n=23; 36.5%) or 3 or more bedrooms (n=67; 28%).

Houseboys

In total 31 houseboys from 31 main family houses were included in the survey. An average ranging from 3.2 boys and young men live in houseboys in the area. There are three times more houseboys in Korona (n=20) than Tongona (n=6) and Koinduna (n=5) and there was no significant difference observed in Mf prevalence (Table 5.1).

5.3.2 Housing/Infrastructure characteristics and prevalence

House type, height and ceiling/roof: There were two main types of houses in the three hamlets surveyed. The majority of people lived in bush material (n=232; 77.0%) houses made from woven sago leaves for roofs, sticks and woven bamboo blinds for walls, split sago or palm trunks for flooring and unscreened windows or in semi-permanent houses (n=66; 21.9%) with corrugated iron roofs and sticks and bamboo blinds for walls, split sago or palm trunks for flooring with unscreened windows. Only three people lived in a permanent house constructed with timber, corrugated iron roofing and screened windows. All houses were built above ground. No individuals living in the permanent houses were found to be positive, compared

with 12.1% and 35.3% recorded for the semi-permanent and bush material houses respectively (Table 5.2). There is significant difference in the house type (p=0.001). (Examples of the different structures of houses are shown in Figure 4.8 of Chapter 4).

Figure 5.9 shows the different house types with a significantly higher Mf prevalence found among individuals living in the bush material houses compared to the other type of houses.

Interpolation maps of Mf positives per house in each hamlet with the house and roof types overlaid (Figure 5.10_A-C) showed Korona to have more semi-permanent houses evenly distributed throughout the hamlet. The Mf positive people per house appeared to be lower around the semi-permanent houses, however no formal spatial statistical analysis was conducted (Fig.5.10_A). Koinduna had only bush material houses (Figure 5.10_B) and Tongona had only one semi-permanent house which was found to be away from the intense Mf positive people per household spot which was located north of the hamlet shown in Figure 5.10_C.

Figure 5.9. Different house types showing differences in Mf prevalence

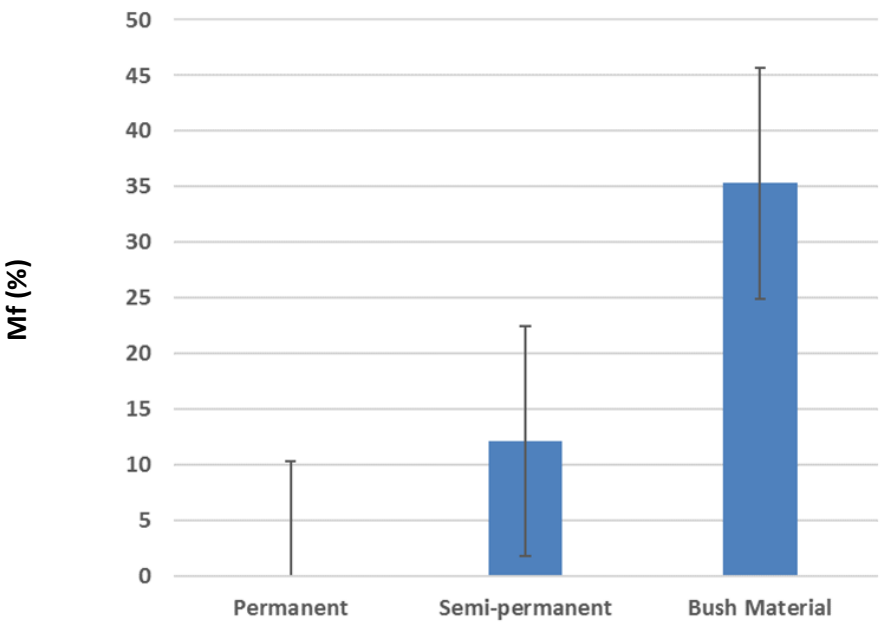


Table 5.2. Summary of Housing and infrastructure characteristics and Mf prevalence

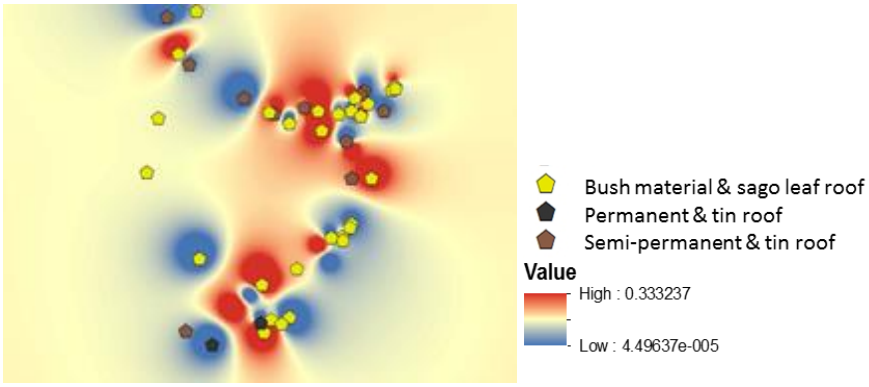
	Total	MF positive (N)	Mf Positive (%)	Pearson Chi-square P-value
Housing/ Infrastructure Characteristics				
House Type				
Permanent	3	0	0	
Semi-permanent	66	8	12.1	
Bush material	232	81	35.3	
				P=0.001**
Roof type				
Sago	232	82	35.3	
Tin	69	8	11.6	
				P=0.000**
Water source *				
Streams/river	113	43	32.8	
Bore hole/rainwater	6	5	83.3	
Tap water (from lake)	118	40	25.3	
Combination				
				P=0.006**
Toilet type *				
Shared toilet	20	7	35.0	
Own toilet next to house	249	72	28.9	
Bush/river				
				P=0.624
Refuse habits *				
River/stream/bush	49	7	14.3	
Common dug out pit	19	11	57.9	
Dugout pits next to house	227	70	30.8	
				P=0.002**
Drainage system *				
Yes	217	56	25.8	
No	78	32	41.0	
				P=0.012**
Proximity to village edge*				
<12m	147	45	30.6	
>12 m	122	34	27.9	P=0.761

* Household level information linked from ICT survey

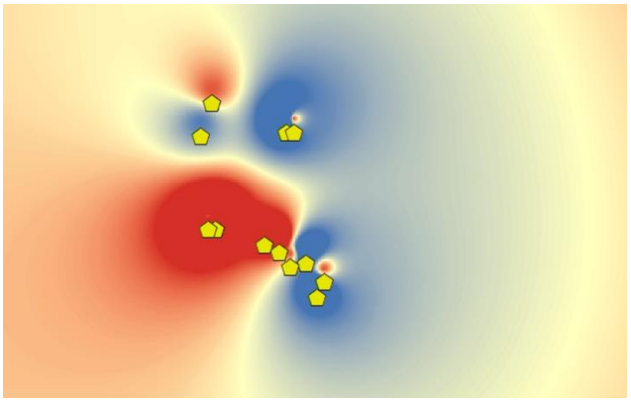
** P-value less than 0.05 indicating significant difference

Figure 5.10 Interpolated maps of Mf positives per house in each hamlet with the house type and roof type highlighted

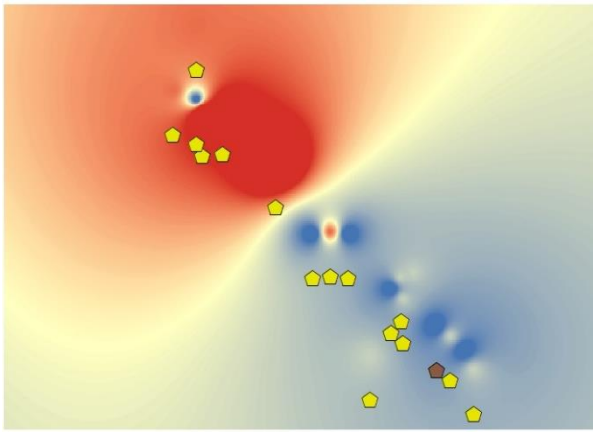
A. Korona hamlet



B. Koinduna hamlet



C. Tonguna hamlet



Water source and toilet type: Households that sourced water predominantly from streams/rivers (n=131) and from main tap water sourced (n=158) through channelled pipes from a nearby lake are similar, and had similar Mf prevalence in people using both these water sources (9.57% and 8.28% respectively). However, a smaller number of people (n=6) (only in Tongona) using borehole water were significantly different from the streams and main supply sources of water, Figure 5.11 shows this clearly. The majority of houses had their own pit toilets (n=270, 87%) which were located close to the main house, and the rest either shared or use the bush and rivers for defecation. No significant differences were found in the types of toilets.

Refuse habits and drainage system: Mf prevalence was similar between people who used their own dug out pits (n=227, 14%) for domestic waste and people who threw their rubbish in the bush or nearby streams and rivers (n=49, 14%), however people who disposed their rubbish in common dump sites had a significantly higher Mf prevalence than the other two groups. Figure 5.12 shows Mf prevalence in refuse habits and the significant difference between the groups. Houses with proper drains (n=56, 25.8%) around them had a significantly lower Mf prevalence than houses with no drainages (n=32, 41.0%) around them. The results are shown in Table 4.2.

Proximity to village

Mf prevalence was similar between people who lived within 12 m and those who lived more than 12 m from the edge of the village.

Figure 5.11. Different water sources showing differences in Mf prevalence

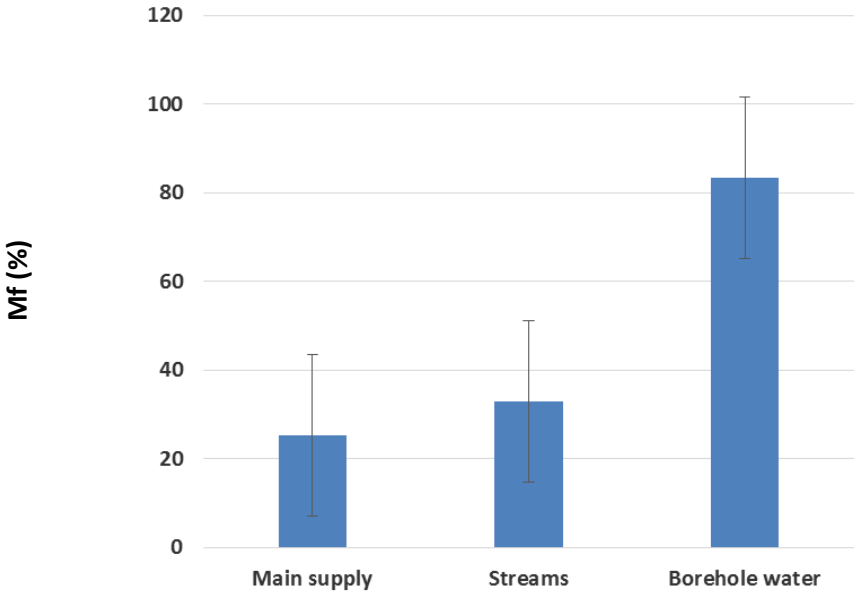
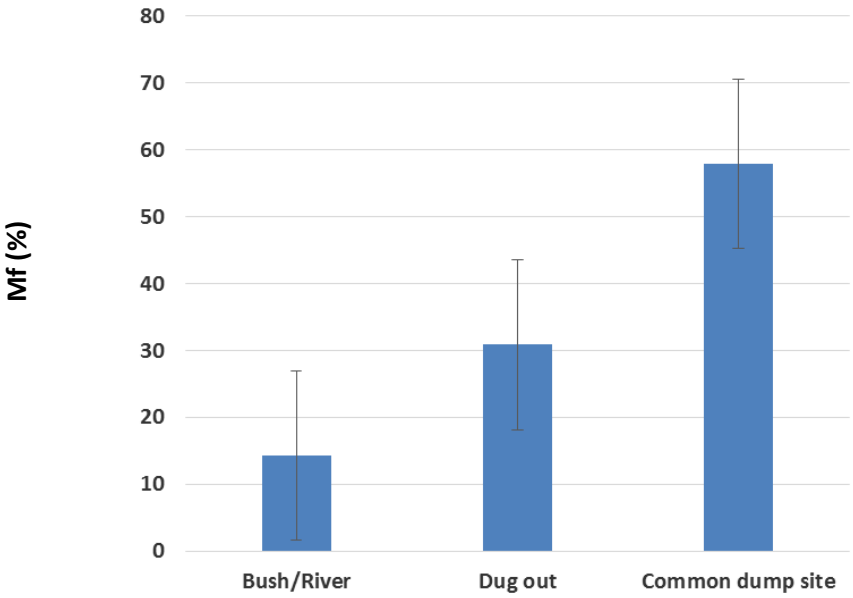


Figure 5.12. Different refuse habits showing difference in Mf prevalence



5.3.3. Use of different types of interventions

LLINs – All participants stated they received LLINs in 2012 as part of a national malaria control campaign. The number of LLINs per household ranged from 1 to 8, with an average of 3.3 LLINs per house. Almost everyone (n=295, 98%) stated they sleep under a mosquito net at night, and there was no statistical difference observed in the owner/use of mosquito nets.

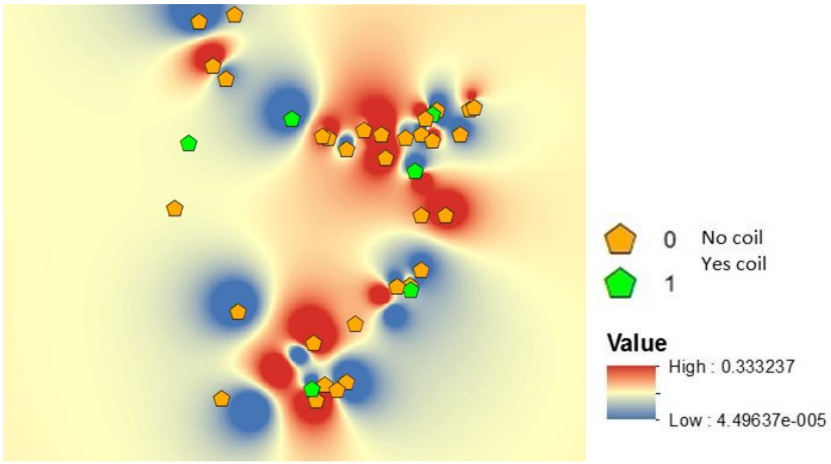
Mosquito coils/repellents: A significant difference was observed for Mf prevalence in people who use mosquito coils/mortein sprays in their house (n=6, 12.2%) while those who didn't use such repellents had twice as much Mf prevalence (n=73, 33.2%) as shown in Table 5.3. The locations of coil houses were overlaid over the interpolated maps of the number of Mf per household to examine if there was any clustering or an association with general hamlet level risk zones. For Korona, in total 10 houses used coils and locations are given in 5.13 A and the location of these on interpolated maps suggested most do not directly occur in the higher risk zone. For Koinduna, no houses used coils, while for Tongona, 4 houses used coils and 3 of these were found to be located in lower Mf prevalence zone (low risk). Interestingly, the one house that used coil in the high zone (Figure 5.6_C) also reported no Mf prevalence as shown in the Figure 5.6_C.

Table 5.3. Use of intervention and Mf prevalence

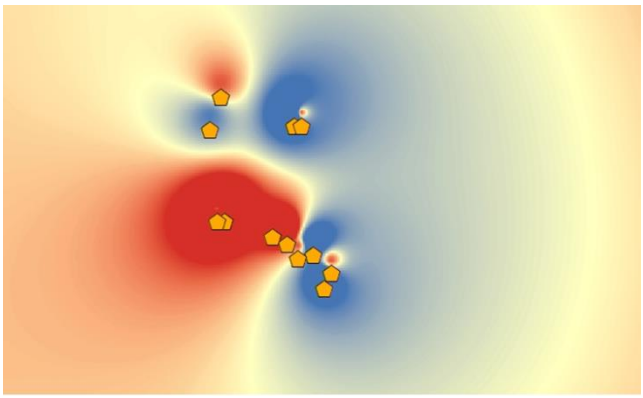
	Total	Mf positive (N)	Mf positive (%)	Pearson Chi-Square P-value
Mosquito coils / spray repellents *				
Yes	49	6	12.2	
No	220	73	33.2	
				P=0.04

Figure 5.13. Household using mosquito coils overlaid the Mf positivity houses

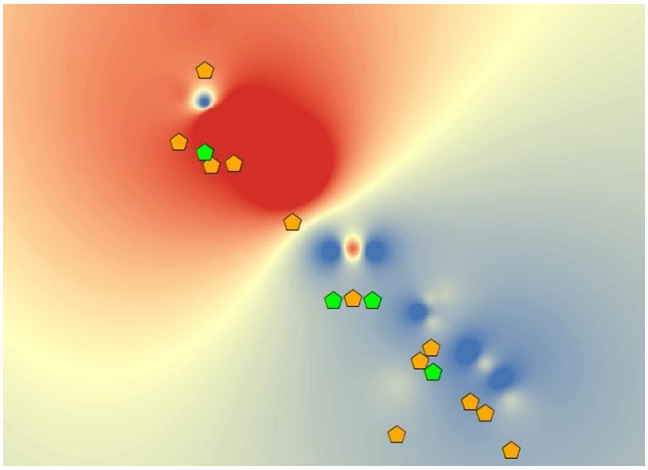
A. Korona hamlet



B. Koinduna hamlet



C. Tongona hamlet



5.4 Discussion

This study is the first of its kind undertaken in PNG to show such variation in LF prevalence within a small geographical area not spanning more than 4kms square. It specifically examined and mapped Mf at individual and household level within a highly endemic village, made up of three hamlets and looked at potential reasons for any variation that may be present within the village. It provides key information to the National LF Programme information on the prevalence and associated risk factors, and will also be an important sentinel site to monitor the impact of any intervention scaled up in this area over time.

Interestingly, there were significant geographical differences in Mf prevalence between hamlets distanced only 1-2 kms apart. The hamlet of Korona had a lower risk compared to the other two hamlets, which may be due to a number of factors. Korona hamlet is situated down closer to a main road connecting two provinces and has less natural vegetation suitable for *Anopheles* vector habitats (reviewed in Chapter 3) with more development including a primary school and a health centre located within its perimeter. It also has more semi-permanent houses with tin roof/ceilings compared to the other hamlets, which mainly had bush houses with sago leaf roof/ceiling. This suggests that slightly better housing, particular a ceiling made of better materials may be more protective as they keep the mosquitos away. This finding is similar to the ICT survey in Chapter 4, where Raikos community members mostly lived in better constructed semi-permanent and permanent houses, and were found to be at low/no risk of LF infection.

There are no studies that refer to housing structure and *Anopheles* transmitting LF, however literature on malaria transmission and housing structures indicate that house design, including screening eaves/ ceilings can be protective against *Anopheles* mosquitoes and reduce transmission (Lwetoijera et al., 2013, Ogoma et al., 2010, Atieli et al., 2009, Lindsay et al., 2003). Better ceilings have been highlighted as an acceptable intervention with netting and insect-screen ceilings in particular substantially reducing biting rates. This is further confirmed by the recent

review by Tusting and others (2017) on Demographic Health Survey (DHS) data who highlighted the importance of proper housing and especially potential protection of tin roofs, which was also found to be significant in this study.

This study has found that over half of the households have two or more infected people hence indicating a highly endemic area. Interestingly, when the Mf prevalence was compared with the previous ICT survey (Chapter 4) data, the ICT positive households were found to have higher Mf rates of infection / risks. This association between Mf and ICT rates may be informative for the LF programmes as it suggests that conducting an ICT may be sufficient to detect the high risk people and households within a village or area. This could save time and resources. The interpolation of Mf positives per household potentially pinpointed areas of higher risk in the hamlets, and may help to identify potential vector breeding sites in close proximity. Studies on LF vectors in PNG showed variation in feeding behaviours and ecological habitats between villages in close proximity to each other (Bockarie et al., 2009; Chapter 3). Further, malaria studies done in Yemen and Tanzania also showed a link between infection and mosquito numbers in certain areas of a village and associated with higher number of people in house (Al-Eryani et al. 2016, Kaindoa et al. 2016). Russell et al., (2013) also showed that different *Anopheles* vector species have different distribution on a micro spatial distribution and were attracted to highly populated houses within a village.

In addition to human household density, the age and sex composition of people within each house may be an important factor for risk as well. Males and older age groups are shown to have higher risks of Mf infection. This is in line with LF studies both in PNG (Desowitz et al., 1993) and in other countries, which have shown males are generally at higher risk than females. This could be due to male socio-behavioural practices where they are more likely to go to sleep later in the night, and not always using a mosquito net to sleep, hence exposing them more to infective mosquito bite. It is relevant in PNG especially due to its unique cultural aspect of houseboys. This highlights the importance of knowing who is at higher risk

for the LF National Elimination Programme, so it can target at risk population with tailored public health messages and raise awareness on potential risk factors.

Other potentially important risk factors found included the household's main water source and refuse habits. However, it is not clear why these two factors should be associated with LF transmission and the risk of Mf infection, so these variables should be looked into in greater detail in relation to local mosquitoes or their breeding habitats. Drainage around houses was also found to have higher Mf transmission associated with it and this may suggest an association with vector breeding habitats. Entomological studies in Madang Province have shown *An. punctulatus* to freely breed in clean rain collected puddles closer to human dwellings (Bockarie et al., 2002). It may be an important vector in Korona, and a better understanding the characteristics of local *Anopheles* larval habitats may help to assess appropriate interventions such as environmental management or vector control (WHO, 2016b).

Under the National Malaria Control Program with the Global Fund support had rolled out a nationwide LLIN campaign in mid-2012, hence the use of LLIN was widespread and every household had in possession at least an LLIN. This can potentially have an impact on transmission as found with other studies in other part of the world (van den Berg et al., 2013). In PNG, Bockarie et al., 2002 found untreated bednets and ITNs associated with a decline in Mf prevalence, also in the 1980s in the Solomon Islands, Webber (1977, 1979) found IRS for malaria control to eliminate LF on the small island country. Moreover, since the recent LLIN distribution, two studies have shown the impact of LLIN on malaria (Hetzel et al., 2016) and filariasis (Reimer et al., 2013) with a decrease in infection and transmission. Most significantly, Reimer et al., (2013) found LLIN to be very effective against *Anopheline* vectors in the absence of other intervention. Therefore, it may be useful to have a follow up study to see the impact of LLIN in this village in the near future as LLINs were only distributed a few months before the survey was conducted, and therefore may potentially be viewed as a pre-intervention baseline study.

This study found that the use of mosquito coils was not as common as LLINs, however they appeared to have potential impact on lowering Mf transmission risks, suggesting they could be an important protective intervention. Maia and others (2013) evaluated the use of coils in Tanzania concluded that a high coverage of repellents would significantly reduce man-vector contact. In the absence of a sustainable and effective LF MDA programme in PNG at this point in time, the use of LLINs in combination with high coverage of mosquito coils may be something that the National LF programme could encourage to increase for protection from vector borne diseases. This could be implemented with specific information, education and communication (IEC) materials, radio awareness or through community health outreach programmes. The LF programme should also work in close collaboration with the Malaria National Program and other health programs that also do outreach into communities and do overlapping interventions for disease control and elimination.

Chapter Six

Distribution and incrimination of *Anopheles* species in a Highly Endemic Village

6.1 Introduction

Previous entomological studies carried out in LF endemic parts of PNG have shown the main vectors for *W. bancrofti* are the members of the *Anopheles punctulatus* complex, made up of nine species. The three main species are the *An. punctulatus, An. koliensis* and *An. farauti* which comprises of 7 sibling species (Cooper and Francis, 2002, Cooper et al., 2002, 2009, Chapter 3).

The high LF transmission rates found in the human populations in the study area of Madang, and specifically in Korona village (Chapter 4 and 5), indicates that there are efficient *Anopheles* vector species present. The review of entomological studies in PNG and specific maps of the Madang study area presented in Chapter 3 highlight that *An. punctulatus*, and *An. farauti* 4 are likely to be the main vectors responsible for transmission.

In this specific area of the Madang Province, there have been no studies incriminating these two *Anopheles* species, however in the other regions of the province, other studies (Bockarie et al., 1998, Reimer et al., 2013) found *An. punctulatus* and *An. koliensis* to be the main species present but *An. punctulatus* as the main vector responsible for LF transmission, which also was found to have reduced infection rates after the introduction of ITNs for malaria control.

Knowing which vectors have their transmission impacted with certain type of vector control is important – not all *Anopheles* species may be the same (Thompson et al., 2016, Reimer et al., 2016). The role of personal repellents on the different mosquito species and their ability to transmit *W. bancrofti* may also be important, for

example, some vectors may not bite inside the house and subsequently be exposed to the ITNs and repellents/mosquito coils – where as others may be.

Understanding the *Anopheles* peak biting times (seasonally as well as by day/night) may be important for understanding environmental associations and also for targeting specific control measures and relevant messages. There are two main seasons in PNG, the wet season is between November and April, while the dry season is between May and October. During the dry season, and the highest abundance/ biting rates and infection rates of the main *Anopheles* species has been found in the wet season (Bockarie et al., 1998, 2000). Knowing when the highest transmission risk occur will help target control at the most optimal time to have impact. Also it is important to also establish when the vectors bite during the day/night to determine if the control measure in place will be effective e.g. early biting vectors vs. ITN use at night.

In addition, understanding the distribution of the different species across the village may be important if a few main vectors are present and they have different ecological habitats (or breeding sites) that may be targeted. In the previous Chapters 4 and 5, there were significant difference in housing structure, which also may be important for vectors and may influence their host seeking behaviour and ability to transmit, and as shown in Chapter 5 the risk of human infection can vary at a fine geographical scale i.e. hamlet level. Therefore similar fine scale variation in the different *Anopheles* species may also be evident as shown elsewhere (Al-Eryani et al. 2016, Kaindoa et al. 2016 . Russell et al. 2013)

Xenomonitoring which is the use of entomological techniques to assess LF transmission in vectors is not a main recommendation of the GPELF, however in the absence of an active LF Elimination Programme in PNG, it may be an activity that can be conducted collaboratively with the malaria control programme, which currently has more funding available and a wider scope. A collaborative effort between programmes may help with surveillance and assess the impact of recent distribution of ITNs/LLINs. Examining the vectors transmitting LF in PNG is

important (especially in highly endemic areas, and when so few studies have been done and only in selected areas (Chapter 3).

6.1.1 Aim

The aim of this chapter was to determine the main vector species, their infection rates, and spatial and temporal distribution patterns in the high risk village of Korona, Madang. Specifically, this work aimed to;

- Identify the main *Anopheles* species
- Determine *W. bancrofti* infection rates in the main *Anopheles* species
- Determine the peak abundance times by comparing seasonal patterns and daily biting times of the main *Anopheles* species
- Map the spatial distribution of each *Anopheles* across the village and within each hamlet
- Examine the positivity of *Anopheles* species and key characteristic found in Chapter 5

6.2. Methods

6.2.1. Adult mosquito sampling – wet and dry season

Landing catch method

Mosquitoes were collected using the all-night landing catch method as previously described by Bockarie et al, (1996). This method of collection was only used outdoors next to MF positive houses (identified in Chapter 5), where adult collectors were seated on benches, with their feet and legs bared to the knee. The collectors, using aspirators and aided by light from battery-operated flash lights captured mosquitoes that attempted to land on them in search of a blood-meal. Collectors worked in pairs, with one starting at 18:00 and was relieved by the second pair at midnight, who then worked till 06:00. Each village was divided into four sections, and mosquitoes will be sampled from a different house in each section on different nights consecutively within a week. These landing collections were performed for four nights, twice in the wet season, and twice in the dry season, in each of the 3 hamlets. Mosquitoes caught were placed in paper cups in cool boxes and taken to a local laboratory to be morphologically identified into species and recorded by date, location (house owner and GPS coordinates), and stored dried for further testing. Hourly biting patterns for each main *Anopheles* species was examined across hamlets

Figure 6.1. Local volunteers using aspirators to collect blood seeking mosquitoes

Ethical clearance for local collectors

All local mosquito collectors gave written consent to take part in landing catch sampling. They were explained the risk and provided with information on malaria prophylaxis. Ethical approval was obtained from LSTM and PNG research ethics committees for this specific work.

6.2.2 Morphological identification of vectors

Captured female mosquitoes were sorted according to species using Belkin's morphological identification keys (Belkin, 1962) for the *An. punctulatus* group of mosquitoes and stored dried in enclosed containers containing silica gel. The three main vectors of the *Anopheles punctulatus* group, *An. punctulatus, An. koliensis* and *An. farauti* are distinguished from each other from the presence/absence of sector spot on the wings and the colour of their proboscis.

Figure 6.2. An *Anopheles punctulatus* morphologically identified using field microscope

6.2.3 DNA extraction using Qiagen DNAeasy

DNA was extracted using Qiagen DNeasy Blood and Tissue Kit (Cat. #69506, Qiagen Inc, Valencia, CA, USA) as described previously by Fischer et al., (2002) with a modification from S. Laney (Williams, S. Laney, S., et al., 2002), where instead of using pestles to ground the mosquitoes, zinc-plated .177 calibre BBs (4.5mm) were used.

Mosquito specimen or part (head or body) were place in 2ml graduated strip tubes with a sterilised BB before adding 180µl of PBS X1 solution. Lids were fastened shut and placed in 96 plate tube boxes and put into a TissueLyser machine (Qiagen) at a frequency of 30 shakes/second for 5 minutes. The tubes were removed and briefly centrifuged to draw away any grounded mosquito sludge from the lids. An aliquot of 200µl buffer AL and 20µl of proteinase K were added to samples, vortexed briefly to mix before samples were put into 70°C oven (Ilumina® hybridization oven) for 10 minutes. After that, the tubes were centrifuged briefly and another 20µl of proteinase K added, briefly vortexed and placed in 56°C temperature oven for an hour. After which, the tubes were centrifuged at maximum speed for 8 minutes in a 96 well plate centrifuge machine (Qiagen) and the supernatant pipetted and mixed with 400µl of Buffer AL/E and placed into mini spin columns. Plates were sealed with airpore tape and centrifuged at 6 000 rpm for 10 minutes and the flow-through and collection tubes were discarded. The mini spin columns were placed over new collection tubes and 500µl Buffer AW1 was added to the mini spin columns, covered with airpore tape and centrifuged at 6 000rpm for 5 minutes. The supernatant and collection tubes were discarded, columns were again placed in new collection tubes and 500µl Buffer AW2 was added. Columns were centrifuged at 10 0000rpm for 5 minutes, the supernatants and collection tubes discarded and columns were transferred to new elusion microtubes (provided in kit), 200µl of Buffer AE added, sealed with airpore tape and left at room temperature on the bench for a minute before centrifuged at 6 000rpm for 2 minutes to collect the extracted DNA samples which were stored at -20°C to be used for mosquito species identification and determine *W. bancrofti* infection in specimens.

6.2.4 Molecular identification of *An. punctulatus* complex species

The restricted fragment length polymorphism (RFLP) PCR assay as described by Benet and others (2004) was used to confirm sibling species within the *An. punctulatus* complex. This was done using PCR -restriction fragment length polymorphism (PCR-RFLP) analysis of the internal transcribed spacer region 2 (ITS2) of the ribosomal DNA using the methods and primers of Beebe and Saul (1995), while *Msp*1 digestion of the ITS1 resolved sibling species within the *An. farauti* complex.

6.2.5 Molecular determination of LF infection rates

LF infection rates were determined using *Wuchereria bancrofti* Taqman protocol adapted from Rao et al, 2006. The mosquito samples were separated into head, thorax and abdomen before DNA extraction. DNA was extracted as described in section 6.2.3 above. One µl of extracted DNA was added to 5µl of SensiMix, 0.45µl of 10µM Wb forward primer, 0.45µl of 10µM Wb reverse primer, 0.125µl Wb probe and 2.975µl of water in an optical 96 well plate. Plates are sealed with optical caps and centrifuged at 2 000rpm briefly to get the reaction to the bottom of the plate. The taqman PCR cycle is programmed as follows 95°C for 10 minutes, then 40 cycles of 92° C for 15 seconds and 60°C for 1 minute.

Figure 6.3. Using the Qiagen DNA extraction kit to extract DNA from mosquito samples

Department of Vector Biology, Liverpool School of Tropical Medicine

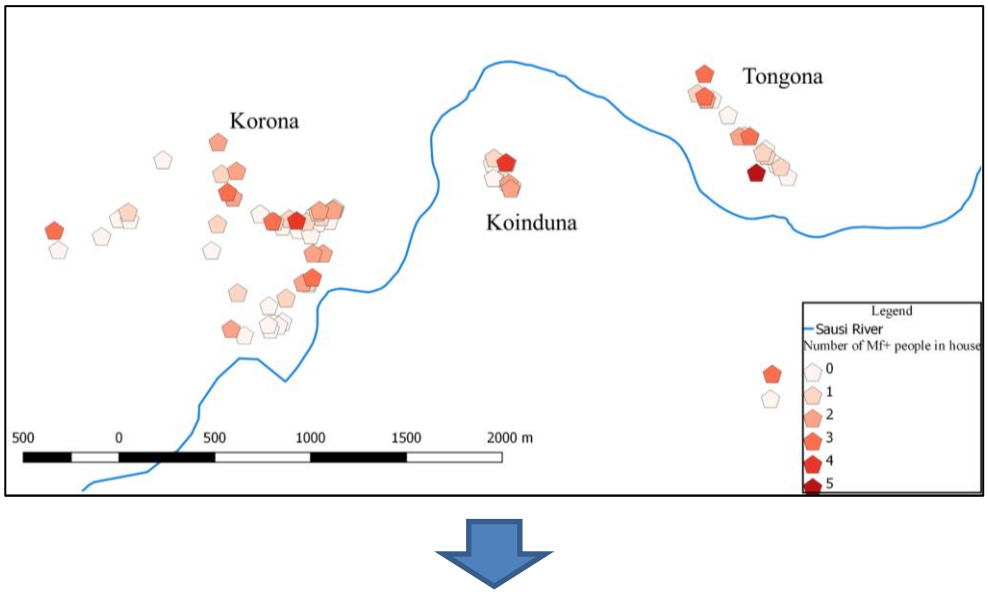
6.2.6 Spatial Distribution of *Anopheles* species and key household characteristics

To examine the spatial distribution of potential vectors, the interpolation tool in QGIS was used to create maps of each species abundance patterns for the village overall, and for within each hamlet. Figure 6.4_A shows the human Mf positive house (from Chapter 5) from which houses were chosen for wet and dry entomological collections; the location of the collection houses is shown in Figure 6.4_B.

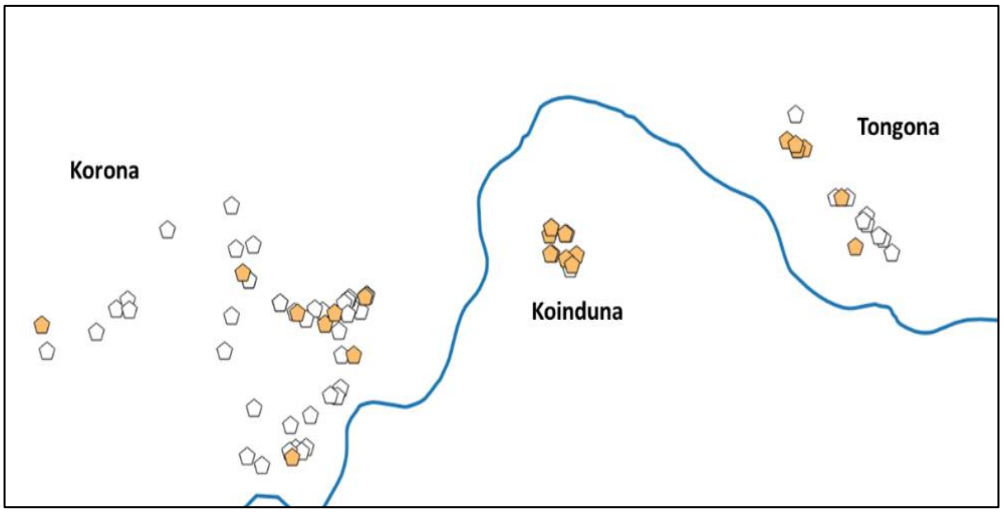
Differences in interpolated patterns of human infection and *Anopheles* abundance were compared. The household characteristics of *Anopheles W. bancrofti* positive houses were compared with negative houses.

Figure 6.4. The study village comprises of three hamlets, coloured houses indicates a) Mf positive houses and b) entomological sampling houses

A. Mf positive people per house (as shown in Chapter 5)



B. Mf positive houses used for the entomological collections



6.3 Results

6.3.1 Adult mosquito collections

A total of 1370 mosquito specimens were collected from the all-night landing catch method over an 11 month period, from November, 2013 to September, 2014. Two 4 nightly collections were conducted in the wet season, one in November, 2013 and the other in March, 2014, which yielded 74.45% (n=1020) of the mosquitoes while another two 4 nightly collections were conducted for the dry season in July, 2014 and September, 2014 which yielded 25.54 % (n=350) of mosquitoes. A total of 26 houses in 3 hamlets were used to conduct all-night outdoor landing catch outside these houses.

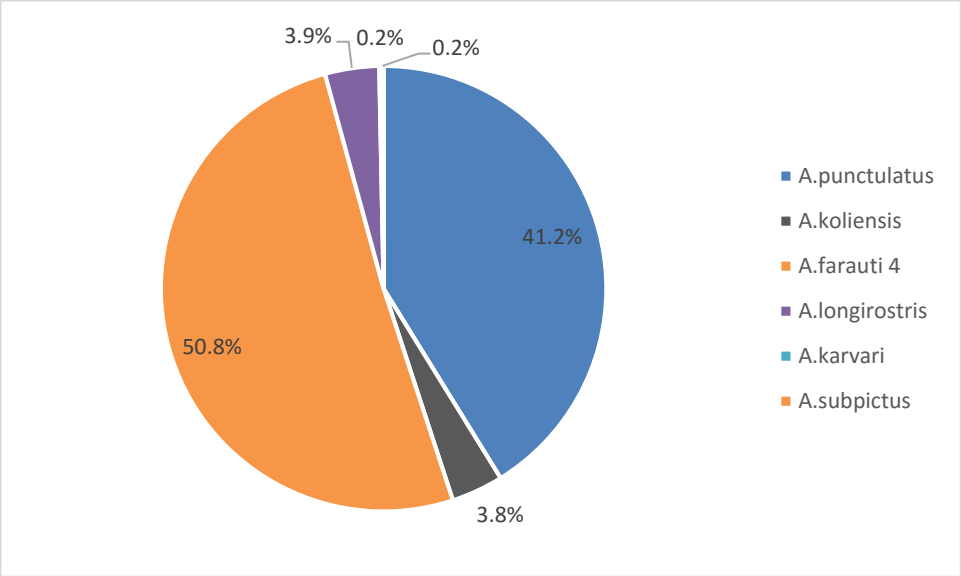
There were six *Anopheles* species morphologically identified from all the mosquitoes collected, *Anopheles punctulatus* (AP), *Anopheles koliensis* (AK), *Anopheles farauti* (later confirmed by RFLP-PCR as AF no.4), *Anopheles longirostris* (AL), *Anopheles karwari* (AK) and *Anopheles subpictus* (AS). Other mosquito species collected included *Culex annulirostris* (Cx.A), *Culex quinquifasciatus* (Cx.Q), Armigeres (Am.) and *Aedes* (Ae.) species. Total number of mosquito species are shown in Table 6.1.

Table 6.1. Mosquito species collected in Korona in 2013 - 2014

Village	No. of mosquitoes spp. collected									
	AP	*AK*	*AF4*	*AL*	*KA*	*AS*	*Cx.A*	*Cx.Q*	*Am. Sp*	*Ae. Sp*
Korona	43	7	268	12	1	1	310	76	71	111
Koinduna	135	4	46	8	0	0	45	9	24	16
Tongona	84	0	22	5	0	5	24	8	11	24
Total	262	11	336	25	1	6	379	93	106	151

Of the 641 Anophelines collected, 95.0% (n=609) were known vector species from the *An. punctulatus* complex, these were *An. punctulatus*, *An. koliensis* and *An. farauti.* The composition of the different Anopheline species are given in Figure 6.5. *An. farauti* makes up slightly over half (50.8%) of the total *Anopheles* collected, followed by *An. punctulatus* (41.2%), while *An. koliensis* only makes up 3.8%. The other Anophelines make up less than 5% of Anophelines collected.

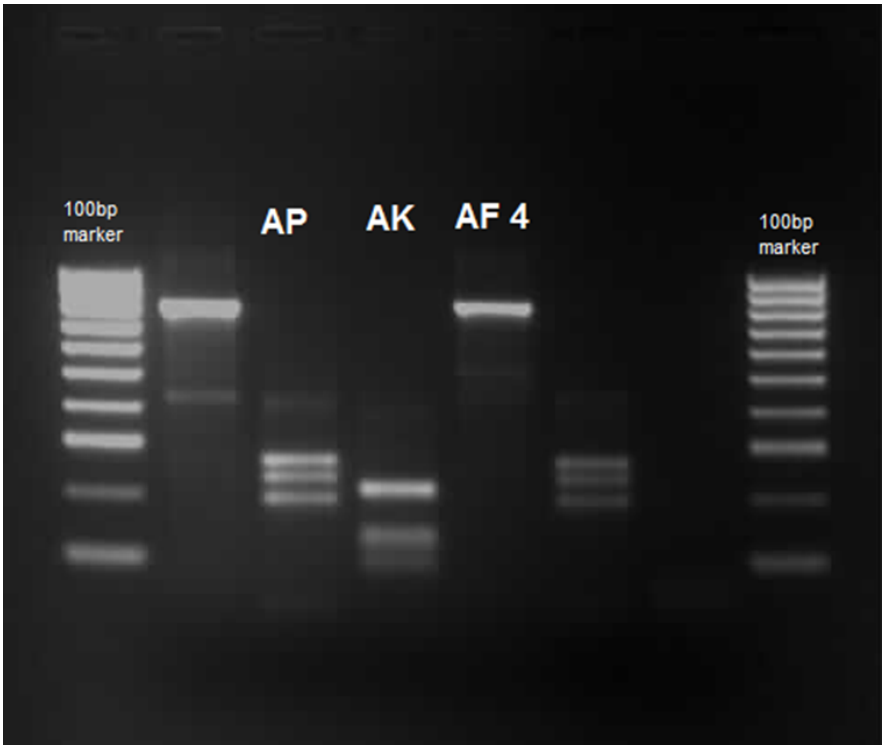
Figure 6.5. Anopheline composition of mosquitoes collected



6.3.2 Mosquito species identification

Known LF vectors are members of the *An. punctulatus* complex which consists of 12 subspecies within the group of which 7 sibling species make up the *An. farauti* complex. The *An. farauti* sibling species are morphologically similar and can be reliably distinguished by RFLP-PCR. All morphologically identified vector species of the *An. punctulatus* complex were re-confirmed by RFLP-PCR (Figure 6.6). Of the 336 *An. farauti* 4, 12 (3.57%) were morphologically identified as *An. punctulatus*, while 3 (0.89%) were morphologically identified as *An. koliensis*. And 1 (0.38%) AP was morphologically identified as *An. farauti*.

Figure 6.6. Gel electrophoresis of RFLP-PCR products



6.3.3 LF infection rates of vectors

By season

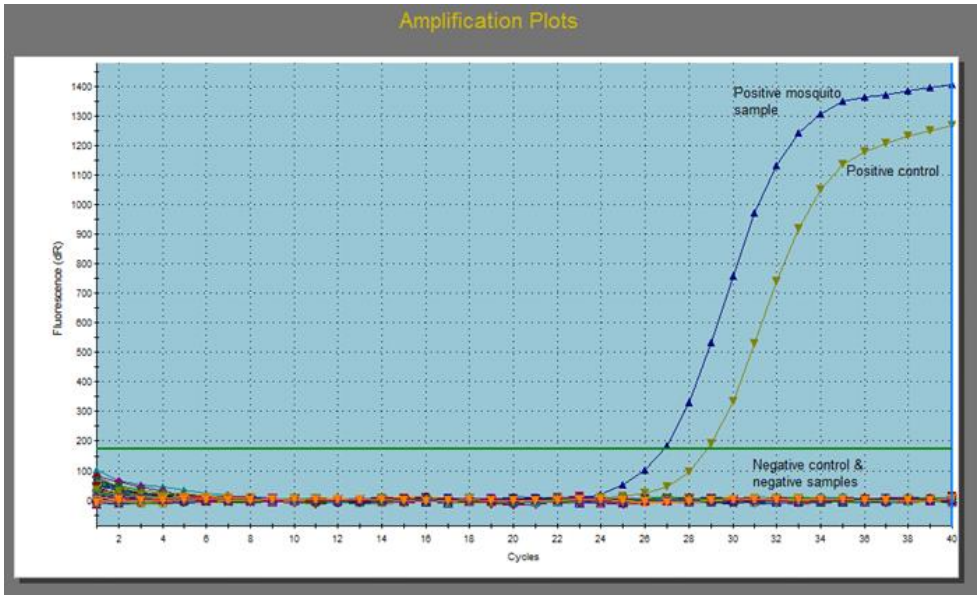
There were a higher number of vectors collected during the wet season (n=431, 70.8%) than during the dry season (n=178, 29.23%). *An. punctulatus* was prevalent throughout both wet and dry season while *An. farauti 4* was most prevalent during wet season. Amplification plot from *W. bancrofti* qPCR showing positive samples is shown in Figure 6.6.

The overall vector infection rate is 6.57% (n=40), with a higher infection rate found in the wet season (n=38, 6.24%) than the dry season (n=2, 0.33%). An infection rate of 14.56% was seen for *An. punctulatus* during the wet season and nil infection during the dry season, an infection rate of 5.66% was observed for *An. farauti 4* during the wet season and 2.82% infection rate during wet season. There were no infected *An. koliensis* specimens observed.

Table 6.2. Infection rates of vectors during wet and dry seasons

Season	No. of mosquitoes								
	AP	Pos	%	AK	Pos	%	AF4	Pos	%
Wet	158	23	14.56	8	0	0	265	15	5.66
Dry	104	0	0	3	0	0	71	2	2.82
Total	262	23	14.56	11	0	0	336	17	8.48

Figure 6.7. Amplification plot from *W. bancrofti* qPCR indicating positive samples



By hamlets

There were a higher number of vectors collected at Korona hamlet (n=318, 52.21%) followed by Koinduna (n=185,22.16%) and Tongona (n=106,17.41%) as seen in Figure 6.7. *An. punctulatus* was the most prevalent in Koinduna (n=135, 51.52%), and also had the highest infection rate of 12.59%. This contrasts to *An. farauti 4* which was significantly more prevalent in Korona (n=268, 79.76%) with a 4.85% infection rate as shown in Figure 6.8. *An. farauti* 4 was also found in Tongona, and while the abundance was low, the infection rates were 13.64%. There were no *An. koliensis* found to be infected with *W. bancrofti* and this vector was also found in very low numbers in only two hamlets as seen in Figure 6.8 and Table 6.3.

Figure 6.8. Vector abundance in the hamlets

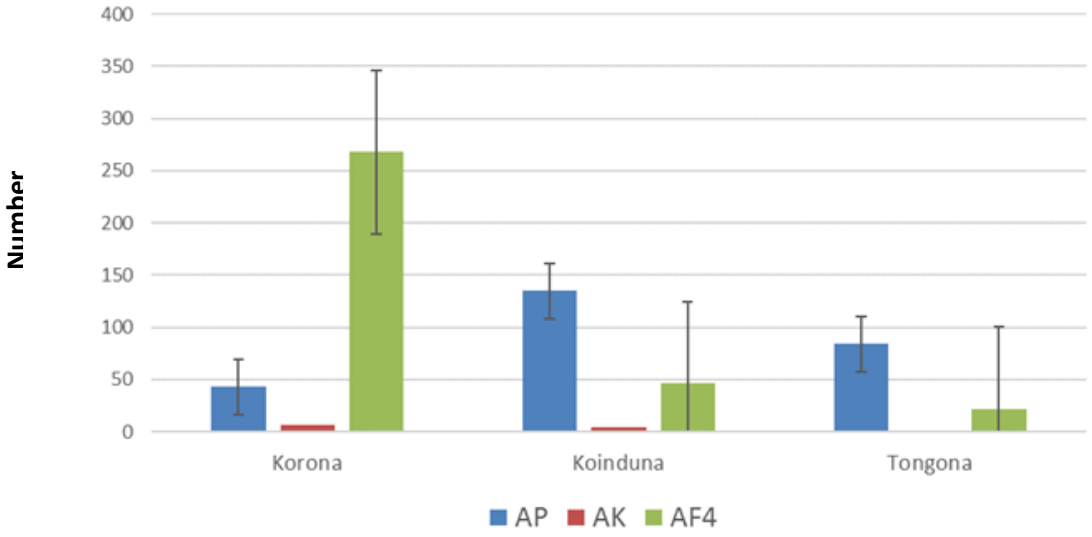


Table 6.3. Infection rates of vectors by hamlets

Village	No. of mosquitoes								
	AP	**Pos**	**%**	**AK**	**Pos**	**%**	**AF4**	**Pos**	**%**
Korona	43	1	0.02	7	0	0	268	13	4.85
Koinduna	135	17	12.59	4	0	0	46	1	2.17
Tongona	84	5	5.95	0	0	0	22	3	13.64
Total	262	23	8.78	11	0	0	336	17	5.06

Biting patterns

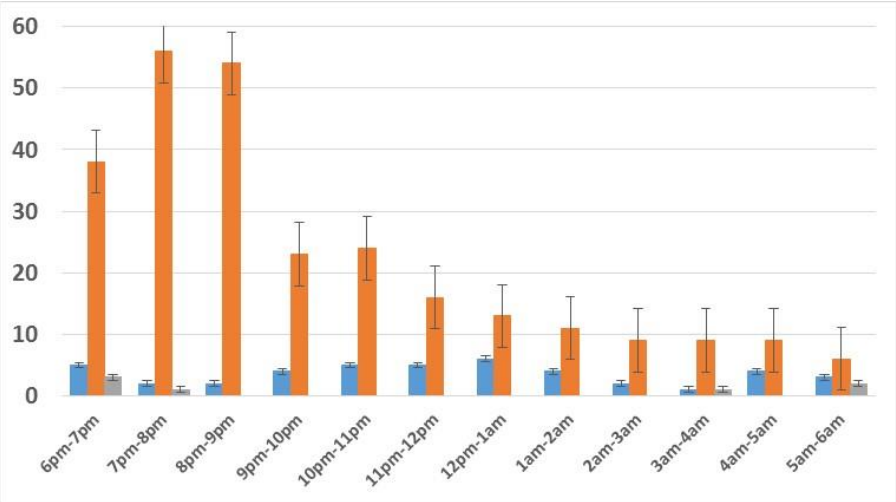
The hourly biting numbers and overnight patterns of the *An. punctulatus, An. farauti 4 and An. koliensis* caught between 18.00 and 06.00 were found to be distinct between the hamlets as shown in Figure 6.9_A-C.

In Korona hamlet where *An. farauti 4* was most dominant, the peak biting times were between 19.00-21.00 when more than 50 mosquitoes were recorded per hour. The biting then drops off to less than 20-30 bites per hour for the rest of the night. The differences between 19.00-21.00 are significantly different to the rest of the night (Figure 6.9_A).

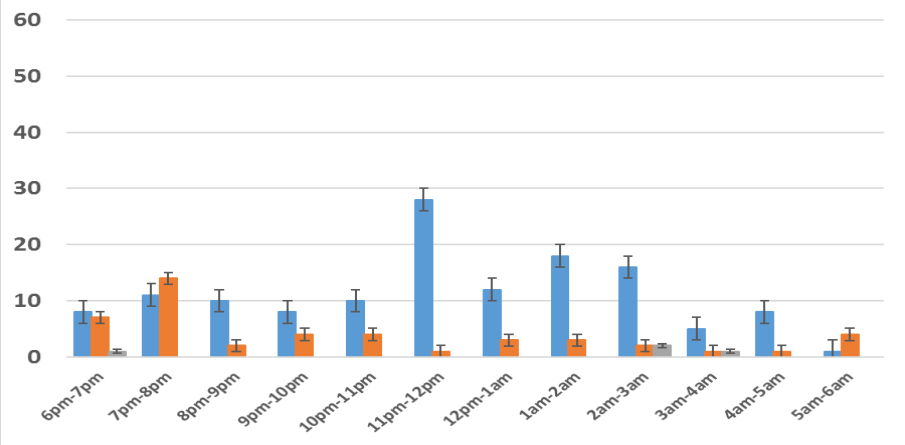
In contrast, the hamlets of Koinduna and Tongona, where *An. punctulatus* was most dominant, the overall number of bites per hour was significantly less than in Korona. Further the peak biting times of *An. punctulatus* in Koinduna was 19.00-20.00 and for *An. farauti 4* in Koinduna was 23.00-03.00 when between 10-30 mosquitoes were recorded per hour, while in Tongona the peak biting time was between 19.00-23.00, with overall low numbers of 11-15 recorded per hour.

Figure 6.9. Outdoor Anopheles species hourly biting numbers by hamlet

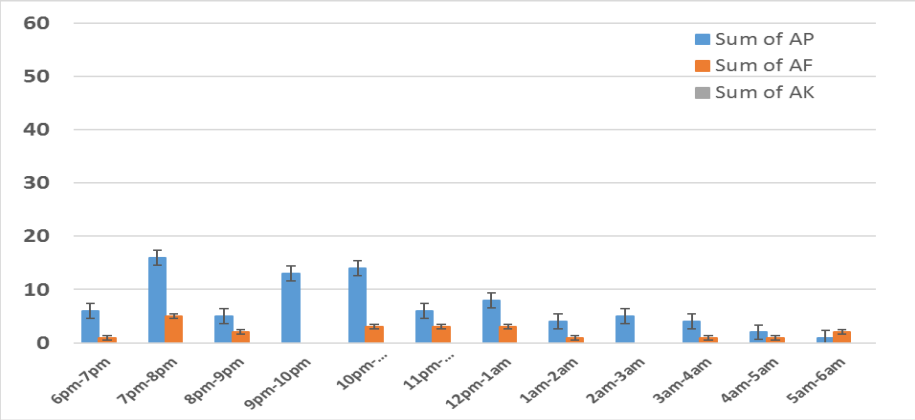
A. Korona hamlet



B. Koinduna hamlet



C. Tongona hamlet

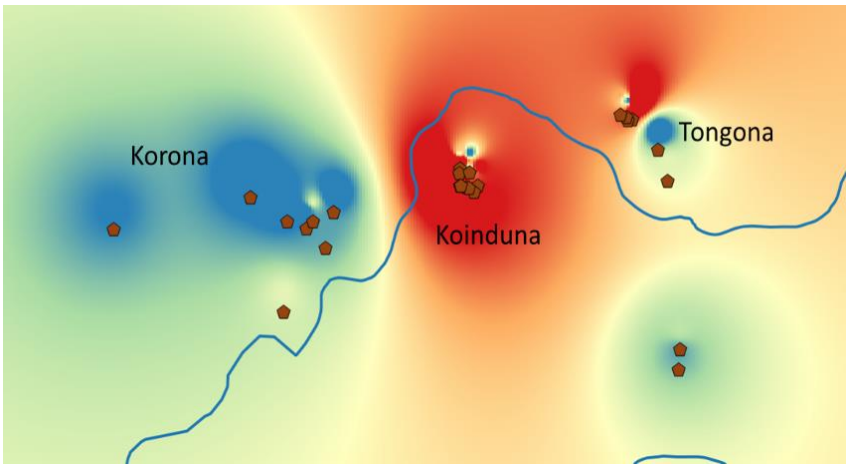


6.3.4 Mapping the spatial distribution of vectors

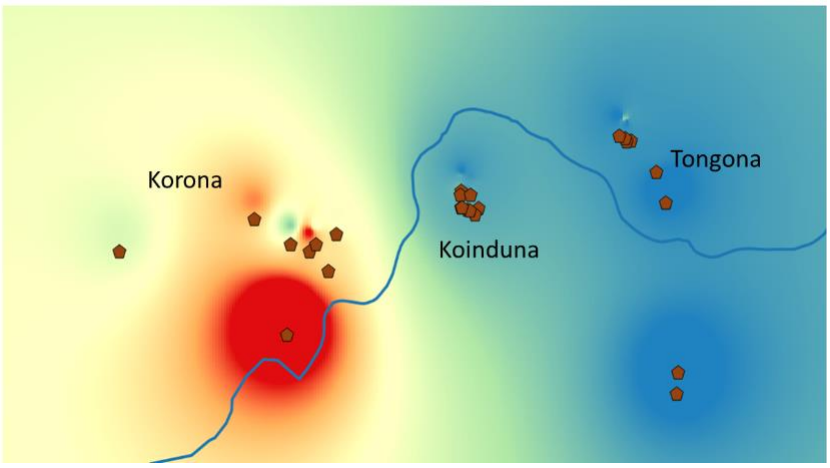
Interpolated maps of the vector distribution across the village of Korona showed distinct distribution patterns. The species *Anopheles punctulatus* was the more dominant vector in the hamlet Koinduna towards Tongona hamlet, and was present in low numbers in Korona as shown in Table 6.1 and seen in Figure 6.10_A. In comparison, *An. farauti* 4 was the more dominant vector in the hamlet Korona as shown on Table 6.1 and seen in Figure 6.10_B.

Figure 6.10 Interpolated maps showing vector distribution across study area

A. Distribution of *An. punctulatus*



B. Distribution of *An. farauti 4*



6.3.5 Spatial vector distribution at hamlet level (species abundance)

Interpolated maps at hamlet level showed similar distribution patterns in Korona where both vector were occurring together as shown in Figure 6.11A. However, *An. farauti* 4 was more abundant than *An. punctulatus*. Positive mosquito samples were collected outside houses found at the edge of high *An. farauti* 4 areas where houses are clustered together.

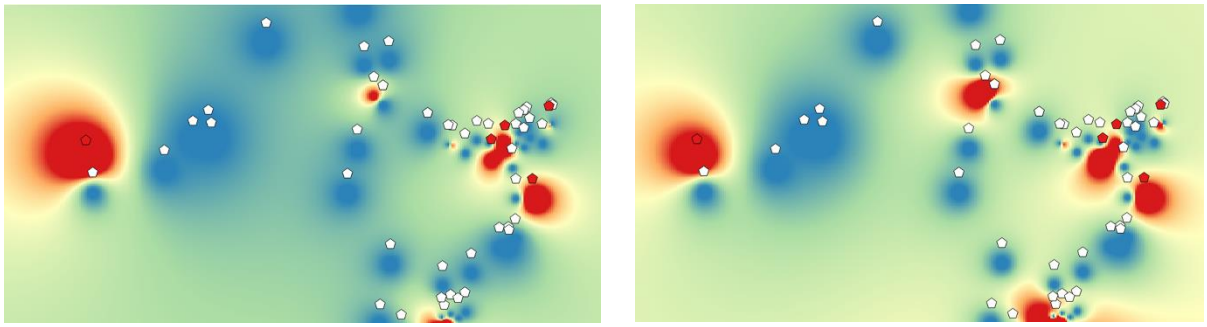
The interpolated maps of distribution patterns in Koinduna, where *An. punctulatus* was more abundant, found that *An. punctulatus* and *An. farauti* 4 have quite different distribution patterns as shown in Figure 6.11_B. *An. punctulatus* appeared to be more dominant in the western part of the hamlet, while *An. farauti* 4, in low numbers (see Table 6.3) appeared to be more prominent in the eastern half of the hamlet.

The interpolated maps of distribution patterns in Tongona shown in Figure 6.11_C, found that both the *An. punctulatus* and *An. farauti* 4 have similar distribution patterns, with both *An. punctulatus* and *An. farauti* 4 dominant in the north westerly part of the hamlet with both positive houses in the cluster of houses. This was found to be in accordance to the spatial patterns of the Mf positives in Chapter 5.

Figure 6.11. Interpolated maps of AP and AF and Mf positives houses in each hamlet

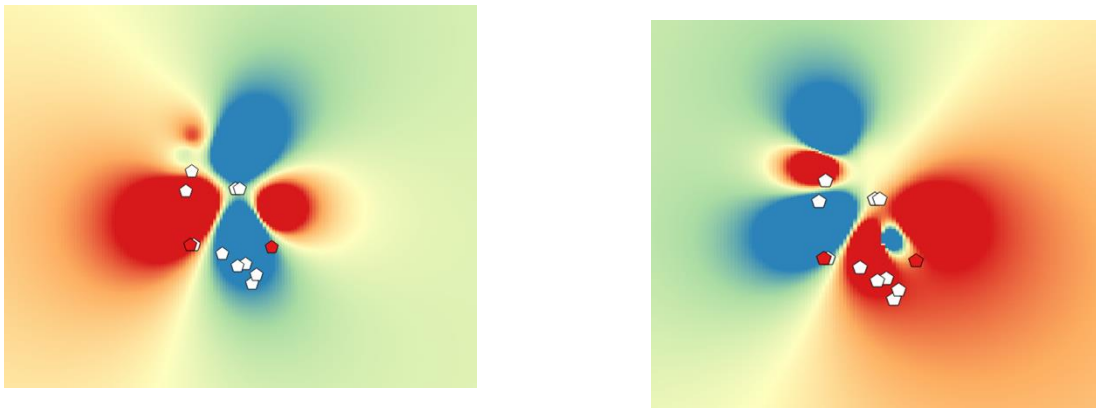
a) Korona hamlet

An. punctulatus ***An. farauti 4***



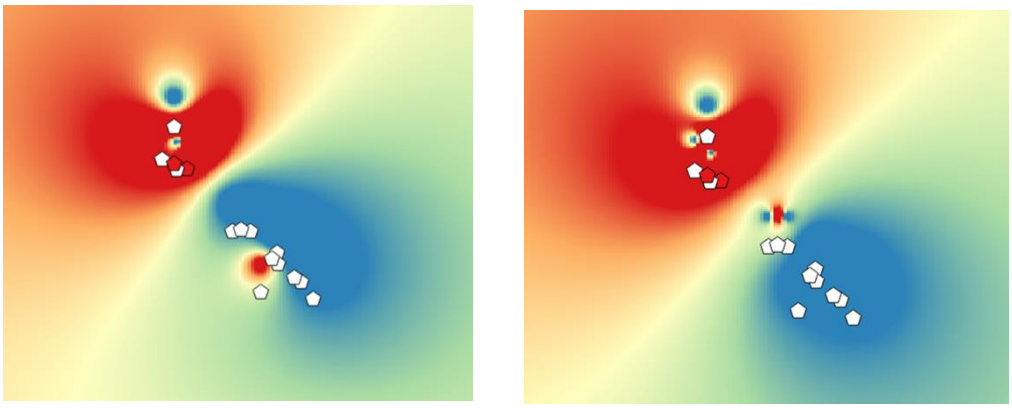
b) Koinduna hamlet

An. punctulatus ***An. farauti 4***



c) Tongna hamlet

An. punctulatus ***An. farauti 4***



6.4. Discussion

Overall *An. punctulatus* and *An. farauti 4* were the most abundant *Anopheles* species found in the village of Korona. The presence of these vectors in this area is in agreement with the maps presented in Chapter 3 (based on Cooper, 2002 national species maps) highlighting distributions in Madang. Interestingly, *An. punctulatus* was found in higher numbers in one hamlet (Koinduna), while *An. farauti 4* was more dominant in another hamlet (Korona), both hamlets are in very close proximity to each other.

The reasons for these differences may be related to micro-epidemiological factors including environmental factors i.e. proximity to river, type of breeding sites in the hamlet, housing infrastructure (also drainage , refuse), intervention use or number of people at different times of the evening/night as found by Al-Eryani et al., 2016, who also suggested that groups of people gathering in the evenings attracted more mosquitoes. This may also explain why in Korona hamlet, which has more people and houses close together where people may socialise more outdoors, there is an earlier biting time of *An. farauti* 4.

Such variation in species distribution at a fine geographical scale has not been widely examined or mapped in PNG– but has been found with different *Anopheles* species elsewhere in a few studies. Kaindoa et al., (2016) found fine scale spatial variations in *Anopheles* species distributions in one village, while Russell et al. (2013) found that in two neighbouring villages that two main *Anopheles* vector species had different distributions on a micro- level (spatial and temporal) and the monthly variations appeared to be related to wet and dry seasons. However, one species had more of a seasonal trend whereas the other species was more steady in numbers year round.

Similarly, in this study in PNG, the *An. punctulatus* had steady numbers during both the wet and dry season, while more *An. farauti 4* was found during the wet season collections. Bockarie et al., (1998) also showed *An. punctulatus* to be a perennial LF

vector in East Sepik. Generally across PNG, these two main *Anopheles* species have been found to be more abundant in the wet season, which may be related to the water/breeding sites available for mosquitoes, however patterns vary in different areas. Understanding the environmental/ecological niches of *An. punctulatus* and *An. farauti* 4 may help to determine the risk of transmission in other areas of Madang. The examination of *W. bancrofti* in this study area highlighted that the wet season is when the greatest risk is.

In general *An. punctulatus* are widespread throughout PNG and known to breed in semi-permanent, shallow pools of water and established flora and fauna (Cooper, et al, 2002, 2009) and its dominance in Koinduna hamlet may be due to all year round established source of water in the nearby river and streams that form pools of water on the edge of the river banks. *An. punctulatus* larva have also been readily collected from temporary sites such as road ruts and wheel tracks.

The ecological niche habitats of *An. farauti 4* are inland, lowland river valleys and commonly found in the northern part of the country. Cooper et al (2002, 2009) also found this species to oviposit in artificial sites and commonly found in associated with *An. punctulatus* and *An. koliensis* larva. This vector was also noted to have a positive association with humans and this may explain why it is more abundant in Korona hamlet where more people live close together

Defining peak transmission can help to target any vector control. Knowledge of when vectors are most abundant and infectious can be used to link with malaria control programmes to ensure distribution of LLIN can be most effective against both diseases in highly endemic areas like this. This highlights the importance of the national malaria program and the LF program to work in close collaboration since the vector control measure being used by the malaria program can greatly benefit both programs.

The national LF program can also target public health messages capturing this information on vectors so the people know when there are higher risks of being

bitten by infected mosquitoes. The used of LLINs as well as mosquito coils may help to reduce transmission, however in some hamlets like Korona where the main vector *An farauti* 4 bite early and people won't be protected, then other control / personal protection measures and public health messages may need to be conveyed. Recently there has been studies on spatial repellency of of transfluthrin-treated hessian strips for outdoor biting malaria mosquitoes in Africa – similar new tools could also be used here in PNG (Govella et al., 2015, Ogma et al., 2012).

In summary this work adds to the literature on LF vectors in PNG confirming that *An. punctulatus* and *An. farauti 4* are key vectors of LF in Madang.

Chapter Seven

Summary of Key Findings and Recommendations

Chapter 3 – Review of LF research in PNG

The LF entomology review highlighted several key factors especially the lack of information of known and potential vectors associated with LF transmission in many endemic areas across the country.

There is only one national institution in the country (PNGIMR) conducting entomological research with international collaborators within selective study areas, resulting in most studies focused in particular region than in others. There were only 17 LF entomological articles produced in the last 70 years, which presents limited vector knowledge in an LF endemic country of vast geographical landscapes. It is unclear if the *An. farauti*, *An. punctulatus* and *An. koliensis* in other regions have the same transmission patterns and also if the interventions of MDA and ITNs will also the same impact.

Information on entomology can assist programmatic purposes where integrated program approaches targeting same endemic population for instance the malaria vector control activities (Issuing of ITN). The review also provides information gap for future entomological research activities to target endemic areas lacking such knowledge.

MDA and ITNs/LLINs are shown to have impact on transmission, hence in areas of intense LLIN coverage without MDA will require future research as vector control has been shown to impact or even eliminate LF such as in The Gambia (Rebollo et al. 2015) and Solomon Islands (Webber 1977, 1979). The scale up of LLIN distribution throughout the country may also result in vector behavioural changes and understanding how these affect different species that drive transmission in different areas will be important to assess the use of LLINs as an intervention that could be use more by the LF Programme.

Chapter 4 – Ag prevalence and risk factor analysis

During the antigenemia survey in four villages of the Usino Bundi district, it was obvious that Ag was more prevalent in one of the four villages although the Ag prevalent village shared boundaries and relations with people in the next nearby village. This may be due to the vector flight range, which cannot fly far without the aid of wind. The high Ag prevalence village also had a river run through the hamlets of the village, where previous studies in Drekikir, East Sepik province have shown similar characters. The community most furthest from the other three villages, located in high altitude and had mostly semi-permanent and permanent houses had no Ag detected.

Key findings included indication of increase prevalence with higher age groups with more positive males than females. As LF is acquired in early childhood years, this may be an expected trend. At this time when LLINs are given to ≥5 year olds and pregnant women, with LLIN efficacy to last up to at least 3-5 years, high LLIN coverage could play an important role in delaying infection in childhood in endemic areas. Public health messages targeting older people living in endemic areas could have an impact of improving personal protection from infective vector bite.

Another important finding from this chapter is house type and prevalence of infection. There is limited information on housing structure and prevalence of LF, specifically for *Anopheles* vectors of bancroftian filariasis, although there is some literature on housing structure and other vector borne diseases like malaria. There is very little information on filariasis transmission and housing structure. Improve housing could reduce infection rates

Almost all the people interviewed never heard of LF, how it's transmitted and the treatment for it, they are not aware of a national LF elimination program either. The lack of basic knowledge on LF presents present a risk in itself, as people are not aware of the presence and prevalence of disease in their communities. The fact that the National LF Elimination Program is supporting MDA activities in only one province and is its initial stage suggest that it will need much support to expand its

activities to other parts of the country may be a reason the general populace are not aware of the national program. This finding shows that a significant scale up of public health awareness on the disease needs to be done, specially targeting people living in high risk areas who have little or no knowledge about the risk of infection and disease.

Specific help for those with clinical conditions also needs to be addressed, as at this stage, no firm statistics is known on the burden of clinical manifestations. However, this study highlighted that more people affected by lymphoedema and hydrocoeles were from the more endemic areas i.e. with highest sero-prevalence. Also the general observation that clinical manifestations of the disease is not common in moderate to high LF prevalent villages, hence people are not aware of what the disease can result in.

The LF programme needs to develop and distribute country specific IEC materials to high risk areas, and these could be adapted from existing WHO materials and help facilitate the elimination process.

Chapter 5 – Mf micro-mapping and spatial analysis

The Mf survey showed that there was active transmission of filariasis in the village of high Ag prevalence. Although the hamlets of the village were in close proximity to each other, there were significant geographical differences in Mf prevalence between the hamlets. Several factors may have had an effect of transmission and prevalence in this hamlet, hamlet situated closest to the main highway had less natural vegetation suitable for the *Anopheles* vector habitats, it also had more semi-permanent and permanent houses compared to the other two hamlets. That suggest that better housing structure may be a key to obstructing transmission.

Mf was more prevalent in males than females and this could be a result of socio-behavioral activities of males like staying up late into the nights without protective clothing or repellents and since the place could be hot in the night can make sleeping inside a mosquito net uncomfortable. The older age groups also had higher Mf prevalence compared to younger age groups and this could have been the result

of consistent infective bites over the years. The roll out of LLINs only occurred a few months before the surveys for this study took place, therefore it would be beneficial to conduct a follow up study to investigate if ITNs/LLINs have impacted on transmission in the absence of MDA.

Although the use of mosquito coils was not as common as ITNs/LLINs, because of monetary security in these areas where subsistence farming sustains their livelihoods, the houses that used coils appeared to have a negative impact on Mf transmission, suggesting coils can be options for protective intervention. In the absence of any rapid scaling up of country-MDA, options like coils and other personal protection measures, should be encouraged to supplement the use of LLINs. This really highlights the need for development of specific IEC materials targeting the different sub-groups of the communities at risk.

Importantly, the LF programme should maintain close collaboration with the Malaria National Program and other health programs that also do outreach into communities and do overlapping interventions for disease control/elimination. This is important for a limited resource programme like the LF programme where only a few international stakeholders are supporting the programme, and the country may need to depend on vector control as its primary intervention.

Chapter 6 -Vector incrimination and analysis

This study highlighted that two main vectors were identified, *An. punctulatus* and *An. farauti 4*, the former was dominant in one hamlet (Koinduna), while AF4 was dominant in the other hamlet (Korona), both hamlets were only separated by the Sausi river, in close proximity to each other. The differences could be due to environmental factors i.e. proximity to river, availability of breeding sites in the hamlet, housing infrastructure (also drainage, refuse), intervention use or number of people at different times of the evening/night also suggested that groups of people gathering in the evenings attracted more mosquitoes. The importance of understanding local and focal hotspots is key to targeting interventions.

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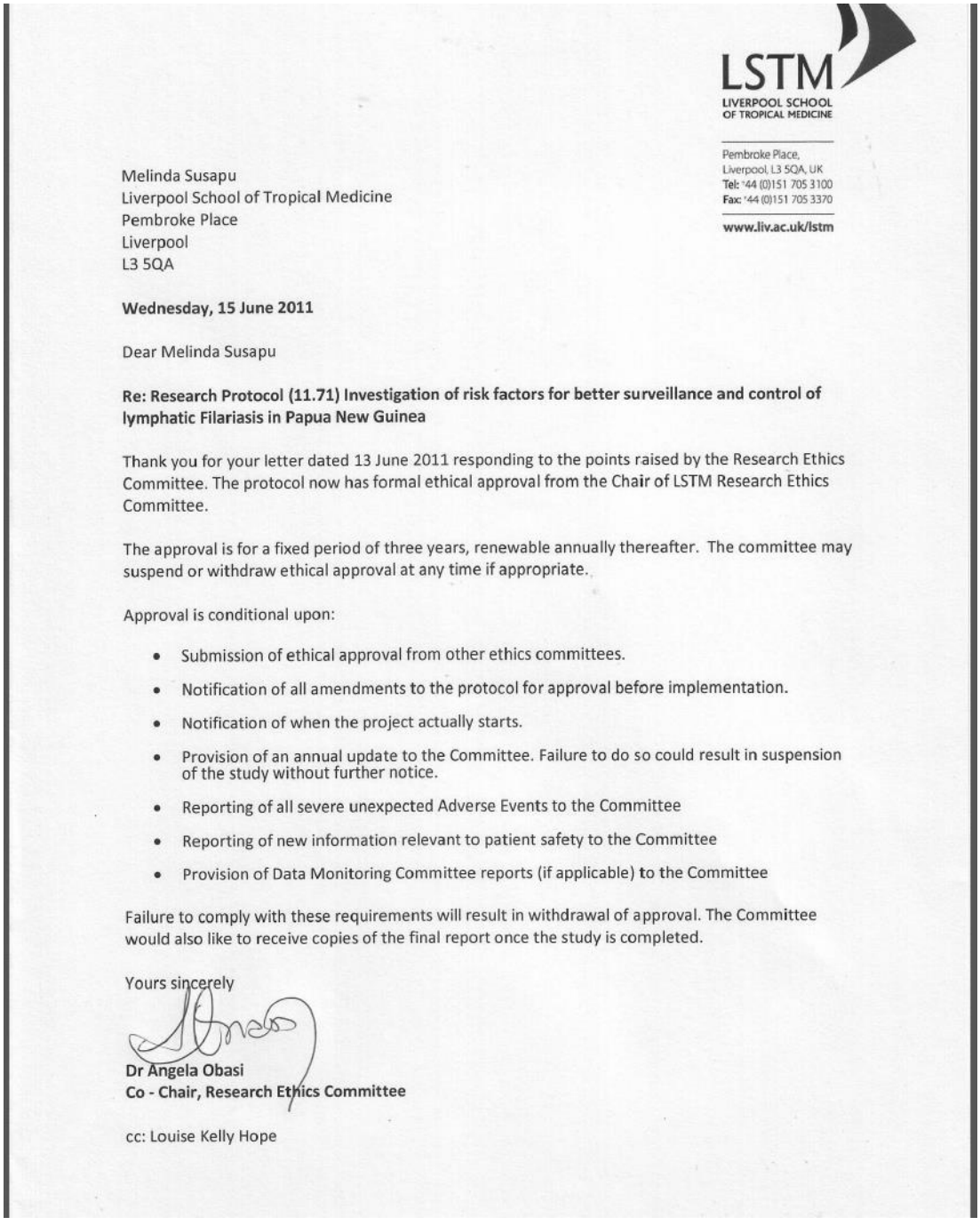
Appendices

1. **LSTM ethics approval letter**
2. **PNG ethics approval letter**
3. **ICT Survey questionnaire**
4. **MF Survey questionnaire**
5. **Conference poster presentation**
6. **LF related co-authored publications**

Appendix 1. LSTM ethics approval letter



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Melinda Susapu
Liverpool School of Tropical Medicine
Pembroke Place
Liverpool
L3 5QA

Wednesday, 15 June 2011

Dear Melinda Susapu

Re: Research Protocol (11.71) Investigation of risk factors for better surveillance and control of lymphatic Filariasis in Papua New Guinea

Thank you for your letter dated 13 June 2011 responding to the points raised by the Research Ethics Committee. The protocol now has formal ethical approval from the Chair of LSTM Research Ethics Committee.

The approval is for a fixed period of three years, renewable annually thereafter. The committee may suspend or withdraw ethical approval at any time if appropriate.

Approval is conditional upon:

- Submission of ethical approval from other ethics committees.
- Notification of all amendments to the protocol for approval before implementation.
- Notification of when the project actually starts.
- Provision of an annual update to the Committee. Failure to do so could result in suspension of the study without further notice.
- Reporting of all severe unexpected Adverse Events to the Committee
- Reporting of new information relevant to patient safety to the Committee
- Provision of Data Monitoring Committee reports (if applicable) to the Committee

Failure to comply with these requirements will result in withdrawal of approval. The Committee would also like to receive copies of the final report once the study is completed.

Yours sincerely

Dr Angela Obasi
Co - Chair, Research Ethics Committee

cc: Louise Kelly Hope

Appendix 2. PNG ethics approval letter

Government of Papua New Guinea
Medical Research Advisory Committee
National Department of Health

PO Box 807
WAIGANI 131, NCD
Papua New Guinea

Phone: + (675) 301 3650
Fax: + (675) 325 1825
Email: urarang_kitur@health.gov.pg

FILE: 54-6-2
DATE: 19/11/2012

Melinda Susapu
Malaria & Vector Bourne Disease
Disease Control Branch
National Department of Health

Dear Ms Susapu,

This is to certify that the proposal:

Investigation of risk factors for better surveillance and control of lymphatic filariasis in Papua New Guinea

Submitted by you has been examined by the Medical Research Advisory Committee of Papua New Guinea and MRAC has approved your study in principle pending you respond to the MRAC recommendations.

The PI is encouraged to address the MRAC recommendations and re-submit to the MRAC Chairman as soon as possible. The recommendations are:

i. PI to notify MRAC who the student's supervisor is (Dr Reimer or Dr Hope). Require confirmation letters from supervisors
ii. PI to submit a Community Consent Form for a local counsellor to sign

The Medical Research Advisory Committee of Papua New Guinea act as the National Ethical Clearance Committee and as the Institutional Ethical Committee for the Papua New Guinea Institute of Medical Research and so there is no further bar to this project being carried out in Papua New Guinea.

Investigators are reminded of the importance of keeping provincial health and research authorities informed about their study and its progress, and of submitting progress and outcome reports to the Medical Research Advisory Committee.

With best wishes.

Yours sincerely,

Dr. Urarang Kitur
Chairperson

SERVICE DELIVERY TO THE RURAL MAJORITY AND URBAN POOR

Appendix 3: Household questionnaire - Chapter 4

Household Survey

Date:_____ Interviewer: _____ Household code:_____

This survey is divided into two parts

i) Household/demographic information

ii) Knowledge, attitude and practice information

Lat/long location:

Elevation:

Village/Ward/District:

Name (owner of house):

Questions/Observations	Code for answers	Response
Part I) Household/Demography Info		
Age / Sex		
Length of residency (if less than 5 yrs, go to Q2a)	01 less than 1 year 02 1-5 years 03 5-10 years 04 10-15 years 05 life time O6 other	
2a) Where did you move from?	Village_____ District_____ Province_____	
Do you (your family) sometimes live in another place?	01 Yes (go to Q4 & Q5) 02 No	
If yes, where else do you (your family) live?	Village_____ District_____ Province_____	
How often do you (your family) move between these places?	01 Daily 02 Weekly 03 Forthnightly (every 2 weeks) 04 Monthly 05 yearly 06 Other (describe)	

Level of Education (owner)	01 No formal education 02 Primary only 03 Secondary only 04 Tertiary Other	
Number of people living in household		
Number of bedrooms		
Intervention related		
Are there any mosquito nets in the house?	01 No 02 Yes (go to Q16)	
) If Yes, how many?		
) What type of nets do you own?	01 Untreated 02 Treated (LLIN) (go to Q18 & Q19)	
) When was LLIN issued to the household?	(put year)	
) How many people in the house use LLINs to sleep at night?		
) Do you use mosquito coils/repellents around the house	No Yes	
House, design, material and surrounding environment		
Type of house	Permanent Bush material Part permanent/part bush materials Cardboards/makeshifts Other	
Ground or raised	01 Ground 02 Raised	
3) Source of water	Rain collected water/tank Borehole Stream/river/lake Main water supply Other	
4) Toilet	01 own 02 shared 03 bush/sea/river 04 other	
) Domestic refuse disposal habits	01 dug out pit 02 bush/sea/river	

	03 common dump site 04 other	
) Presence of drainage system around house	No Yes	
Proximity of house to edge of bush/water/sea (estimate in meters)		
Closest Health Centre/aid or health post		
Part II) Knowledge, Attitude & Practice Survey		
Have you heard of lymphatic filariasis?	**No** **Yes**	
Do you know what causes it?	Don't know By mosquitoes 02 By other bugs 03 03 By sorcery/ 'puripuri' 04 04 By touching 05 05 Hereditary 06 Other	
How is it prevented?	Don't know Sleeping in mosquito nets Eating the right food Getting help from sorcerer Going to church Drinking medicine Other	
How is it treated?	00 Don't know 01 drink medicine 02 operation (swelling) 03 go see the sorcerer 04 going to church 05 other	
Any individual in house with elephantiasis?	No (go to Q16) yes	
6) What are the symptoms	01 swollen arm Swollen leg Swollen breasts Hydrocele Other	

) How many years living with swollen condition	1-3 years 3-6 years 6-9years 10 years or more	
Does he/she need assistance with day-to-day care?	No Yes	
Has she/he received treatment for filariasis?	00 No 01 Yes	
) Do you know anyone in this village with elephantiasis?	01 No 02 Yes	
) Have you heard of the National Program to Eliminate LF?	**No** **Yes**	
) Have you ever received treatment for LF?	**No** **Yes**	
13) If yes, where and when?	Place_____ Year_____	
4) If no, would you like to receive treatment?	**01 No** 02 Yes	

Thank you very much for your participation and cooperation

Any notes/comments

_____
_____
_____
_____
_____
_____

Appendix 4 Individual questionnaire – Chapter 5

Date:_____ Interviewer: _____ House #_____Participant ID#:_____

Name:_____-

_____

Household information (applies to all people in one house)

Lat /long location _____Elevation:

_____

Village/Ward/District:_____-

_____

No. of people (usually) in household_____ _____No. of bedrooms -

_____

Main type (e.g. wood, tin) _____ Ceiling (yes/no)

_____

Questions/Observations	Code for answers	Response
Part I) Household/Demography Info		
) Age		
) Sex		
) Length of residency	Put number of years (If < 5 years then go to Q3a)	
) Where did you move from?	Village_____ District_____ Province_____	
Do you sometimes live in another place?	01 Yes (go to Q4a) 02 No (go to Q5)	
4a) If yes, where else do you live?	Village_____ District_____ Province_____	
4b) How often do you move between these places?	01 Daily 02 Weekly 03 Forthnightly (every 2 weeks) 04 Monthly 05 Other (describe)	
5) Do you always sleep in this house in this village?	**1 No** **2 Yes**	
5a) If no, where else do you sleep?	Describe where else and how often live in another house/place (e.g. house boys)	
rt 2) Intervention related		
Do you sleep under a mosquito net?	01 No (go to 6a) 02 Yes (go to 6b)	
6a) If no net, describe why not	Describe	
6b) If yes, describe type of net	Untreated_____ Treated (LLIN)_____ (go to 6c)	
6c) When did you get the LLIN?	(put month/ year of issue if possible)	

Do you have LF morbidity (swelling limb, breast female, scrotum males)	01 No 02 Yes (describe severity/stage of disease)	
8) Have you received medication for the swelling?	01 No 02 Yes (go to Q9)	
9) What medication did you take for the condition?	**01 DEC & Albendazole** **02 DEC alone** **03 Don't know** **03 Other (specify)**	

Any notes/comments (write over page if necessary)

_____

_____

_____

_____

_____

Sero-prevalence and risk factor survey for lymphatic filariasis in Papua New Guinea

Melinda Susapu[1,2], Moses Bockarie[2], Lisa Reimer[2], Louise A. Kelly-Hope[2]

[1] *National Health Department, Papua New Guinea*
[2] *Centre for Neglected Tropical Diseases, Department of Parasitology, Liverpool School of Tropical Medicine, Liverpool, UK*

Introduction

Human lymphatic filariasis (LF) is one of humanity's oldest diseases caused by thread-like nematode worms which are transmitted by mosquitoes. Infection with the parasite can cause chronic disabling diseases such as lymphoedema, elephantiasis, and hydrocele.The disease is a major cause of morbidity in most tropical and subtropical countries of Africa, South East Asia, the Pacific and limited areas of Central and South America.

In Papua New Guinea (PNG), LF is caused by the parasite *Wuchereria bancrofti* and is mainly transmitted among humans by the Anopheles mosquitoes, the same vectors that transmit malaria. PNG has an estimated population of 7 million inhabitants; of which 4 million are predicted to be at risk of LF. Some national and published data exists, however, the geographical distribution, burden of disease and associated risk factors are currently not well defined.

This study investigated the prevalence and potential risk factors of LF in one endemic province of PNG through the administration of a prevalence survey and household questionnaire related to the LF transmission and the national LF programme.

Methods

➢ ***Study site:*** In the Usino Bundi district of Madang Province, four villages (Waput, Yakumbu, Korona, Reikos) were first selected to determine the presence of LF using the ICT rapid test kits to check for filarial antigen. 100 people in each village were tested in accordance with WHO protocol .

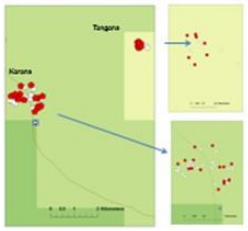
Figure 1. Map of four study villages in Usino District

➢ ***Night blood microfilaraemia (MF) survey*** A night time blood survey was conducted in the village with the highest ICT positives to determine true transmission potential and if the pattern of infection were similar at a finer within-village scale.

➢ ***Household survey:*** A survey was carried out to determine residence status, peoples knowledge of the disease, disease risk by sex, age and house type, usual time of bed time, number of people in house, use of control measures like sleeping under a long lasting insecticide nets (LLINs).

ICT results

Figure 2. Map of ICT positive households in Korona village

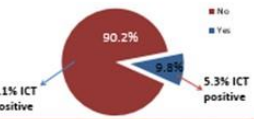


The ICT survey showed that Korona had 28.9% prevalence other villages were low (Waput 3%, Yakumbu 5%, Reikos 0%). Most people were permanent residents and recently have acquired LLINs.

Mapping the GPS coordinates of Korona ICT positives showed the LF infected individuals to be fairly scattered in the village and not clustered at one location.

During the ICT survey, ~90% stated that they didn't know what caused LF, and of this group 9.1% were ICT positive compared to 5.3% of the other group who stated they knew what caused LF (Figure 3).

Figure 3. Knowledge of LF



MF results

The MF study was conducted in 3 small sub-villages of Korona, called Korona which also had the highest MF infection rate (50.5%), followed by Tongona (32%) and Koinduna (17%).

Figure 4. MF by age and sex

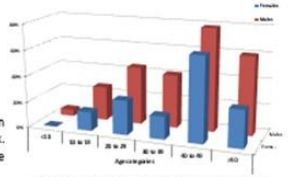


Figure 4 shows the distribution of MF positives by age and sex. The majority (42%) were in the 10-19 age category. A higher average of MF positive individuals were above age 40 and were mostly males.

Figure 3. Average % of MF by house type

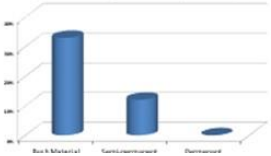
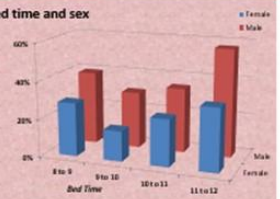


Figure 5. shows a higher average MF infection rate among people living in traditional bush material houses compared to other houses. None in permanent.

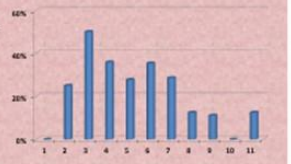
MF results

Figure 6. MF prevalence by bed time and sex



When the time of bed was examined, it was found that a higher number stayed up after 10pm and were also found to have MF infection rates which may be related to the local mosquitoes biting time and the fact that many men sit outside late at night.

Figure 7. MF prevalence by number of people living in house



MF infections rates were found to be high where 2 to 7 people were living in the same house. It was distinctively in houses where only 3 or 4 males where living, which is related to the cultural separation of 'houseboys'.

Conclusions

➢ This region of Madang Province is highly endemic for LF. The highest rates of infection occurred in older males, and among those living in male dominated household, lived in houses made of traditional bush materials and among those who went to bed late -- all which were likely to increase the risk of exposure to peak biting time of local mosquitoes.

➢ The PNG LF Elimination Programme will need to raise public awareness of its activities and scale up its activities in this region, specifically targeting health messages at young male individuals to increase their knowledge on the causes of disease, the importance of trying to improve household structure to keep it mosquito-proof and using personal protection late at night.

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Appendix 6. LF related co-authored publications

Graves et al. Parasites & Vectors 2013, 6:7
http://www.parasitesandvectors.com/content/6/1/7

Parasites & Vectors

RESEARCH Open Access

Lymphatic filariasis in Papua New Guinea: distribution at district level and impact of mass drug administration, 1980 to 2011

Patricia M Graves[1,8*], Leo Makita[2], Melinda Susapu[2,3], Molly A Brady[4], Wayne Melrose[1], Corinne Capuano[4], Zaixing Zhang[3], Luo Dapeng[3], Masayo Ozaki[5,9], David Reeve[1], Kazuyo Ichimori[6], Walter M Kazadi[3], Frederick Michna[1], Moses J Bockarie[7] and Louise A Kelly-Hope[7]

Abstract

Background: Lymphatic filariasis (LF) caused by *Wuchereria bancrofti* is present at high prevalence in some parts of Papua New Guinea. However, there has been no rigorous data-based representative assessment of nationwide prevalence of LF. The LF programme has been daunted by the scope of the problem, and progress on mass drug administration (MDA) has been slow and lacking in resources.

Methods: A systematic literature review identified LF surveys in Papua New Guinea between 1980 and 2011. Results were extracted by location, time period and test used (blood slide, immunochromatographic test (ICT) or Og4C3 ELISA) and combined by district. Three criteria schemes based on the Global Programme to Eliminate Lymphatic Filariasis guidelines, with modifications, were developed to classify and prioritize districts by prevalence level. Results of repeated surveys in the same sites were used to investigate the impact of MDA on LF prevalence over the time period.

Results: There were 312 distinct survey sites identified in 80 of the 89 districts over the 31-year period. The overall LF prevalence in the sites tested was estimated at 18.5 to 27.5% by blood slide for microfilariae (Mf), 10.1% to 12.9% by ICT and 45.4% to 48.8% by Og4C3. Biases in site selection towards areas with LF, and change in type of assay used, affected the prevalence estimates, but overall decline in prevalence over the time period was observed. Depending on the criteria used, 34 to 36 districts (population 2.7 to 2.9 million) were classed as high endemic (≥5% prevalence), 15 to 25 districts (1.7 to 1.9 million) as low endemic (<5%) and 20 to 31 (1.3 to 2.2 million) as non-endemic. Nine districts (0.7 million) had no information. The strong impact of MDA, especially on microfilaria (Mf) prevalence, was noted in sites with repeat surveys.

Conclusions: This analytical review of past surveys of LF in Papua New Guinea enables better estimation of the national burden, identifies gaps in knowledge, quantifies and locates the population at risk, and can be used to predict the likely impact of MDA and/or vector control. Better targeting of districts by level of prevalence will strengthen the control programme, facilitate monitoring of the disease trend and increase the likelihood of reaching the target of LF elimination by 2020.

Keywords: Lymphatic filariasis, Papua New Guinea, Mapping, Mass drug administration

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The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Insecticidal Bed Nets and Filariasis Transmission in Papua New Guinea

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ABSTRACT

BACKGROUND

Global efforts to eliminate lymphatic filariasis are based on the annual mass administration of antifilarial drugs to reduce the microfilaria reservoir available to the mosquito vector. Insecticide-treated bed nets are being widely used in areas in which filariasis and malaria are coendemic.

METHODS

We studied five villages in which five annual mass administrations of antifilarial drugs, which were completed in 1998, reduced the transmission of *Wuchereria bancrofti*, one of the nematodes that cause lymphatic filariasis. A total of 21,899 anopheles mosquitoes were collected for 26 months before and 11 to 36 months after bed nets treated with long-lasting insecticide were distributed in 2009. We evaluated the status of filarial infection and the presence of *W. bancrofti* DNA in anopheline mosquitoes before and after the introduction of insecticide-treated bed nets. We then used a model of population dynamics to estimate the probabilities of transmission cessation.

RESULTS

Village-specific rates of bites from anopheline mosquitoes ranged from 6.4 to 61.3 bites per person per day before the bed-net distribution and from 1.1 to 9.4 bites for 11 months after distribution ($P<0.001$). During the same period, the rate of detection of *W. bancrofti* in anopheline mosquitoes decreased from 1.8% to 0.4% ($P=0.005$), and the rate of detection of filarial DNA decreased from 19.4% to 14.9% ($P=0.13$). The annual transmission potential was 5 to 325 infective larvae inoculated per person per year before the bed-net distribution and 0 after the distribution. Among all five villages with a prevalence of microfilariae of 2 to 38%, the probability of transmission cessation increased from less than 1.0% before the bed-net distribution to a range of 4.9 to 95% in the 11 months after distribution.

CONCLUSIONS

Vector control with insecticide-treated bed nets is a valuable tool for *W. bancrofti* elimination in areas in which anopheline mosquitoes transmit the parasite. (Funded by the U.S. Public Health Service and the National Institutes of Health.)

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