Expansion of lymphatic filariasis transmission assessment surveys (TAS) in Oceania as a pragmatic platform for key insights into the epidemiology of communicable diseases including intestinal parasite infections

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

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Dedication

To my family

Declaration

This thesis is my own work and the contents have not been presented, nor are currently being presented, either wholly or in part for any other degree or qualification.

The data set I analyzed in chapter 4, 5, 6, 7 and 8 came from the studies for which I was one of the co-investigators, and I took the lead to mobilize the resources of the studies, to develop the study protocols, to organize and train the survey teams, and to coordinate the survey implementation and data collection/management, as well as statistical analyses. The studies were conducted in collaboration with the National Filariasis Unit, Fiji Centre for Communicable Diseases Control (FCCDC), as part of the Project for Control of Soil-transmitted helminthiases in Fiji —a joint initiative comprising researchers at Seoul National University College of Medicine in Korea, and the Fiji Ministry of Health and Medical Services(MHMS), and with the Department of Health, Government of Wallis and Futuna.

This data set had, up to the time of this analysis, not been analyzed and results presented elsewhere.

Acknowledgement

It has been a long journey, and I would not have made it through without the support and guidance of many.

Firstly, my sincere appreciation goes to my supervisors at Liverpool School of Tropical Medicine and Seoul National University College of Medicine, Professor J. Russell Stothard, Dr Louise Kelly-Hope, Professor Sung-Tae Hong and Professor Min-Ho Choi, who provided endless support. This thesis would not have been possible without your guidance.

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Abbreviations and Acronyms

AIC	Akaike information criterion
ANOVA	Analysis of variance
BAZ	Weight-for-height Z-score
BC	Baermann concentration technique
BD	Birth dose
BIC	Bayesian information criterion
CFA	Circulating filariasis antigen
CI	Confidence interval
DALYs	Disability-adjusted life years
DEC	Diethylcarbamazine citrate
DNA	Deoxyribonucleic acid
DPS	Division of Pacific technical Support
EAs	Enumeration areas
ELISA	Enzyme-linked immunosorbent assays
EPI	Expanded Programme on Immunization
EPG	Eggs per gram
EUs	Evaluation Units
EVI	Enhanced vegetation index
FAO	Food and Agriculture Organization
FCCDC	Fiji Centre for Communicable Diseases Control
FEC	Formol-ether-acetate concentration
FSM	Federated States of Micronesia
GIS	Geographic information systems
GPS	Global positioning system
GPELF	Global Programme to Eliminate Lymphatic Filariasis
HAZ	Height-for-age Z-score
HBcAg	Hepatitis B virus core antigen
HBsAg	Hepatitis B virus surface antigen
HBV	Hepatitis B virus
HepB	Hepatitis B virus vaccine
ICT	Immunochromatographic test
IDA	Ivermectin- Diethylcarbamazine citrate-albendazole
IPI	Intestinal parasite infections

IRR	Incidence rate ratio
IU	Implementation unit
KK	Kato-Katz
LF	Lymphatic filariasis:
LOWESS	Locally-weighted scatterplot smoother
LQAS	Lot quality assurance sampling
MDA	Mass drug administration
Mf	Microfilaraemia
MHMS	Ministry of Health and Medical Services
NA	Not available
NDVI	Normalized difference vegetation index
NIMS	National iron and micro-nutrient supplementation
NPLF	National programme for LF elimination
NTDs	Neglected tropical diseases
OR	Odds ratio
PacELF	Pacific Programme to Eliminate Lymphatic Filariasis
PCR	Polymerase chain reaction
PCT	Preventive chemotherapy
PICTs	Pacific island countries and territories
PhHV 1	Phocine herpesvirus 1
POC	Point-of-care
pre-SAC	Preschool age children
RDT	Rapid diagnostic test
RT	Reverse transcriptase
SAC	School-aged children
SD	Standard deviation
SDGs	Sustainable Development Goals
SE	Standard error
SIDS	Small island developing states
SNUCM	Seoul National University College of Medicine
SS	*Strongyloides stercoralis*
STH	Soil-transmitted helminths
TAS	Transmission assessment surveys
TAS 1, 2, or 3	1st, 2nd, or 3rd transmission assessment survey

UNICEF	United Nation Children's Fund
US	The United States
WAF	Wallis and Futuna
WASH	Water, sanitation, and hygiene
WAZ	Weight-for-age Z-score
WCBA	Women of childbearing age
WHO	World Health Organization
WPRO	Western Pacific Regional Office

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Abstract

Introduction: Lymphatic filariasis (LF) and intestinal parasite infections are most prevalent neglected tropical diseases (NTDs) in Oceania. Since 1999, Pacific Programme to Eliminate Lymphatic Filariasis (PacELF) has successfully addressed critical issues and countries are able to stop mass drug administration (MDA) after a decade of the implementation. Transmission assessment surveys (TAS) is a standard methodology to assess whether MDA has reduced the LF prevalence to levels equal to or below the critical cut-off threshold. The aim of this thesis is to present the successful examples of expanding TAS as a pragmatic platform in providing insights into the epidemiology of the key communicable diseases in Oceania.

Method: In Wallis Futuna (WAF), grade 2-5 students for the TAS and grade 4-5 for the hepatitis B virus (HBV) infection prevalence assessment were enrolled. The timeliness of vaccination was defined as being vaccinated no later than 4 weeks in comparison to the recommended immunization schedules. In Fiji, all class 1 and 2 students were targeted in 77, 82, and 50 selected schools for the Western TAS 2, Central TAS 1 and Northern Division TAS 2, where same students of 30, 20, and 20 schools were subsampled for stool sample collection. For the diagnosis of intestinal parasite infections, stool samples were examined by coproscopy. In the Eastern, stool samples of class 1-8 students in 24 schools were additionally collected. For the selected stool samples of the Western Division, the Baermann-concentration technique (BC) was added to diagnose strongyloidiasis. All positive samples for intestinal parasites and a systematic sub-sample (every 10^{th}) from the Western Division were transferred to the reference laboratory for real-time PCR.

Results: In Wallis Futuna (WAF), TAS 1 had a passing result. The overall HBsAg prevalence was close to 2%. The HBV vaccine coverage for 3 doses was 96%, and the proportion of timeliness was 65%. In the Western Division of Fiji, 7 children in an island out of 3,242 were circulating filariasis antigen (CFA) positive. In the Central and Northern Divisions, the results were all passing, with 2 and no CFA positives, respectively. As for the assessment of intestinal parasite infections in Fiji, 12.4% of children in 3 Divisions were found positive either with *Ascaris* or hookworm eggs. The overall any intestinal protozoan infection prevalence was 4.8%, and the *Giardia* infection prevalence was 1.6%. The spatial analysis confirmed that there are still areas with high endemicity at school levels, where *Ascaris* infection was more clustered in the Central, and Eastern Division and hookworm in the Northern, and hotspots of *Giardia* infections were clustered around in the urban centres of the Western Division. As for associated factors with STH infections, shoes wearing and main water supply at home from Fiji Water Authority had protective effects. For strongyloidiasis, BC found 1 positive and the overall *S. stercoralis* infection prevalence via real-time PCR was 3.5 %, from which also higher levels of polyparasitism were reported as well as per species specificity.

Discussion and conclusion: The study shows that LF programmes in WAF and in Fiji are on track towards achieving the global goal of LF elimination as a public health problem. For WAF, the HBV infection prevalence is close to meet the regional goal of elimination, but timely vaccination should be more actively encouraged. As for STH infections in Fiji, a low-level of endemicity was observed, but the infections at schools were clustered. Overall any intestinal protozoan infection prevalence was not high, but there were hotspots of *Giardia* infection clustered, possibly due to contaminated water or foods following the floods caused by a cyclone. An analysis of factors associated STH infections in Fiji suggesting that there is a need to urgently resume preventive chemotherapy for the population at risk as well as to deliver integrated WASH interventions. Given that the estimated prevalence of *S. stercoralis* infections is endemic (>1 %) in Fiji, some 1.5K children are likely to have strongyloidiasis, implying the needs for curative measures, which was further assessed by molecular technique. In conclusion, LF TAS is a practical platform for assessing epidemiological features of the key communicable diseases in Oceania and should be actively utilized in accessing school-aged children in monitoring and evaluating public health interventions.

Chapter 1

General overview

Chapter 1. General overview

1.1. Introduction

Lymphatic filariasis (LF) is caused by infection with mosquito-borne filarial nematodes and is also known as elephantiasis when the clinical disease is advanced. This parasitic disease is one of the leading causes of morbidity in the world; an estimated 120 million people in tropical and sub-tropical regions are infected, having 25 million men with hydrocele and another 15 million with either lymphoedema or elephantiasis of the leg (WHO 2017c). Transmission of the disease can be curtailed by preventive chemotherapy approaches and globally, LF accounts for at least 2.8 million Disability adjusted life years (DALYs), excluding significant co-morbidity of mental illness experienced by patients and their caregivers (WHO 2017c). Of the total population requiring preventive chemotherapy, 57% live in the South-East Asia Region (9 countries) and 37% live in the African Region (35 countries) (WHO 2017c).

In Oceania, a region of tropical and sub-tropical islands in the South Pacific Ocean (Kline et al. 2013), LF and elephantiasis have been historically endemic and of the highest occurrence in the world . The region also has a long history of efforts to control the diseases. In 1999, the Pacific Programme to Eliminate Lymphatic Filariasis (PacELF) was launched under the auspices of the World Health Organization (WHO) (Huppatz et al. 2009), which was as a regional programme driven by the countries and constituted a network of the 22 island countries and territories in the Pacific (Ichimori and Graves 2017). The main strategy for achieving the goal was with preventive chemotherapy by annual mass drug administration (MDA) using diethylcarbamazine (DEC) and albendazole to stop LF transmission. As onchocerciasis is not endemic in this region, use of ivermectin has been precluded (WHO 2011c). Over the next decades, the programme successfully addressed a number of critical issues and many countries were able to stop MDA in the mid-to-late 2000s out of 16 countries which commenced MDA in 1999-2001 (See Figure 1.1a and 1.1b) (Ichimori and Graves 2017).

Figure 1.1a LF in the Pacific—countries and territories endemic in 2000 (Ichimori and Graves 2017)

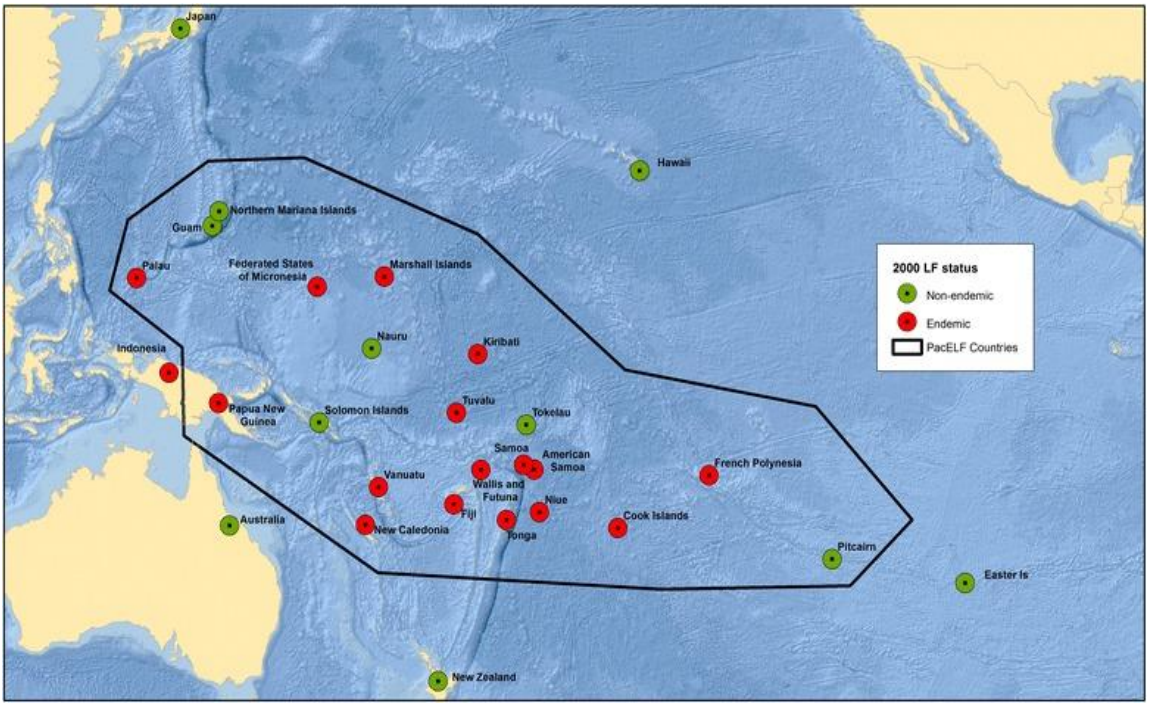
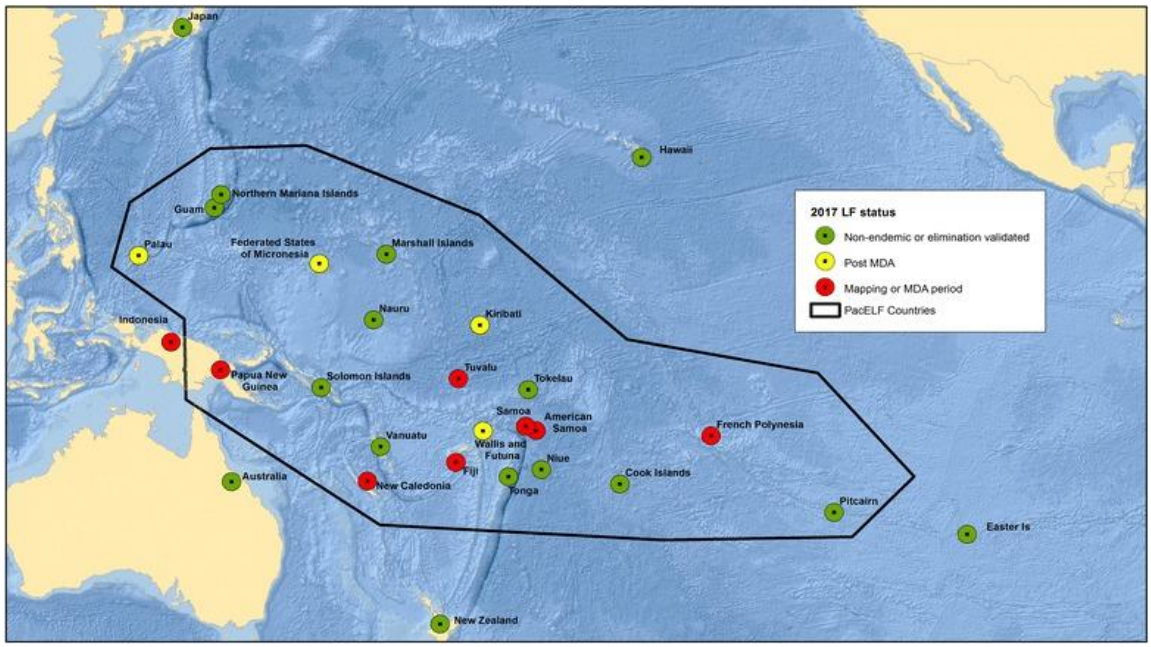


Figure 1.1b LF in the Pacific—countries and territories endemic in 2017 (Ichimori and Graves 2017)



In order to make stopping MDA decisions, the global programme guidelines require the country programme to demonstrate that prevalence of the infection in indicator groups is below acceptable thresholds and low enough to recommend cessation of annual MDA (WHO 2011c). In addition, countries will have to conduct further surveys to show a sustained reduction of the infection below the threshold no earlier than 4 years even after MDA is stopped (WHO 2017d). In this regard, WHO has published a standard survey methodology called the Transmission Assessment Survey (TAS), to assess whether annual rounds of MDA have successfully reduced the prevalence of infection to levels equal to or below the critical cut-off threshold (WHO 2012c). TAS provides a simple, robust survey design for documenting that the prevalence of LF, as ascertained by a rapid diagnostic test, among 6-7-year-old children is below 1% or 2% according to the type of mosquitoes, an important predetermined threshold derived from mathematical modelling (WHO 2012c). It is also recommended as a method of post-MDA surveillance to detect whether recrudescence of LF transmission has occurred, by repeating surveys at least twice, with an interval of 2–3 years (WHO 2012c).

Intestinal parasitic infection (IPI), especially soil-transmitted helminthiases are some of the most common parasitic infections, affecting more than 1.5 billion people worldwide (WHO 2017h). The three-main species are the roundworm (*Ascaris lumbricoides*), the whipworm (*Trichuris trichiura*) and hookworms (*Necator americanus* and *Ancylostoma duodenale*). The infection is transmitted by eggs that are passed in the faeces of infected people, which in turn contaminate soil in areas where sanitation is poor; faecal oral transmission for roundworms and whipworm infections, but percutaneous transmission for hookworm infections (WHO 2011a). Infected children can be nutritionally and physically impaired, as the worms feed on host tissues, including blood, and cause general inflammation which leads to a loss of iron and protein (WHO 2017h). Given that safe and effective medicines, i.e. albendazole and mebendazole, are available to control these infections, the control programme is principally based on the regular provision of preventive chemotherapy. The target of control is to ensure that at least 75% of preschool and school-aged children at risk of soil-transmitted helminth infections in 100% of countries are covered by 2020 (WHO 2012a). It is also recommended to implement health education to prevent re-infection and to improve sanitation to reduce soil contamination with infective eggs (WHO 2011a) as part of water, sanitation, and hygiene (WASH) measures in public health.

With approximately 35 million people living in Oceania and non-negligible levels of poverty (World Bank 2017), there are other neglected tropical diseases (NTDs) and communicable diseases of public health importance on top of LF. Soil-transmitted helminth infections may represent the most prevalent NTDs in Oceania, followed by strongyloidiasis and LF (Kline et al. 2013). Hookworm infection is possibly more prevalent compared to ascariasis and trichuriasis in the region, with an estimated 5.5 million cases, comprising roughly 1% of the world's cases of hookworm infection (de Silva et al. 2003). Strongyloidiasis is an important STH infection in Oceania with possible potential of public health importance, although no overall prevalence data are available (Kline et al. 2013).

1.2. Justification and rationale of the present study

With the remarkable success achieved in implementing MDA under PacELF (Kline et al. 2013; Ichimori and Graves 2017), it was mandated for the national programme in Oceania to conduct a series of population-based surveys, following the standard format of monitoring and evaluation for LF elimination programmes as recommended by the global programme guideline (WHO 2011c). Many of the countries that reduced LF prevalence low enough to stop MDA had entered or are entering into several years of the post-MDA surveillance phase during which they are required to conduct further TAS to validate elimination. At this stage, given the broader anthelminthic action of the drugs used in MDA, it would be wise to explore how best to integrate these surveys for the surveillance of another helminths infections to obtain more in-depth information about the epidemiological and putative treatment needs such as soil-transmitted helminthiases.

As WHO recommends, an integrated approach for the control of neglected tropical diseases is needed in order to avoid duplication of effort and to reduce costs (WHO 2006; WHO 2012a). In particular, it is important for STH infection control programme to determine whether administration of albendazole and mebendazole for specific age groups should be continued, in the absence of further community-based mass drug administration against LF targeting all age groups (WHO 2015a). In this regard, it was recommended by WHO that TAS could be used as an evaluation platform to assess STH infection epidemiology by collecting stool samples and conducting analysis through faecal microscopy e.g. Kato-Katz and to decide whether interventions for STH infection control, specifically mass deworming, should be implemented (WHO 2015a). Additional information collected on STH infections during TAS can also help programme managers to improve the effectiveness of STH infection control (WHO 2015a) such as water, sanitation, and hygiene.

Moreover, TAS–STH surveys provide good opportunities to collect additional data on the population or other biological specimens (WHO 2015a). For example, once stool specimens have been collected, the prevalence of infection not only of STH but also of other parasitological diseases such as intestinal protozoa or *Strongyloides stercoralis* can be determined using alternative methods to Kato-Katz, namely formal-ether concentration (FEC) or the Baermann concentration technique. Typically, data collected from these latter methods are not routinely undertaken as special laboratory equipment or techniques are required (WHO 2015a). Similarly, if additional blood samples are collected, it is also feasible to obtain information on serological data of other diseases, i.e. antigen or antibody prevalence. All in all, by assembling diagnostic data from the TAS platform it is possible to estimate the prevalence of several infectious or communicable diseases alongside surveillance for LF; collectively, a more integrated package of disease monitoring and surveillance could help national programmes establish or modify their control interventions and recommendations (WHO 2015a). Therefore, the rationale of the investigations presented in this thesis is to widen the application of TAS as an access platform for surveillance of other diseases of public health significance in Oceania, with a particular focus on Wallis and Futuna, as well as Fiji.

1.3. Aims of the thesis and study objectives

1.3.1. Aims

The overall aim of the thesis is to explore the possible expansion opportunities of LF TAS as a pragmatic platform for providing insights into the epidemiology of the key communicable diseases with public health importance, with attention on soil-transmitted helminth (STH) infections in Oceania. Once MDA has stopped, TAS can be used as a surveillance tool not only to determine that infection levels of LF are sustained below target thresholds (WHO 2015a) but also to monitor, evaluate and assess the impact of different control and intervention measures being implemented against other parasitic and communicable diseases.

1.3.2. Objectives

Specific study objectives of the thesis are:

(i) To determine whether 5–6 rounds of MDA using albendazole and diethylcarbamazine citrate (DEC) had interrupted transmission of *Wuchereria bancrofti* filariasis in Wallis and Futuna and in Fiji, by measuring the prevalence of circulating filariasis antigenemia among school children who were born after the initiation of the mass drug administration;

(ii) To assess the impact of the introduction of the hepatitis B vaccines into the national immunization programme for the infants in Wallis and Futuna, through measuring the prevalence of hepatitis B surface antigen, and the coverage and timeliness of the hepatitis B vaccination among school children in order to provide the Public Health Agency to improve their hepatitis B vaccination delivery for children under 1 year;

(iii) To explore the geographical distribution of the intestinal parasite infection among school children on Fiji by assessing the infection prevalence of the key parasite species and conducting spatial analysis on the clustering of the infection, especially for assessment of the impact of the implementation of nation-wide preventive chemotherapy via MDA rounds against LF and micronutrient supplementation programme for the STH infections;

(iv) To understand the impact of school and household WASH conditions, as well as behavioural components, on STH transmission and infection intensity among schoolchildren of Fiji, to be served as evidence-based information to guide future

STH infection control strategies for the Ministry of Health and Medical Services in Fiji;

(v) To conduct a pilot assessment of the epidemiology of *Strongyloides stercoralis* infections in the Western Division of Fiji and application of molecular techniques as a diagnostic quality control for soil-transmitted helminths and protozoan infections; and

(vi) To explore the possibility of the expansion of LF TAS as a platform to monitor, evaluate and assess the impact of different public health interventions, especially for the control and elimination of key communicable diseases in Oceania.

The thesis consists of the first-ever TAS in Wallis and Futuna having a whole country as one evaluation units (EU), as well as studies conducted in conjunction with the TAS. In Fiji, the thesis summarizes the survey results from 3 different TAS covering two main islands of Fiji by having 3 non-overlapping EUs in Fiji and other studies conducted together with these TAS.

This thesis therefore examines the extent to which these objectives can be achieved when the assessment is carried out not as a stand-alone initiative, but in conjunction with TAS, utilizing locally available techniques and resources. This assessment is carried out to provide evidence to policymakers: 1) when most of the disease burdens have been lightened and interventions at national level can be scaled down and questions arise whether there is sufficient evidence to maintain current policy decision or any room to improve the current strategy; or 2) when the distribution and extent of the disease burden are not previously well documented, but the information is needed to develop effective control strategies or to raise awareness on the necessities of developing the strategies. The former applies to LF and hepatitis B elimination programmes in WAF and Futuna as well as LF elimination programme of Fiji, while the rather would be the case for the STH infection control programme, where the components of the interventions can be tailored based on the risk factors identified but there are limited resources compared to LF elimination programme which limits the options to adapt for the national programme and is in needs of trade-off among the complementary interventions on top of preventive chemotherapy. Lastly, as for the wider applicability of LF TAS which provides a pratical platform to minimise the financial expenditure of general disease assessments (Chu et al. 2014), the thesis will investigate the benefits of this approach with the focus given to epidemiological surveillance of the communicable diseases.

1.4. Thesis layout

The thesis chapters for this Ph.D. will be laid out per the following structure:

Chapter 1 General overview: Provides a general overview of the thesis, with the rationale and the objectives of the study.

Chapter 2 Background and literature review: Describes the current knowledge of LF and TAS, as well as of the epidemiology of other key communicable diseases in Oceania including hepatitis B virus infections, soil-transmitted helminthiases, intestinal protozoan infections, and strongyloidiasis.

Chapter 3 General methods: Details about the region of Oceania and the study sites, Fiji and Wallis and Futuna, and introduces the standard methodology of LF TAS which was used as a platform to collect the epidemiological data and its steps for field implementation. The chapter also describes how the survey methods had been transferred to the study sites by the writer, and the important role of the writer in the survey organization, coordination, implementation and data analysis.

Chapter 4 Assessing the impact of mass drug administration (MDA) against LF via TAS in Wallis and Futuna and Fiji: Describes recent progress and the impact achieved from MDA rounds with DEC and albendazole on LF infections which are assessed by TAS, a standard methodology for the impact evaluation of MDA, in two different countries in Oceania.

Chapter 5 Expanded communicable diseases surveillance: Assessing the impact of hepatitis B virus vaccination in Wallis and Futuna utilizing LF TAS as a platform: Describes the impact of the hepatitis B virus vaccine introduction on hepatitis B infections in the country assessed in conjunction with LF TAS as described in Chapter 4.

Chapter 6 The geographical distribution of intestinal parasite infection on Fiji: Describes the distribution of intestinal parasite infections on Fiji, based on data compiled in conjunction with LF TAS as described in Chapter 4. Also, data from the sentinel site surveys on the STH infections of the remaining Eastern Division was included to provide national figures of STH infections on Fiji. The results of the spatial analysis of the school level intestinal parasite infection status to see whether the infection is clustered are presented here.

Chapter 7 Factors associated with soil-transmitted helminth infection on Fiji: Explores factors associated with the infection including water, sanitation, and hygiene (WASH) related, environmental, and socioeconomic factors that can help developing tailored public health interventions.

Chapter 8 Pilot assessment of the epidemiology of *Strongyloides stercoralis* infections using LF TAS as a platform and application of molecular techniques as a diagnostic quality control tool for soil-transmitted helminth and intestinal protozoan infections: Describes results from a pilot study on the distribution of *Strongyloides* infections in the Western Division of Fiji, assessed in conjunction with part of TAS as described in Chapter 4. It also details the results of the pilot quality control of classic parasitological examination of soil-transmitted helminth and intestinal protozoan infections using real-time PCR.

Chapter 9 General discussion: Provides policy implications of the studies presented, related to control or elimination of the key communicable diseases mentioned, and outlines suggestions for a future work.

Chapter 2

Background and literature review

Chapter 2. Background and literature review

2.1. Lymphatic filariasis (LF)

Lymphatic filariasis (LF) is a mosquito-borne parasitic disease caused by *Wuchereria bancrofti*, *Brugia malayi*, and *Brugia timori*, causing morbidity such as elephantiasis or hydroceles in humans (Ottesen 2006). The infection is a major public health problem in many tropical and sub-tropical countries and one of the most common parasitic diseases for human beings (Kimura 2011). The major vectors of *W. bancrofti* are *Culex* mosquitoes in urban and peri-urban areas, *Anopheles* in rural areas of Africa and elsewhere, and *Aedes* in the Pacific islands. The parasites of *B. malayi* can be transmitted by *Mansonia* spp. and anopheline mosquitoes are responsible for transmitting the infection. Brugian spp. are only found in the southeast Asia, namely Indonesia, Malaysia, India, Timor-Leste, and Thailand (WHO 2017c).

Lymphatic filariasis is known as elephantiasis, as it causes disfiguring disease most commonly in the lower extremity. If acquired during childhood, its visible manifestations may occur later in life, such as lymphoedema of the limbs, and genital diseases including hydrocele, chylocele, and swelling of the scrotum and penis. The majority of those infected with the parasites are without symptoms, but eventually they will develop subclinical damage and overt clinical symptoms yielding major social and economic impact with the loss estimated to be $ 1 billion (WHO 2017c).

2.1.1. Global Programme to Eliminate Lymphatic Filariasis (GPELF)

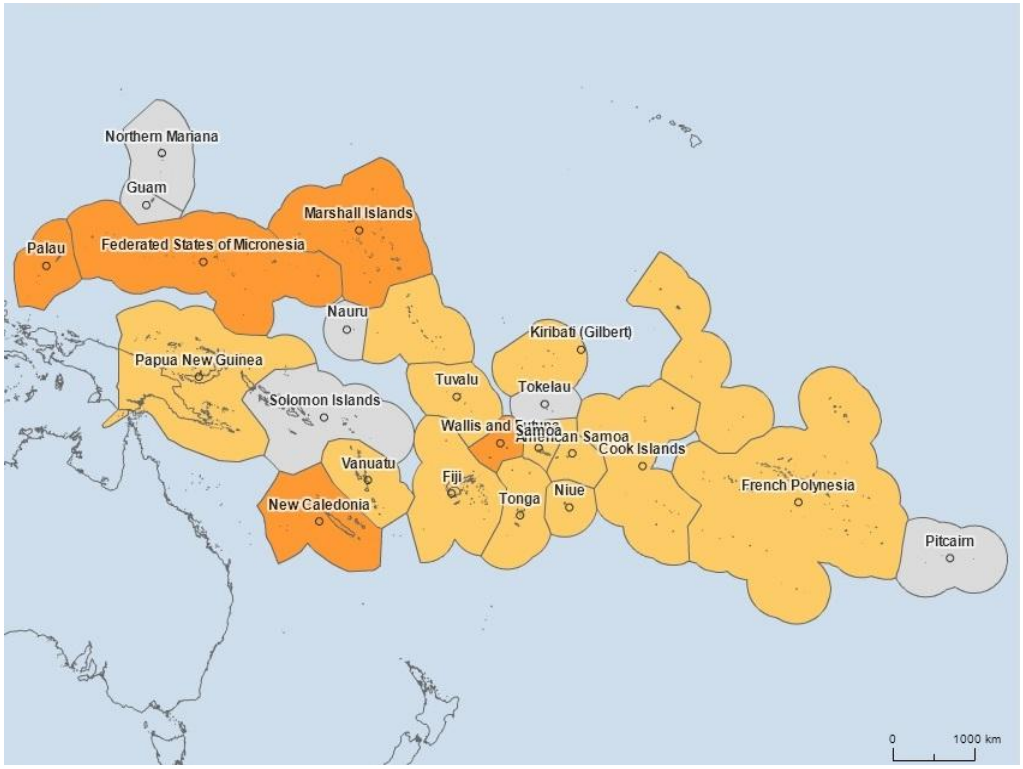
Following the 1997 World Health Assembly Resolution to eliminate LF as a public health problem by 2020 (WHA 50.29), the Global Programme to Eliminate Lymphatic Filariasis (GPELF) was launched in 2000. Annual mass drug administration (MDA) of either diethylcarbamazine citrate (DEC, 6mg/kg body weight) or ivermectin (150 μg/kg body weight) with albendazole (400mg per person) to the whole population at risk was a major tool (Burkot and Ichimori 2002) in stopping LF transmission. The drug combination reduces the density of microfilariae in the blood and the level of disease transmission in endemic areas. The strategy needs to be repeated for at least 4-6 rounds with effective epidemiologic coverage, namely minimum of 65%, corresponding to the life-span of the adult worms (Derua et al. 2018).

2.2. Lymphatic filariasis (LF) in Oceania

In Oceania, LF is solely caused by *Wuchereria bancrofti* and has long been highly endemic, with infection rates amongst the highest in the world (Ichimori and Graves 2017). With different ecologies based on the locality, there are three species of mosquito vectors (*Anopheles*, *Culex*, or *Aedes*) with the varying periodicity (time when microfilariae are at highest density in the blood) (Ichimori and Graves 2017).

2.2.1. Pacific Program to Eliminate Lymphatic filariasis (PacELF)

Figure 2.1 Sketch map of the countries in the Pacific, with its LF endemicity in 2000 according to PacELF classification



NB: Yellow coloured countries were classified as endemic, while orange for partially endemic countries (WHO 2011c).

The Pacific Program to Eliminate Lymphatic Filariasis (PacELF) was created as a regional counterpart of GPELF and has been coordinated by the WHO Western Pacific Regional Office (WPRO) based in Suva, Fiji, with the goal of eliminating LF in the 16 endemic Pacific island countries and territories (PICTs) (Figure 2.1) (Burkot and Ichimori 2002). The principal strategy for achieving LF elimination goal was annual mass drug administration (MDA) using diethylcarbamazine (DEC) with albendazole to stop transmission, together with clinical management of infections to minimize progression of pathology in individuals already

infected adapting the global strategy (WHO WPRO 2006). Since its creation 1999, PacELF has been supported by many partners including WHO, WPRO, the Government of Japan, Australia, the United Kingdom, the United States (US), New Zealand, the Republic of Korea, the NTD support centre at the Task Force for Global Health, James Cook University, Nagasaki University, the US Centres for Disease Control and Prevention, and GlaxoSmithKline (Ichimori and Graves 2017).

2.2.2. Lymphatic filariasis (LF) in Wallis and Futuna

The French overseas territory of Wallis and Futuna is one of the island countries in Oceania and has been long considered to be *Wuchereria bancrofti* endemic, for which the main vector is *Aedes polynesiensis* (WHO WPRO 2006). In the 1800s, lymphatic filariasis was common and it was reported that half of the adult population suffered from elephantiasis; in 1954, the rate of microfilaraemia was 40.0%, 20.4% in 1959, and 21.9% in 1977 (Fauran et al. 1981; WHO WPRO 2006). Monthly diethylcarbamazine citrate (DEC) distribution, targeting the entire population officially began in 1978 by the French army and continued until 1987 (WHO WPRO 2006). The impact of these interventions was demonstrated as microfilaraemia prevalence decreased from 5.3% in 1978 to 3.2% in 1985 in Wallis, and from 1.7% to 0.4% in Futuna over the same period of time (WHO WPRO 2006) and distribution of DEC was decreased to every six months until 2001 (Public Health Agency of Wallis and Futuna, data unpublished).

2.2.3. Lymphatic filariasis (LF) in Fiji

Elephantiasis was first reported in Fiji in 1876 by the British Medical Officer as early as 1876 (Sasa 1976). Opportunistic testing of patients at the Colonial War Memorial Hospital found 25.7% of microfilaremia (Mf) (WHO WPRO 2006). A nationwide survey of 57,000 people in 1956 found a 14.2% of Mf positivity (Sasa 1976). In Vanua Levu, much higher rates were reported on the windward wet side than on the leeward dry side (WHO WPRO 2006). Control measures with DEC were experimented in the 1950s which reduced the number of circulating microfilaria in the peripheral blood (Mataika et al. 1971). A pilot MDA was started in 1961 which was followed by a nationwide MDA programme from 1969 to 1975, where 5mg/kg of DFC was given weekly for 6 weeks and monthly for 22 months (WHO WPRO 2006). Mf prevalence levels had fallen less than 1% in some areas, but they had returned to their original levels or higher ones (WHO WPRO 2006).

From 1984 to 1991 a pilot project was carried out by Mataika et al. with the objective of comparing DEC efficacy between 5 annual single-dose MDA at 6mg/kg (total 30mg/kg) and very intensive 28-dose MDA (5mg/kg once a week for 6 weeks, then monthly for 22 months; total 140mg/kg) (Kimura 2011). The annual scheme reduced the Mf prevalence from 6.5% before treatment to 0.9% after 5 rounds of single DEC treatment, while in the multi-dose scheme, the prevalence dropped from 11.6% to 0.8% in 1987, and to 0.9% at 5 years (93% reduction) (Kimura and Mataika 1996). This indicates the effectiveness of annual single-dose MDA which is easier and more practical to implement, than the multi-dose scheme (Kimura 2011). Surveys between 1991 and 1995 determined the overall prevalence of Mf to be around 5.1%, without treatment for more than 2 years, suggesting the needs of continued treatment (WHO WPRO 2006).

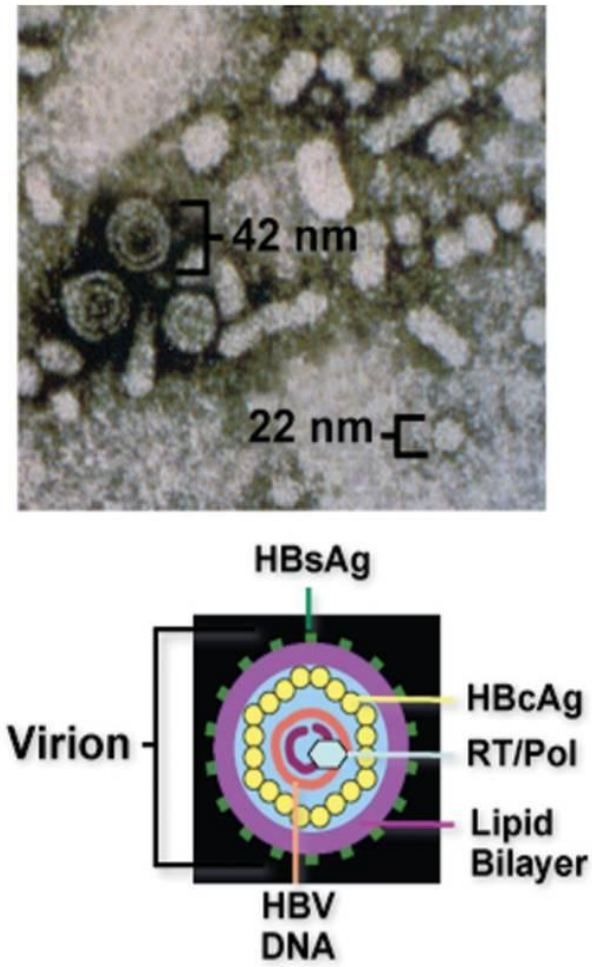
Fiji joined PacELF in 1999. A nationwide baseline blood survey was carried out on a sample of 5,983 people in 2000-2001 showed that up to 16.6% of the survey participants were positive with circulating filariasis antigen (CFA), and the country was classified as endemic under PacLEF (WHO WPRO 2006). An annual MDA round using the combination of DEC (6mg/kg) and albendazole 400mg was commenced in 2002.

2.3. Epidemiology of Hepatitis B virus (HBV) infections in Oceania

2.3.1. Hepatitis B virus

The hepatitis B virus (HBV) is an enveloped, double-stranded DNA virus which belongs to Hepadnaviridae family, and replicates by reverse transcription similar to retroviruses (Liang 2009). The genome of HBV is a circular DNA of about 3.2 kilobase (kb) pairs, and the viral polymerase is covalently attached by base pairing to the 5' end of the minus strand (Gerlich and Robinson 1980). The infectious HBV virion is also called Dane particle and has a spherical, double-shelled structure 42 nm in diameter (Tan 2016). It consists of a lipid envelope containing hepatitis B surface antigen (HBsAg) and an inner nucleocapsid core, which is composed of hepatitis B core antigen (HBcAg), complexed with virally encoded polymerase (Pol) and the viral DNA genome (Figure 2.2) (Liang 2009).

Figure 2.2 Diagram of Hepatitis B virus particle structure (Liang 2009)



N.B.: RT: reverse transcriptase; Pol: viral polymerase

The virus can be transmitted via percutaneous or mucosal exposure to infected blood and various body fluids, such as saliva, vaginal, and seminal fluids (Tan 2016). However, in highly endemic areas, the most common mode of transmission is from mother to child at birth (perinatal transmission), or through exposure to infected blood during the first 5 years of life (WHO 2016). The virus can survive outside the body for at least 7 days (WHO 2016), and still can cause infection if it enters human bodies of those who are not previously immunized (WHO 2010a). The clinical course of an HBV infection includes an incubation period (generally 4-12 weeks), acute illness (2 weeks-3 months) and recovery for individuals who resolve their infection (WHO 2001). The development of chronic infection is more common when they are infected in young age, mainly before the age of 6 years (Liang 2009; Tan 2016). An individual in whom HBsAg is present in his/her blood for more than six months are considered chronically infected with HBV and is therefore potentially infectious (WHO 2001).

HBV infection leads to a wide spectrum of liver diseases ranging from acute hepatitis, including fulminant hepatic failure, to chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma (Liang 2009). An estimated 257 million people are living with hepatitis B virus infection (defined as hepatitis B surface antigen positive) and about 780,000 people die each year due to consequences of hepatitis B worldwide (WHO 2016). Chronic hepatitis B is one of the leading causes of liver diseases and represents a serious public health problem (Hyams 1995; WHO 2010a). The prevalence of chronic HBV infection varies in different parts of the world (Desalegn et al. 2016), ranging from high (above 8%) in the most resource-limited settings, to low (below 2%) in most of developed countries (Alter 2003; WHO 2016).

2.3.2. Diagnosis of hepatitis B virus infection

A number of blood tests are available to diagnose and monitor people with hepatitis B, but laboratory diagnosis of hepatitis B virus infection focuses on the detection of the HBsAg (WHO 2016). An enzyme-linked immunosorbent assay (ELISA) is the gold standard to detect HBsAg, but requires a high-quality laboratory, expensive equipment, trained technicians, and a sustained supply of electricity (Njai et al. 2015). In contrast, point-of-care (POC) tests are easier to use and inexpensive compared with ELISA (Njai et al. 2015) in which a positive result is indicated by the appearance of a coloured dot or line (WHO 2001). These POC tests provide a flexible, technically undemanding, and relatively inexpensive approach to diagnostic testing (Lin et al. 2008).

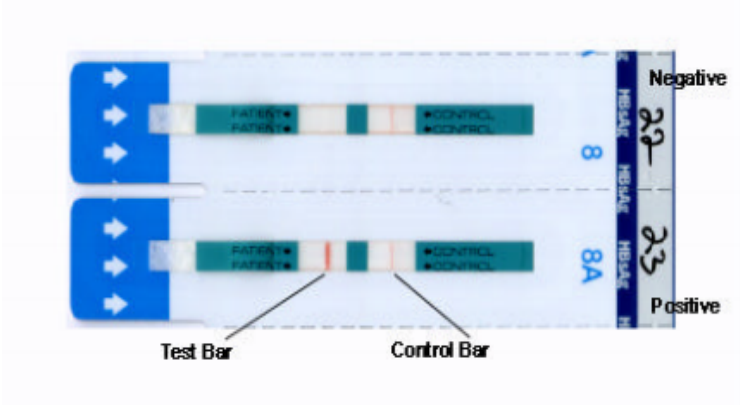
There are many rapid POC tests for HBsAg are commercially available, which have a range of diagnostic accuracy and generally do not have the same sensitivity and specificity (Table 2.1) (WHO 2001; Gish et al. 2014). One of the most widely used kits is Determine™ HBsAg (Abbott Laboratories) (Figure 2.3), a visually read, qualitative immunoassay for the detection of HBsAg in human serum, plasma and whole blood using immunochromatography (Alere 2017), and a meta-analysis reported high performance of the test, with a pooled sensitivity at 98.2% and a specificity at 99.9% (Njai et al. 2015). However, there have been false-negative results reported, in association with a low HBsAg concentration and HBsAg mutants (Njai et al. 2015). The minimum detection threshold is reported as low as HBsAg of 1 IU/ml (Lin et al. 2008).

Table 2.1 List of commercially available POC assays for HBsAg detection and its characteristics (WHO 2001)

Rapid Assay (Company)	Price/test USD (year)	Sensitivity (%)	Specificity (%)	Indeterminate results*(%)
ADVANCED QUALITY™ One Step HBsAg Test (Bionike Inc.)	0.75 (1999)	99.0	95.5	2.9
Determine™ HBsAg (Abbott Laboratories)	1.20 (1999)	99.0	99.4	0
Doublecheck HBs Antigen (Orgenics)	1.00 (1999)	99.0	96.1	1.4
Rapid HBsAg Test (Genelabs Diagnostics Pte Ltd.)	0.63 (1999)	99.0	97.8	0.7
HEPACARD (J.Mitra & Co. Ltd.)	0.63 (1999)	99.0	97.8	0.7
ImmunoComb® II HbsAg 90' (Orgenics)	0.90 (1999)	99.0	95.5	0.7
SERODIA® - HBs.*PA* (Fujirebio Inc.)	1.30 (2001)	99.0	100.0	0.0
Uni-Gold™ HBsAg (Trinity Biotech plc)	2.1 (1999)	99.0	100.0	0
GENEDIA® HBsAg Rapid Device (Green Cross Life Science Corp)	0.35 (2001)	99.0	100.0	0
HEP B STAT-PAK *ULTRA FAST* (Chembio Diagnostic Systems Inc.)	0.70 (2001)	99.0	100.0	0

N.B.: *: When test results could not be interpreted as clearly positive or negative

Figure 2.3 Photos of Determine™ HBsAg test kit showing negative and positive results (Alere 2017)



2.3.3. Control and prevention strategy for hepatitis B virus infection

Since the 1980s, a highly effective vaccine has been available to prevent HBV infection. The recombinant hepatitis B vaccine is a preparation of purified HBsAg, that has been produced by recombinant DNA techniques, and the antigen may be formulated with a suitable adjuvant (WHO 2010c). The complete vaccine series induces protective antibody levels in more than 95% of infants, children and young adults, and protection lasts at least 20 years and is lifelong (WHO 2016). Worldwide, in 2015, the estimated prevalence of HBV infection in 5 years old group was about 1.3%, compared with about 4.7% in the pre-vaccination era (WHO 2016).

In 1992 the WHO has made a recommendation that countries introduce three doses of hepatitis B virus vaccine (HepB), to be administered with diphtheria, pertussis, and tetanus vaccine (DPT), to their national immunization schedules (WHO 2010a). In 2009, WHO's recommendation was further specified to administer the first dose as soon as possible after birth, preferably within 24 hours, emphasizing the importance of preventing mother-to-child HBV transmission in controlling HBV infection (WHO 2010a). The World Health Assembly resolutions on viral hepatitis, WHA63.18 in 2010 and WHA67.6 in 2014, called for the Member States to develop and implement multi-sectoral national strategies to prevent, diagnose and treat viral hepatitis based on the local epidemiological context and for WHO to support these efforts (WHO WPRO 2016a).

2.3.4. Hepatitis B virus infection in Oceania

In Oceania, HBV infection prevalence has been historically high (Wong et al. 1979). According to the WHO Western Pacific Regional Office (WPRO), it is home to almost half of the estimated 350 million people with chronic HBV infections worldwide, even if the region contains only 28% of the global population (Rani 2009). Chronic HBV infection prevalence is the highest in the WHO Western Pacific Region as well as in the WHO African Region, where 6.2% and 6.1% of the adult population is still infected (WHO 2016). The rate was as high as 25 – 30% in many of the Pacific island nations in the pre-vaccination era (Rani 2009). Still, there are an estimated 160 million people with chronic HBV infections and more than 360 000 HBV related deaths occur annually in the region (Rani 2009).

Given that a highly effective vaccine has been available to prevent HBV infection, and WHO recommended that countries introduce the hepatitis B 3-dose vaccination series to their national immunization schedules, as well as the first dose, be administered as soon as possible after birth (WHO 2010a), the regional hepatitis B control goal was initially set to reduce the prevalence of chronic hepatitis B virus infection to less than 2% in children at least 5 years of age by 2012 (Rani 2009). In 2013 the goal was reaffirmed by setting a target date of 2017 for reducing chronic hepatitis B infection rates to less than 1% among 5-year old children (WPR/RC64.R5).

2.4. Intestinal parasite infections in Oceania

2.4.1. Soil-transmitted helminth infections

2.4.1.1. Epidemiology of soil-transmitted helminthiasis

Infections with soil-transmitted helminths (STH), namely *Ascaris lumbricoides*, hookworm (*Ancylostoma duodenale* and *Necator americanus*), and *Trichuris trichiura* are among the most common infections worldwide, particularly in the disadvantaged population, and designated as one of 20 NTDs of public health concern (WHO 2017e). More than 1.5 billion people are infected with STH infections worldwide, with the greatest numbers in sub-Saharan Africa, the Americas, China and East Asia (WHO 2017h). The parasites are also known as the geohelminths, as their mode of infection is transmitted through the ground (WHO 2012b); eggs (or larvae) are excreted from infected persons in their faeces, which then contaminate the soil (WHO 2011a). Ingestions of these infective eggs in food, hands, or utensils will transmit the infection for *As. lumbricoides* and *T. trichiura*, whereas contacts with contaminated soil results in penetration of infective larvae through the skin for *Ancylostoma duodenale* and *Necator americanus*

(WHO 2011a; Strunz et al. 2014).

Table 2.2 Characteristics of the Kato-Katz and Mini-FLOTAC (WHO 2015a)

Characteristic	Kato-Katz	Mini-FLOTAC
Standard	Used regularly in endemic countries	Relatively new
Specimen	Fresh faeces	Fresh or fixed faeces
Sensitivity	Good sensitivity for moderate- or heavy-intensity infection Poor sensitivity for very light-intensity infection	Good sensitivity for moderate- or heavy-intensity infection Poor sensitivity for very light-intensity infection
Equipment	Requires equipment that is simple to use, low cost and recyclable; readily available in endemic countries	Requires equipment that is simple to use, low cost and recyclable; not readily available in endemic countries
Ease of implementation	Very Easy	Easy
Requirement for fixative	No fixative required	2 ml of 5% solution of formalin required to fix each faecal sample
Personnel	Should be trained	Should be trained
Timing	Examination of faecal specimens within 4–6 h of collection	Faecal specimens can be fixed after collection and examined within 1–2 weeks.
Logistic implications	If the site at which specimens are collected is more than 3–4 h from a laboratory, processing, and examination should be done on site.	Specimens can be collected on site, fixed and transported to a central laboratory for examination.
Personnel required	If the specimens are analyzed on site, one to three people are required to expedite examination so as not to delay the TAS team.	One staff should join the TAS team to collect and fix specimens while blood samples for lymphatic filariasis testing are collected.

It is known that adult worms live in the human intestine, where they produce thousands of eggs each day, and these eggs are passed in the faeces of infected people where the soil are contaminated (Muller 2002). In areas with lack of adequate sanitation, eggs that are attached to vegetables or are from contaminated water sources are ingested (WHO 2017h). Likewise, eggs can be ingested by children who play in the contaminated soil and then put their hands in their mouths without washing them (WHO 2017h). In addition, hookworm eggs hatch in the soil, then released larvae which mature into a form that can actively penetrate the skin (WHO 2017h), with which people become infected by walking barefoot on it (Muller 2002).

Soil-transmitted helminths impair the nutritional status of infected human hosts in multiple ways: The adult worms feed on host tissues, including blood, which leads to a loss of iron and protein (WHO 2017h). Hookworms, in addition, cause chronic intestinal blood loss that can result in anaemia (Muller 2002). The worms also increase malabsorption of nutrients and cause loss of appetite, leaving the hosts with reduced nutritional intake and physical fitness (WHO 2017h). Morbidity is directly associated with the worm burden: People with infections of light intensity usually do not suffer from the infection (WHO 2011a). Heavier infections can cause a range of symptoms including intestinal manifestations, malnutrition, general weakness, and impaired growth and physical development (WHO 2017h).

The two laboratory techniques recommended for collecting data on STH especially during a TAS are the Kato-Katz and mini-FLOTAC tests (WHO 2015a). Details of the techniques are provided in Table 2.2.

2.4.1.2. Prevention and control of soil-transmitted helminthiasis

The principal strategy for control of STH infections is to control morbidity through the periodic treatment of at-risk people (preschool children, school-age children, and women of childbearing age including pregnant women in the second and third trimesters and breastfeeding women) via reducing the intensity of infection (WHO 2017h). WHO recommends periodic medicinal treatment (deworming) without a previous individual diagnosis to all at-risk people living in endemic areas, and treatment should be given once or twice a year based on the baseline prevalence of STH infections (WHO 2017g). In addition, health and hygiene education reduces transmission and reinfection, by encouraging healthy behaviours such as hand washing and the use of footwear (WHO 2017h). Provision of adequate sanitation is considered important as seen in the recent meta-analysis (Strunz et al. 2014). The recommended medicines are albendazole (400mg) and mebendazole (500mg), which are effective, inexpensive and easy to administer by non-medical personnel (WHO 2011a).

In 2001, at the World Health Assembly, a resolution was endorsed urging endemic countries to tackle STH infections (WHA54.19). The global target is to eliminate morbidity due to STH infections in children by 2020 by regularly treating at least 75% of the children in endemic areas (WHO 2012a).

2.4.1.3. Soil-transmitted helminth infections in Oceania

Soil-transmitted helminth (STH) infections may represent the most prevalent NTDs in Oceania (Kline et al. 2013). Currently, up to 5.5 million is estimated to have hookworm infections as well as another 1.2 million each with trichuriasis and ascariasis, which yields over 20% of 35 million population in Oceania being STH infected (de Silva et al. 2003; Kline et al. 2013). The prevalence levels will be higher for those living in the Pacific island countries, considering that 26.7 million populations in Australia and New Zealand with improved access and sanitation would have a very low risk of the infection except the aboriginal Australians.

Most of the hookworm-infected cases are found in Papua New Guinea (PNG), where three-quarter of the population is being infected (de Silva et al. 2003). The infection is also prevalent in other Melanesian countries, such as Fiji, the Solomon Islands, and Vanuatu (Kline et al. 2013). *Necator americanus* was the predominant species in PNG (Pritchard et al. 1990). In contrast, both *N. americanus* and *An. duodenale* may have been present in Australia (Hopkins et al. 1997). Compared to hookworm infections, ascariasis and trichuriasis are less common in Oceania (Kline et al. 2013). Historic data shows that trichuriasis was common in Fiji, also in the Solomon Islands and in Vanuatu (de Silva et al. 2003). A large number of ascariasis cases were found in PNG (King and Mascie-Taylor 2004) followed by other Melanesian countries (Kline et al. 2013). Nevertheless, up-to-date data on the epidemiologic profile of STH infections in the Pacific Island countries is scarce (WPRO 2008). The only recent study on STH infections covering the Pacific island countries was a multi-country survey conducted in 2001-2002 by Hughes et al. In each country, a team of environmental health specialist, a nutritionist, and a parasitologist visited 2 schools per country, one urban and one rural as selected by the government. In total 1,996 samples were analyzed and the overall any STH prevalence was 32.8% and the range of school-level prevalence was 0-96.7% (Table 2.3) (Hughes et al. 2004).

Table 2.3 School prevalence of any STH infection in 14 countries (Hughes et al. 2004)

Country	School Name	Number examined	Any STH infection prevalence (%)		
			Boys	Girls	Total
Am. Samoa	Matafau	29	11.1	0	6.9
	Taputapu	31	0	0	0
Cook Islands	Arorangi	78	2.3	5.7	3.8
	Avarua	78	2.9	2.3	2.6
Fiji	Veiuto	58	18.8	7.1	10.3
	Rishikul	176	8.3	10.1	9.1
French Polynesia	Mamao	68	12.5	0	5.9
	Raiarii Tane	93	15.2	10.6	12.9
FSM - Pohnpei	RSP	61	8.6	15.4	11.5
	Ohmine	97	36.2	41	38.1
FSM - Yap	Gaanelay	108	39.2	28.1	33.3
	Gagil	59	40.5	18.2	32.2
Kiribati	Nabeina	39	89.5	100	94.9
	Bareaumai	90	97.8	95.5	96.7
Marshall Islands	Rita	28	83.3	70	78.6
	Laura	92	91.5	75.6	83.7
Nauru	Nauru College	36	41.2	10.5	25
	Location	53	45.5	50	47.2
Niue	Niue Primary	139	1.3	0	0.7
Solomon Islands	St. John's	59	34.5	46.7	40.7
	Lunga	40	43.8	45.8	45
Tonga	Fanga'o	96	7	9.4	8.3
	Mu'a	120	11.8	15.4	13.3
Tuvalu	Tutasi	32	90.5	81.8	87.5
	Nauti	86	100	100	100
Vanuatu	Fresh Wota	77	39.4	25.0	31.2
	Eratap	73	63.9	67.6	65.8

N.B.: FSM denotes the Federated States of Micronesia

According to WHO preventive chemotherapy databank, in as early as 2003, there were 12 countries which reported national deworming coverage (Table 2.4) (WHO 2017f). However, these were all LF endemic countries under PacELF and deworming was covered as part of LF MDA (Figure 2.1). Upon stopping MDA against LF, most of the countries were still considered as endemic for STH infections and several of them were able to switch to mass deworming against STH infections (Table 2.4), namely Kiribati, Marshall Islands, and Vanuatu. Solomon Islands initiated mass deworming targeting schools children and pre-school children without prior LF MDA rounds, while French Polynesia, FSM, and Tuvalu conduct it as part of LF programme (WHO 2017f).

Table 2.4 Changing endemicity of STH infections among the Pacific Island Countries as of 2003 and 2016 (WHO 2017f)

Country	2003	2016
American Samoa	yes	-
Cook Islands	yes	-
Fiji	yes	yes
French Polynesia	-	Deworming not required
Kiribati	yes	yes
Marshall Island	yes	yes
Federated States of Micronesia	yes	yes
Nauru	-	yes
Niue	yes	-
PNG	-	yes
Samoa	yes	No data available
Solomon Islands	-	yes
Tonga	yes	yes
Tuvalu	yes	yes
Vanuatu	yes	yes
Wallis and Futuna	yes	No data available

2.4.2. Intestinal protozoan infections

2.4.2.1. Epidemiology of intestinal protozoan infections

Table 2.5 Protozoa associated with intestinal illness in humans (eMedicine 2017)

Name	Mode of Transmission	Symptoms
Flagellates		
Giardia duodenalis	Contaminated water, faecal-oral	Nausea, bloating, gas, diarrhoea, anorexia
Dientamoeba fragilis	Faecal-oral, associated with *Enterobius*	Previously thought commensal; may cause diarrhoea, abdominal, pain, nausea
Amoebas		
Entamoeba histolytica	Contaminated water, faecal-oral, contaminated food	Colitis, dysentery, diarrhoea, liver abscess, other extra intestinal diseases
Spore-forming (Coccidia)		
Cryptosporidium parvum	Contaminated water, swimming pools, faecal-oral	Immunocompetent patients: Self-limited diarrhoea Immunosuppressed patients: Severe and interminable diarrhoea
Isospora belli	Faecal-oral	Same as in *Cryptosporidium*
Cyclospora cayetanensis	Faecal-oral, contaminated water and food	Same as in *Cryptosporidium*
Microsporidia (*Septata intestinalis*, *Enterocytozoon bieneusi*)	Faecal-oral, contaminated water	Same as in *Cryptosporidium*
Ciliates		
Balantidium coli	Faecal-oral (frequently associated with pigs)	Colitis, diarrhoea
Other		
Blastocystis hominis	Faecal-oral	May cause mild diarrhoea

Intestinal protozoan infections remain as a major health concern in tropical and subtropical areas of the world, where poor sanitary conditions and the unavailability of effective water treatment have contributed their transmission (Sterling and Adam 2004). Several species of intestinal protozoa are associated with acute and chronic diarrheal illnesses in humans (Table 2.5) (Fletcher et al. 2012). More specifically, giardiasis and cryptosporidiosis are important causes of diarrhoea in children, which are associated with severely debilitating conditions such as malabsorption and growth retardation (Kenny and Kelly 2009). *Entamoeba histolytica* can cause dysentery and liver abscess (Sterling and Adam 2004). Infection usually occurs through ingestion of cysts in water (including both unfiltered drinking-water and recreational waters) or food contaminated by the faeces of infected humans or animals (Fletcher et al. 2012). Infections can be asymptomatic, whereas they are intestinal, characterized by chronic diarrhoea (watery initially, then loose greasy stools), abdominal cramps, bloating, fatigue and weight loss, when symptoms occur (Table 2.5).

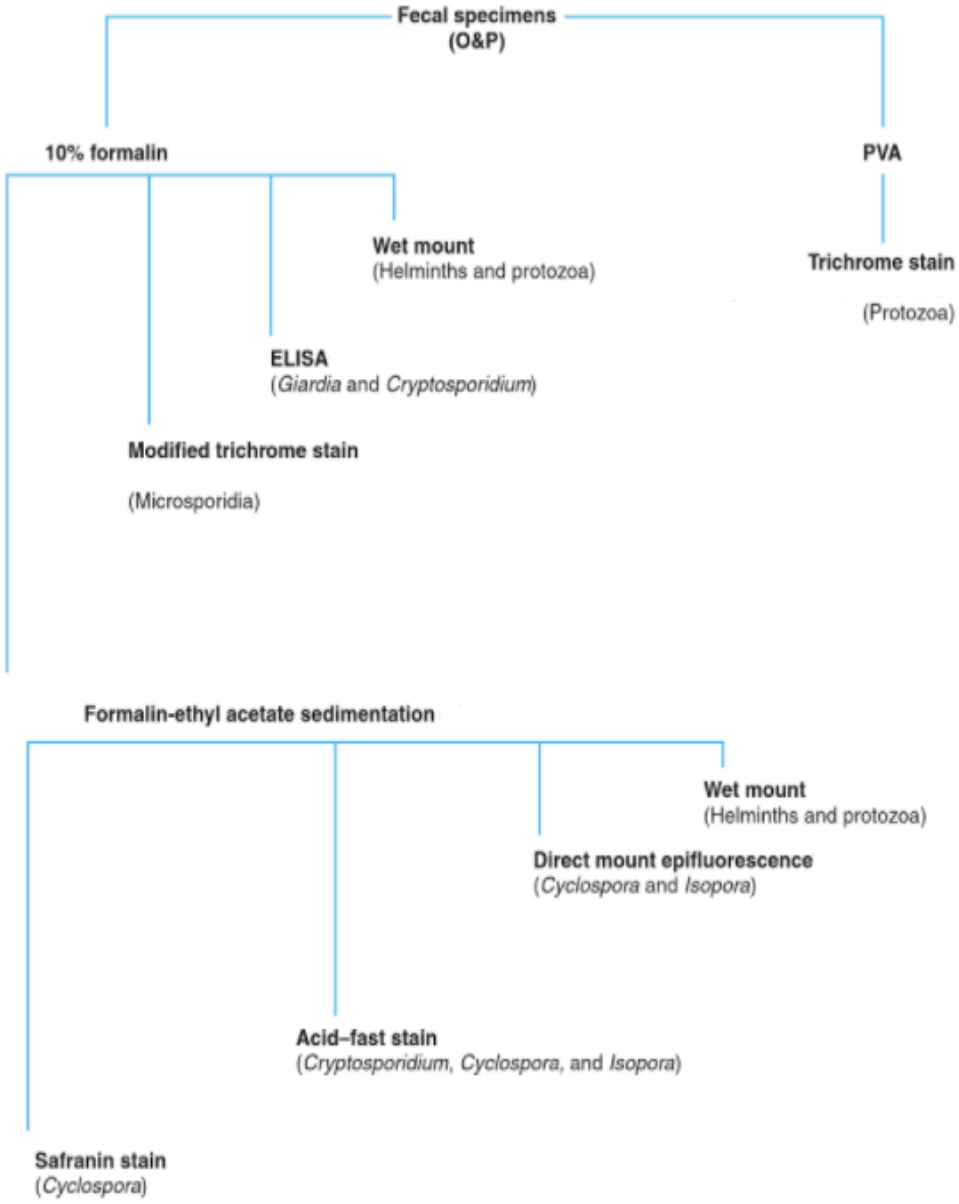
2.4.2.2. Diagnosis of intestinal protozoan infections

Although treatment for amoebiasis and giardiasis is available, diagnosis of infections with intestinal protozoa is difficult than that of intestinal helminths (WHO 1987). Most currently-used techniques either rely on seeing cysts or vegetative stages in faeces (Figure 2.4) or use specific antibodies as a means of capturing parasite antigens from faeces (Bundy et al. 1992). The routine laboratory diagnostic methods being used for the detection of cysts include direct smear and formalin-ethyl acetate concentration (FEC) techniques (Figure 2.4). These techniques are simple, rapid, and inexpensive, but direct smear method alone would be insufficient to diagnose the diseases particularly in cases with low-intensity infections where diagnostic stages of parasites will be only intermittently released into stool samples (Charuchaibovorn 2016). FEC would be more beneficial, but it is labour-consuming and requires specific instruments such as centrifuges (WHO 1991). Moreover, trophozoites or cysts of some intestinal protozoa could be destroyed during the process of FEC (Charuchaibovorn 2016). Nevertheless, it is still widely practised in making diagnosis for intestinal protozoa infections.

It should be noted that assessing the accuracy of the diagnostic test is important if the results are to be used for the control programme (Bundy et al. 1992). Current diagnostic techniques are neither very sensitive nor very specific (WHO 1987), and it will be challenging particularly in epidemiological surveys because the microscopic techniques require highly skilled personnel which is not usually available in resource-limited settings (Bundy et al. 1992). There are a number of rapid diagnostic tests now available as an alternative for the test of

Entamoeba histolytica, Giardia duodenalis, and *Cryptosporidium parvum* either by using fresh or fixed stool samples, though these are costlier than the conventional microscopic techniques. Molecular methods such as real-time PCR have been recently added to the list, but they require more advanced equipment and skills. Which are not readily available in developing country settings for stool examinations (Verweij and Stensvold 2014).

Figure 2.4 Processing of faecal specimen for the diagnosis of intestinal parasite infections (Tille 2014)



N.B.: O&P denotes ova and parasites; PVA: Polyvinyl alcohol

2.4.2.3. Prevention and control of intestinal protozoan infections

For the prevention, the most commonly reported strategies include hand-washing and improvement of personal hygiene (Alum et al. 2010): Hand-washing is one of the most important interventions that has proven to effectively intervene with faecal-oral transmission of diseases (Alum et al. 2010), highlights the importance of an integrated approach for the effective delivery of WASH interventions (WHO 2015e). Where high prevalence levels of intestinal protozoan infections are expected with the morbidity they cause, measures aimed at their prevention and control focusing on WASH should be strengthened (WHO 1987). However, the conditions to promote these strategies fully are not readily available in underprivileged communities where the infection would be more prevalent (Fletcher et al. 2014).

The major constraint on developing, implementing, and evaluating the control strategy of intestinal protozoan infections is that the evidence base for targeted interventions for the prevention is not sufficient (Fletcher et al. 2014), and there is a need to identify pathogenic species and strains as commensal protozoans are ubiquitous and often morphologically very similar to pathogens (Alum et al. 2010). Species such as *E. histolytica* exist as strains with differing pathogenicity, thus effective diagnosis is a key to the establishment and evaluation of the control programme of intestinal protozoan infections (Alum et al. 2010).

2.4.2.4. Intestinal protozoan infections in Oceania

With its impoverished pockets in the region and uneven distribution of access to safe water and sanitation, Oceania is not the exception for the burden of intestinal protozoan infections (Kline et al. 2013). The major intestinal protozoan infections in Oceania are amoebiasis, balantidiasis, cryptosporidiosis, and giardiasis, but few studies have been available outside of Australia, where infections are more prevalent with Aboriginal populations, or New Zealand (Lake et al. 2009; Kline et al. 2013; Fletcher et al. 2014). One study from New Caledonia reported cases of liver abscess from *E. histolytica* among hospitalized patients (Guittet et al. 2004) as well as another study from Fiji (Ram 2014), while an outbreak of balantidiasis was described from swine producing areas in PNG, and following contamination of surface water after a typhoon in Truk with pig faeces (Schuster and Ramirez-Avila 2008; Kline et al. 2013). Giardiasis is considered to be common in Oceania, but a true estimate of the disease burden is not available to date (Kline et al. 2013). Chagas diseases are not endemic in any of the nations in the region (Kline et al. 2013). Nevertheless, programmes aimed at the prevention and control of the infection are not readily available in the region, even in Australia (Fletcher et al. 2014).

2.4.3. Strongyloidiasis

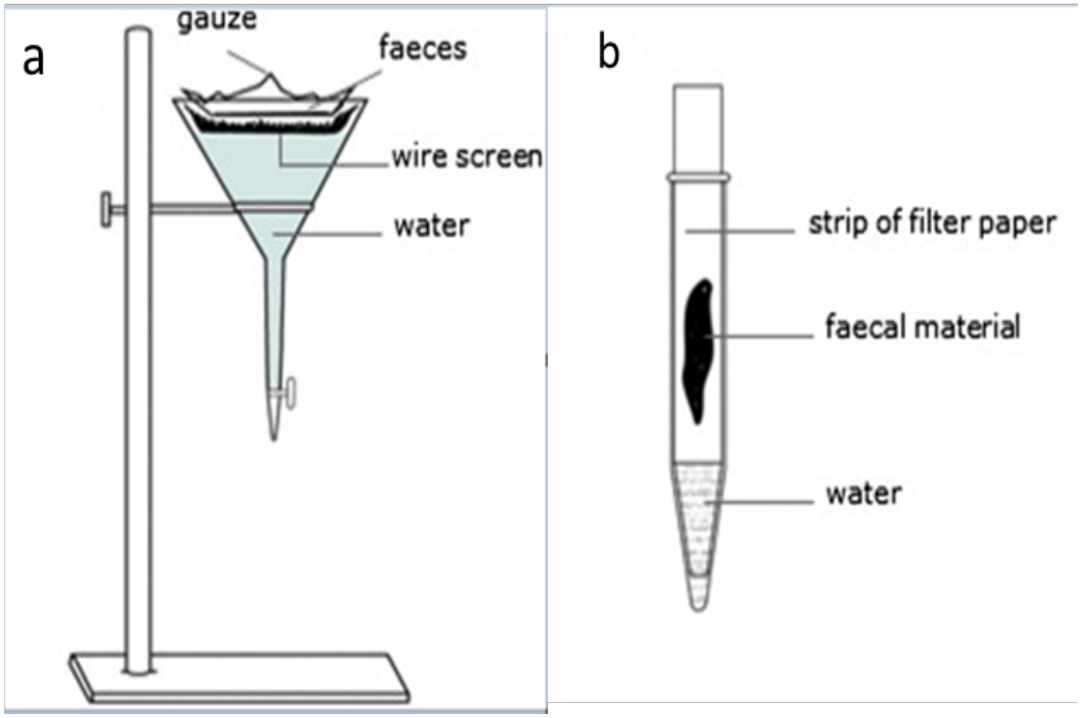
2.4.3.1. Epidemiology of strongyloidiasis

Strongyloidiasis is a chronic infection of humans caused by a nematode, *Strongyloides stercoralis*. It presents mainly in tropical and subtropical regions but also in temperate climates (Muller 2002). Currently WHO estimates that some 30–100 million people are infected worldwide (WHO 2015d). Strongyloidiasis is transmitted through direct penetration of human skin by infective larvae, and similar to hookworm infection, walking barefoot is a major risk factor for acquiring the infection (Muller 2002). The larvae then reach to the human intestine, where they can mature into adults and produce eggs (Muller 2002). These eggs will hatch into larvae in the intestine and most of the larvae will be excreted in the stool, but some of the larvae may re-infect the host either by penetrating into the intestinal wall or the perianal skin, which causes 'auto-infection' (Buonfrate et al. 2013). Strongyloidiasis may be asymptomatic in among healthy individuals, but it may cause intermittent symptoms such as abdominal pain, intermittent or persistent diarrhoea, chronic bronchitis or urticarial (Muller 2002). In cases of immunodeficiency, such as haematological diseases or those under immunosuppressive therapies, the infection can be life-threatening (Buonfrate et al. 2013).

2.4.3.2. Diagnosis of strongyloidiasis

Diagnosis of strongyloidiasis is not standardized, and even frequently used procedures may not often yield positive results when the disease is present (Requena-Méndez et al. 2013). The presence of rhabditiform first-stage larvae in a fresh faecal sample provides positive diagnosis, and Baermann or formol-ether-acetate concentration can be also used to concentrate the larvae (Muller 2002). In the Baermann concentration technique (BC), up to 10 g faeces is spread on a layered gauze pad on a coarse sieve and placed in a sedimentation flask that has been filled 25 mm below the rim with saline or water at 37°C or on top of a funnel with a length of plastic tubing clamped at the bottom (Figure 2.5a). After 2 hours, 5 ml of the bottom sediment can be removed with a Pasteur pipette and transferred to a 5 cm Petri dish, which is examined under a dissecting microscope for first-stage larvae (Muller 2002). If no larvae are found, the flask can be left overnight at room temperature and re-examined (Muller 2002).

Figure 2.5 Schematic diagram of the Baermann concentration (a) and Harada-Mori technique (b) (Toledo et al. 2015)



Various culture methods can be alternatively used if no larvae are found in the concentration techniques. In the Harada-Mori technique, filter paper containing fresh faecal samples is placed in a test tube with water that continuously soaks the filter paper by capillary action (Figure 2.5b) (Harada and Mori 1955). Incubation at 30°C provides temperatures suitable for the development of larvae, which can then migrate to either side of the filter paper. The technique is less sensitive than Baermann or Koga-agar culture but more sensitive than single stool-smears (Khieu et al. 2014). However, it is not frequently used as a standard procedure in clinical parasitology laboratories (Requena-Méndez et al. 2013). In the Koga agar culture method, the stool is placed on a nutrient agar plate, incubated in a humid chamber at 28°C for at least 48 hours and examined for visible tracks created as larvae carry bacteria over the agar (Koga et al. 1991). Mobile *S. stercoralis* larvae can also be seen with the aid of a dissecting microscope (Khieu 2014). Although the agar culture method has a higher sensitivity (96%) it is more time consuming and laborious than direct faecal smears or the Baermann concentration (Requena-Méndez et al. 2013).

2.4.3.3. Prevention and control of strongyloidiasis

For treating the infection, ivermectin is the drug of choice but is not widely available in all endemic countries (WHO 2015d). Moreover, the optimal schedule has yet to be defined. Up to now, no public health strategy has been developed to control strongyloidiasis (Olsen et al. 2009; WHO 2015d). However, in areas where mass treatment with ivermectin has been used to control onchocerciasis or lymphatic filariasis, it is assumed that the prevalence of strongyloidiasis is probably reduced, but further investigation is warranted to test the hypothesis (WHO 2015d). Strongyloidiasis has almost disappeared in countries where sanitation and human waste disposal have improved (WHO 2015d), which shows the potential of controlling transmission by establishing the linkage to the integrated approach for more effective delivery of WASH interventions (WHO 2015e).

2.4.3.4. Strongyloidiasis in Oceania

Helminth infections are considered as the most prevalent NTDs in Oceania, led by hookworm and LF, although significant numbers of cases of strongyloidiasis are also present (Kline et al. 2013). However, there are no overall prevalence data available, except a study conducted in PNG where prevalence rates of *Strongyloides fuelleborni kellyi* infections were reported as high as 60% (King and Mascie-Taylor 2004). In PNG, *S. stercoralis* infections were also reported (Kline et al. 2013), but outside of PNG even sporadic cases of *S. stercoralis* infections are not often documented (Pattison and Speare 2008). Strongyloides-infected populations may benefit from MDA programme using ivermectin (Kline et al. 2013), but there is no country in the Pacific where LF and onchocerciasis are co-endemic or has introduced the ivermectin-DEC-albendazole regimen (IDA) against LF (WHO 2017f).

Chapter 3

General methods

Chapter 3. General methods

3.1. Approach

Monitoring and evaluation of the public health programmes is an essential tool to ensure that health services are implemented as planned, and to assess whether the desired results are being achieved (Milstein and Wetterhall 1999). Data collection is a critical component of monitoring and evaluation, and it is important to follow several key principles (Soucie 2012): Firstly, the objectives should be clearly defined in order to guide the choice of data elements; and data elements also should be well defined and easily available to collect. Identification of the proper target population and sampling strategy is instrumental (Soucie 2012). Once the data have been collected, it is important to have secure database with proper data management system and quality control procedures. Data should be evaluated for their accuracy and completeness (Teutsch and Thacker 1995). The analysis of the data includes descriptive information of the population, and the disease outcomes as well as associated factors, which in turn will help to estimate the burden of the disease in the population and which section of the population is at risk of having diseases. The results will be reviewed by the health authorities and policy makers for the development of the public health interventions to address the risk factors, as well as assessing the effectiveness of the interventions (Soucie 2012).

The principal methodology used to assess the impact of mass drug administration (MDA) against lymphatic filariasis (LF) was based on the global programme guidelines developed by WHO's GPELF, as described in the "Monitoring and epidemiological assessment of mass drug administration in the global programme to eliminate lymphatic filariasis: a manual for national elimination programme" (WHO 2011c) and "Assessing the epidemiology of soil-transmitted helminths during a transmission assessment survey in the global programme for the elimination of lymphatic filariasis" (WHO 2015a). These are specifically designed methods for the national LF elimination programme to be able to monitor MDA effectively and to assess whether infection has been reduced to levels whether transmission is assumed to be no longer sustainable or not (WHO 2012c).

3.2. Study sites

Oceania is the region located in the central Pacific Ocean and spans over 8.5 million km^2 (Columbia University Press 2005). The region includes Australia, New Zealand, Melanesia, and the Polynesian and Micronesian islands (Kline et al. 2013) (Figure 3.1). More broadly it is the entire insular region between Southeast Asia and the Americas, sometimes including Australasia and the Malay Archipelago but not Indonesia and the Philippines (Columbia University Press 2005). The name "Oceania" is used because it is the ocean rather than the continent, which links the nations together (New World Encyclopaedia 2015). Approximately 40 million people are spread across 30,000 islands of Oceania, where up to 23.8 million lives on the Australian continent, followed by 7.9 million in Papua New Guinea, 4.6 million in New Zealand, and 0.9 million in Fiji (Table 3.1).

Figure 3.1 Map showing countries in Oceania (Worldofmaps.net 2017)

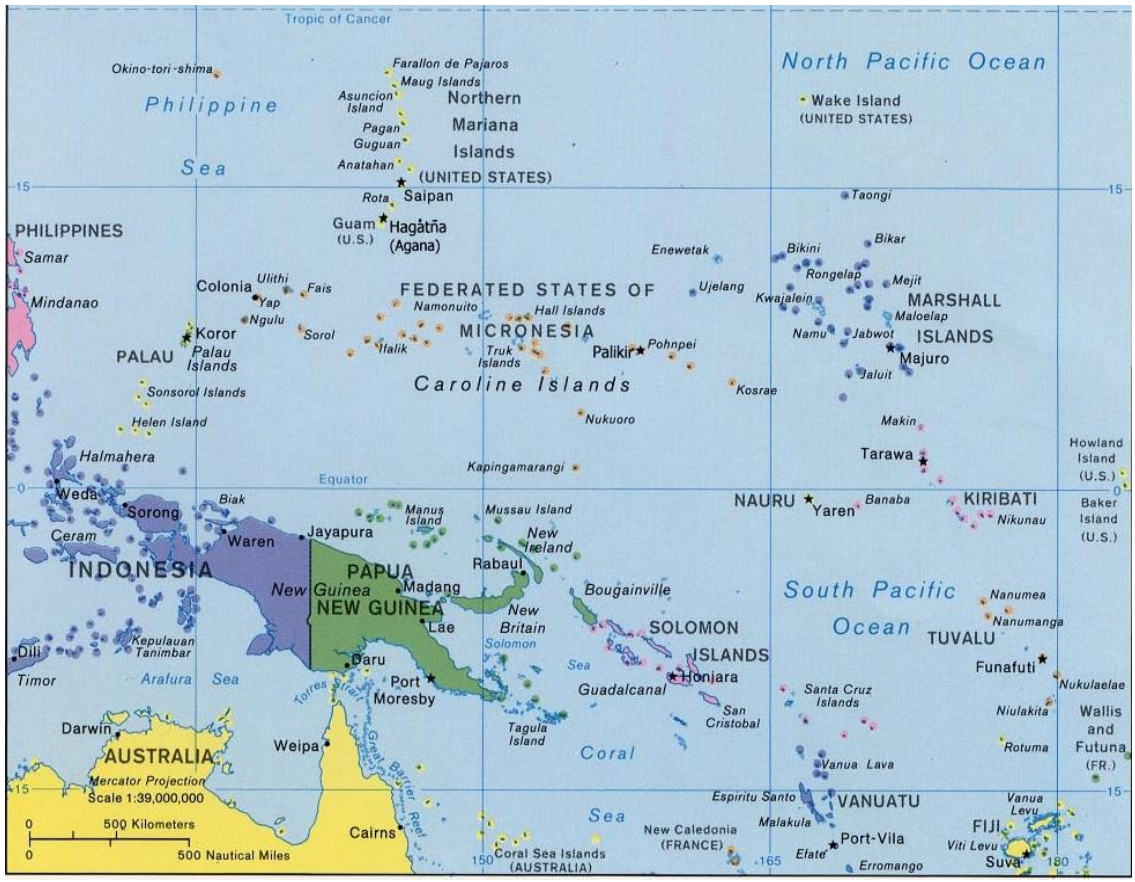


Table 3.1 The countries and territories that are classified as part of Oceania and its population in thousands (UN 1999; Population Division - United Nations 2015)

Australasia	Australia	23800
	New Zealand	4615
Melanesia	Fiji	892
	New Caledonia	269
	Papua New Guinea	7920
	Solomon Island	587
	Vanuatu	265
Micronesia	Federated States of Micronesia (FSM)	104
	Guam	162
	Kiribati	112
	Nauru	11
	The Northern Mariana Islands	55
	Palau	21
	Republic of Marshall Islands	53
Polynesia	American Samoa	56
	Cook Island	17
	French Polynesia	278
	Niue	2
	Pitcairn Island	N/A
	Samoa	194
	Tonga	106
	Tokelau	1
	Tuvalu	11
	Wallis and Futuna	12

This region has a diverse array of economies (Kline et al. 2013) from the highly developed market such Australia and New Zealand (UNDP 2016), to the much less developed economies, particularly those of island nations. For instance, Australia has the 2nd highest human development index in 2015, while Fiji and Papua New Guinea marks 91th and 153rd in the ranking (UNDP 2016). Many of the smaller island nations rely on work in the primary sector and trade with Australia and New Zealand for exports and imports (New World Encyclopaedia 2015). At the regional level, agriculture constitutes only 5% to 10% of the total economy (New World Encyclopaedia 2015), but this is mainly due to having Australia and New Zealand in the figure, which dilutes the data from the less developed island nations. In fact, small island country economies heavily rely on tourism, as well as on foreign assistance for development. (New World Encyclopaedia 2015). The economies of small island developing states (SIDS) are further constrained by high costs of service and transport, and challenges in human resources (UN-OHRLLS 2011).

There are 2 eco-zones in the region. Firstly, Australia, New Zealand and all Melanesia except Fiji constitute the Australasia eco-zone (New World Encyclopaedia 2015). All remaining areas such as Micronesia, Fiji, and Polynesia except New Zealand comprise another zone called Oceania eco-zone (New World Encyclopaedia 2015). This is the youngest eco-zone in the world and the islands in the zone range from coral atolls to mountainous islands, such as Fiji (New World Encyclopaedia 2015).

Most of Oceania is divided into two climate zones: Australia and New Zealand are within the temperate zone while remaining island countries are considered as either within tropical or subtropical, which ranges from humid to seasonally dry (New World Encyclopaedia 2015). Tropical cyclones occur mostly during the month of November till April and can cause catastrophic damages to countries and islands in the region. The area is also perceived to be highly vulnerable to climate change, particularly in small island countries, where land areas are limited and levels of elevation are low (UN-OHRLLS 2011). Rising temperatures is of particular concern in Oceania, as the human settlement is largely coastal and is exposed directly to sea-level rise (New World Encyclopaedia 2015).

Figure 3.2a Proportion of urban, rural, and total population in the island countries of Oceania using improved drinking water sources, 1990 and 2015 (WHO WPRO 2016b)

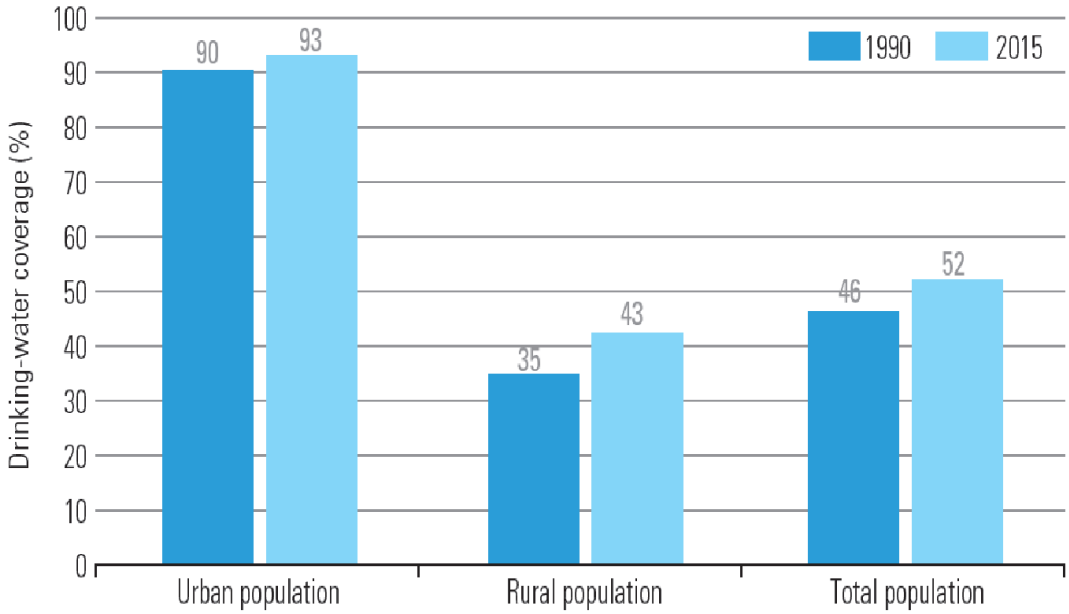
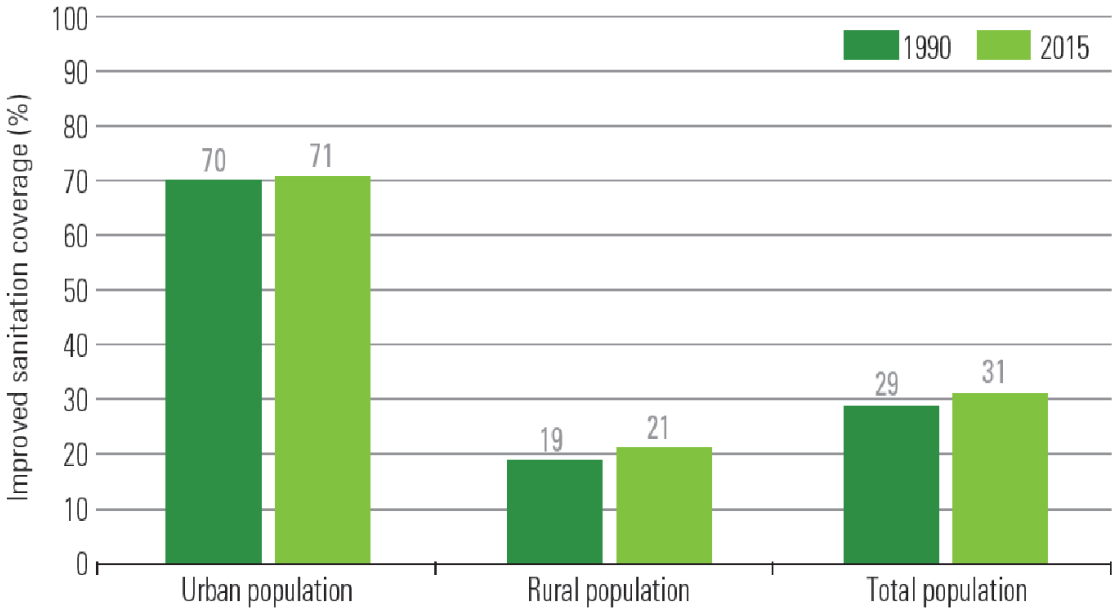


Figure 3.2b Proportion of urban, rural, and total population in the island countries of Oceania using improved sanitation, 1990 and 2015 (WHO WPRO 2016b)

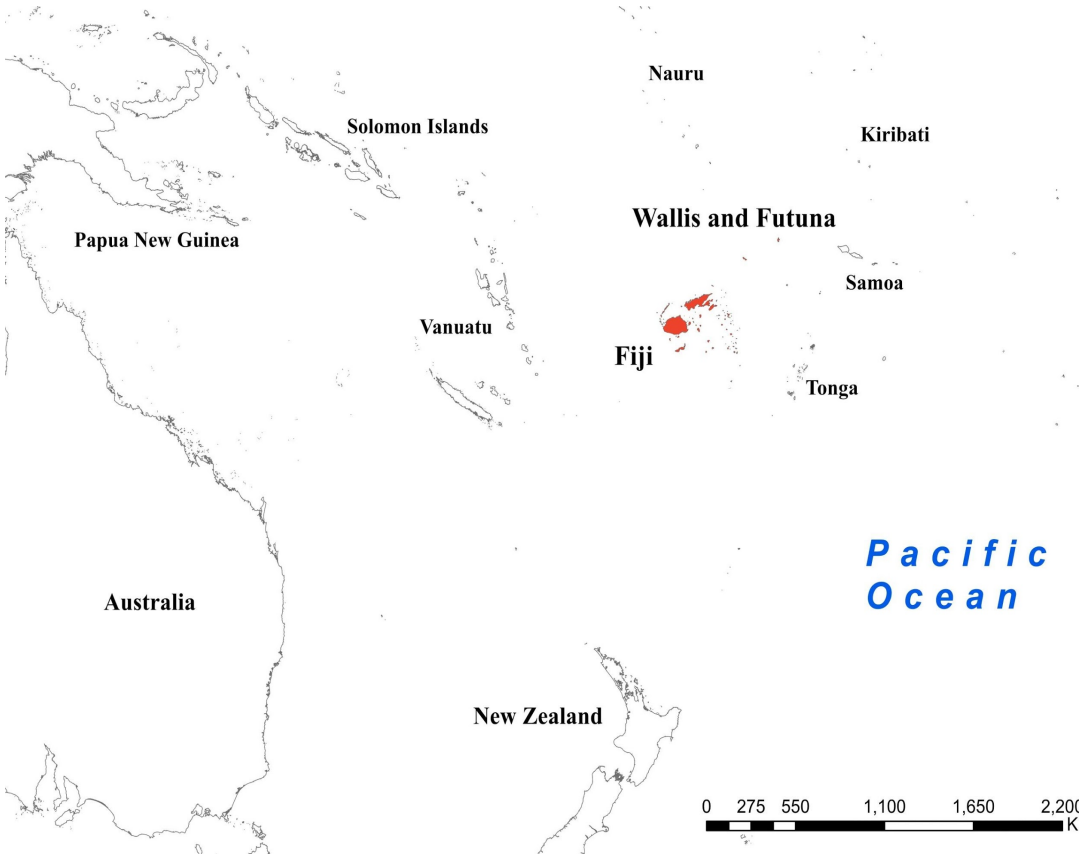


The small size and geography of island countries pose unique challenges in water and sanitation service provision (UNICEF 2013). Only half of the population in island countries have access to improved drinking-water sources, whereas one-third of the population to

improved sanitation (Figure 3.2a and 3.2b) (WHO WPRO 2016b). The proportion of the population with an unprotected water source and unsanitary means of excreta disposal in a rural area is even higher, resulting in overall low coverage of improved water and sanitation services for the region (UNICEF 2013). However, improved drinking-water coverage exceeds 90% in most island countries, except in Papua New Guinea where the coverage is less than 50% (WHO WPRO 2016b). As for improved sanitation, only Papua New Guinea has coverage below 25% and five countries have coverage above 90% (WHO WPRO 2016b).

3.2.1. Wallis and Futuna

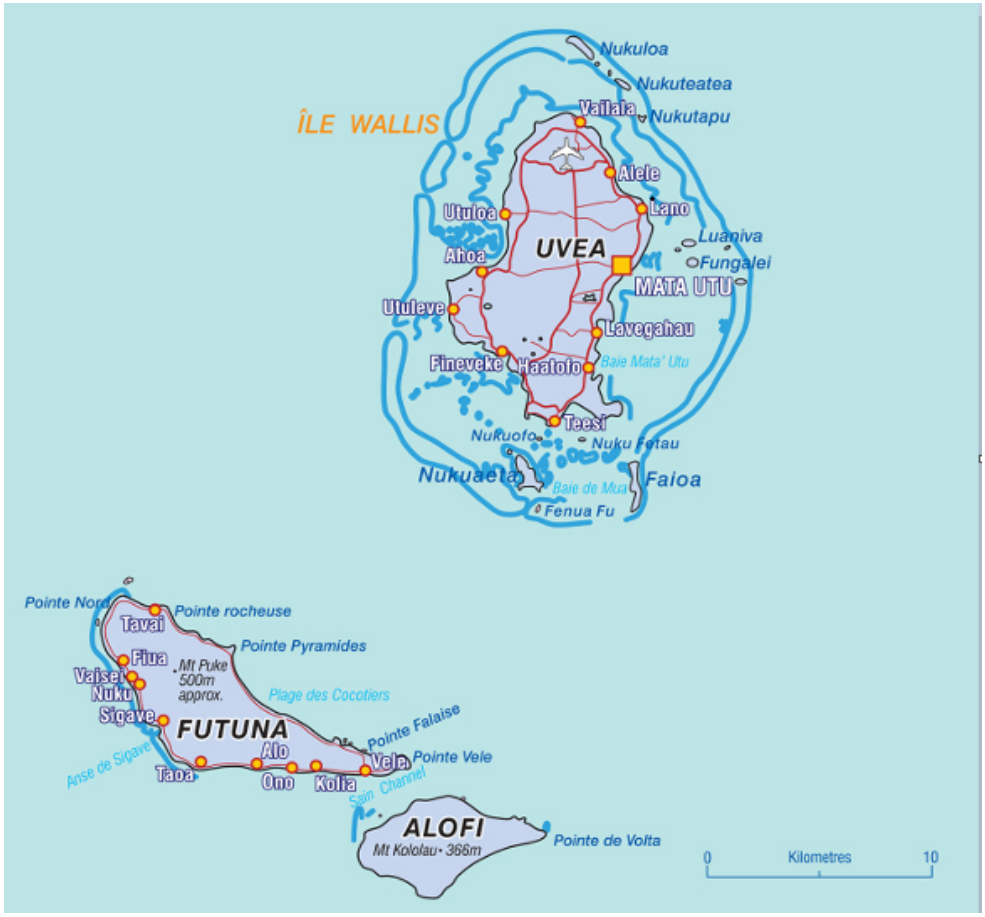
Figure 3.3 Sketch map of southern Oceania, showing the locations of Wallis and Futuna, and Fiji



The French overseas territory of Wallis and Futuna (WAF) is located at about two-thirds of the way from Hawaii to New Zealand, (west of Samoa and north-east of Fiji) in the South Pacific (Figure. 3.3). It is made up of three volcanic islands along with 20 islets, which are further divided into two island groups that lie about 260 km apart, namely Wallis Islands and Futuna. The territory occupies a land area of 145 km^2 and is one of the smallest countries

in the world, with a total population of 14,000 (Wallis and Futuna Statistics Department 2000). Most of the inhabitants reside within two major islands of Wallis and Futuna proper (SPC 2016) and of Polynesian ethnicity.

Figure 3.4 Map of Wallis and Futuna (SPC 2017)



There are two seasons in the islands: a rainy season from November to April with associated sporadic attacks of tropical cyclones, and a dry season from May to October. Average annual precipitation is 2,500 to 3,000 millimetres with rainfall likely on at least 260 days per year (New World Encyclopaedia 2015). The average temperature is 26.6 °C, rarely falling below 24.0 °C and ranging between 28.0 °C and 32.0 °C during the rainy season. The islands belong to Oceania eco-zone together with Fiji (New World Encyclopaedia 2015). Traditionally there were three kingdoms: Uvéa, on the island of Wallis, Sigave, on the western part of the island of Futuna, and Alo, on the island of Alofi and on the eastern part of the island of Futuna. Currently, the capital of the territory is Matā'utu on the island of Uvéa, the most populous area of the Wallis Islands (Figure 3.4). Uvéa is again further divided into three Districts, while Futuna is into two areas, namely Sigave in the west and Alo in the west (Figure 3.4). Health services are provided at one hospital on each island and four clinics. On the island

of Wallis, primary care is also provided by the three dispensaries (one per district). Selected health statistics are presented in Table 3.2.

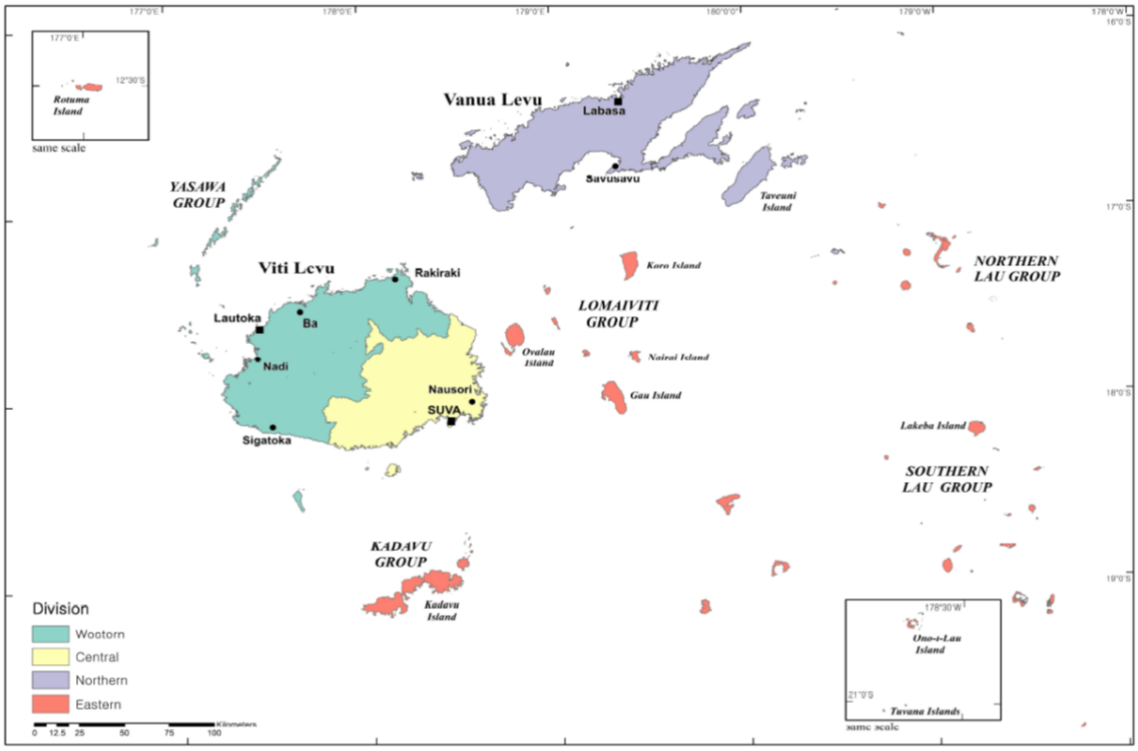
Table 3.2 Selected health indicators of Wallis and Futuna (WHO/WPRO 2016)

Indicator	Year	Value
Population (in thousands)	2013	12.2
Population aged 5-14 years (%)	2013	19.9
Life expectancy at birth (years)	2012	75.8
General government expenditure on health as % of total government expenditure	2008	24.0
Per capita GDP at current market prices (US$)	2004	3,800.0
Literacy rate among adults aged = 15 years (%)	2003	78.8
Maternal mortality ratio (per 100 000 live births)	1996	0.0
Population using improved drinking water source (%)	2008	100.0
Population using improved sanitation facilities (%)	2008	96.0

3.2.2. Fiji

Situated in the Pacific between 16˚S to 19˚20'S in latitude and 178˚W to 177˚E in longitude, Fiji is a county comprised of 332 islands where one-third of them are inhabited, with a total area of 17,870 km^2 (New World Encyclopaedia 2015). The country lies east of Vanuatu, west of Tonga, and south of Tuvalu (Figure 3.3). Its two main islands are named as Viti Levu and Vanua Levu, (Figure 3.5), where 95% of the population out of total 837,271 reside (Fiji Bureau of Statistics 2009). Overall 57% were ethnic Fijians, 37% Indo-Fijian and the remainder of other ethnic groups, such Chinese based on the national census 2007.

Figure 3.5 Sketch map of Fiji showing 4 Health Divisions and their boundaries



On the windward (south-eastern) side, average annual rainfall can be up to 3,000 millimetres, resulting in the dense tropical forest in the mountainous areas as reported by Food and Agriculture Organization (FAO) (FAO 2009). Lowlands on the west of each of the main islands are sheltered by the mountains and have a reliable dry season (FAO 2009). The country has a tropical climate with an only slight seasonal variation of temperature (New World Encyclopaedia 2015). Tropical cyclones can occur mostly from November to January, sometimes February. The major environmental issues are deforestation and soil erosion (FAO 2009).

Table 3.3 Selected health indicators of Fiji (WHO/WPRO 2016)

Indicator	Year	Value
Population (in thousands)	2013	859.2
Population aged 5-14 years (%)	2010	20.8
Life expectancy at birth (years)	2007	67.5
Under-five mortality rate (probability of dying by age 5 per 1000 live births)	2013	17.9
Total health expenditure as % gross domestic product (GDP)	2011	3.8
General government expenditure on health as % of total government expenditure	2011	9.1
Human development index	2011	0.7
Per capita GDP at current market prices (US$)	2012	6,557.2
Literacy rate among adults aged = 15 years (%)	2005	94.4
Maternal mortality ratio (per 100 000 live births)	2013	19.1
Population using improved drinking water source (%)	2015	96.0
Population using improved sanitation facilities (%)	2015	91.0

Health services are managed and administered through four Divisional Health Services offices (Figure 3.5) under the governance of Ministry of Health and Medical Services (MHMS): The Central Division covers east half of Viti Levu, while the Western Division does west of Viti Levu and Yasawa island group. The Northern Division administers all of Vanua Levu as well as Taveuni Island. Remaining island groups including Kadavu, Lomaloma, Lau, and Rotuma Island belong to the Eastern Division. National Referral Hospital based at Suva serves as the Central and Eastern Division, and two other Divisional hospitals for the Northern and

Western Division, respectively. There are five subdivisions in the Central Division, four in the Eastern Division, six in the Western Division and four in the Northern Division, and 16 sub-Divisional hospitals in total. Subdivisions are further divided into 81 Medical Areas, served by 78 health centres and 101 nursing posts. Selected health statistics are presented in Table 3.3.

Fiji is the most urbanized island country in Oceania, having the rural and urban populations comprised 49% and 51% respectively (New World Encyclopaedia 2015). However, the proportion of the population having access to improved water sources is lower than other countries, partly due to large urban-slum populations and a dispersed rural population (WHO WPRO 2016b).

3.3. Lymphatic filariasis (LF) Transmission Assessment Survey (TAS) as a decision-making tool of stopping mass drug administration (MDA)

3.3.1. Background

The surveillance strategy to assess the impact of MDA against LF after at least 4-6 effective (coverage > 65% in the total population) rounds has changed over time (WHO 2006; WHO 2011a). Yet the principal concept is to explore whether circulating filarial antigen (CFA) prevalence among children who were born after the commencement of MDA has been decreased to levels equal to or below the critical cut-off threshold, which will not allow transmission of LF to be sustained (WHO 2011c). Transmission Assessment Survey (TAS) is the recently proposed survey design to test this hypothesis and to provide evidence for the national programme in deciding whether to stop or to continue MDA (WHO 2011c).

The study area selected for the TAS is designated as an evaluation unit (EU) (WHO 2011c) which should have had at least five effective MDA rounds with the similar epidemiological background. In general, EUs should have no more than 2 million population (WHO 2011c), but this would not be an issue for the countries in Oceania with small population sizes. It is recommended that assessment, which is called as 'pre-TAS' should be carried out six months after the last round of MDA (WHO 2011c) in all ages greater than five years in sentinel sites and spot-check sites. The results of the pre-TAS should show the prevalence of circulating LF antigen less than 2%, in order to move to implementing the TAS (WHO 2011c). In EUs which meet the conditions, the TAS is accordingly planned by the national programme.

As for the detection of *W. bancrofti* antigen, immunochromatographic card tests (ICT, BinaxNow® Filariasis, Alere, Scarborough, ME, United States), a rapid-format filarial antigen

test, has been used widely by LF elimination programmes (Figure 3.6) (WHO 2011c). The test detects soluble *W. bancrofti* antigens that circulate in the blood of infected humans (Weil et al. 1997). Detecting microfilaria via night-blood surveys is not recommended for TAS (WHO 2011c). However, as the adult worms and microfilariae die and disintegrate, those treated with antifilarial medicines can retain antigen in the blood sometimes for years (Schuetz et al. 2000) and their ICT tests may be positive. The national programme may opt to combine night blood collections in order to see the infectivity, as being ICT test positive includes past and present infections (Weil et al. 1997). A new version of the ICT test is also available as of 2016: Filariasis Test Strip (Alere, Scarborough, ME, United States), which can be stored at room temperature unlike the ICT.

Figure 3.6 Photos of BinaxNow® Filariasis showing (a) a sample pad where serum or plasma being inoculated and (b)a positive result (Weil et al. 1997)

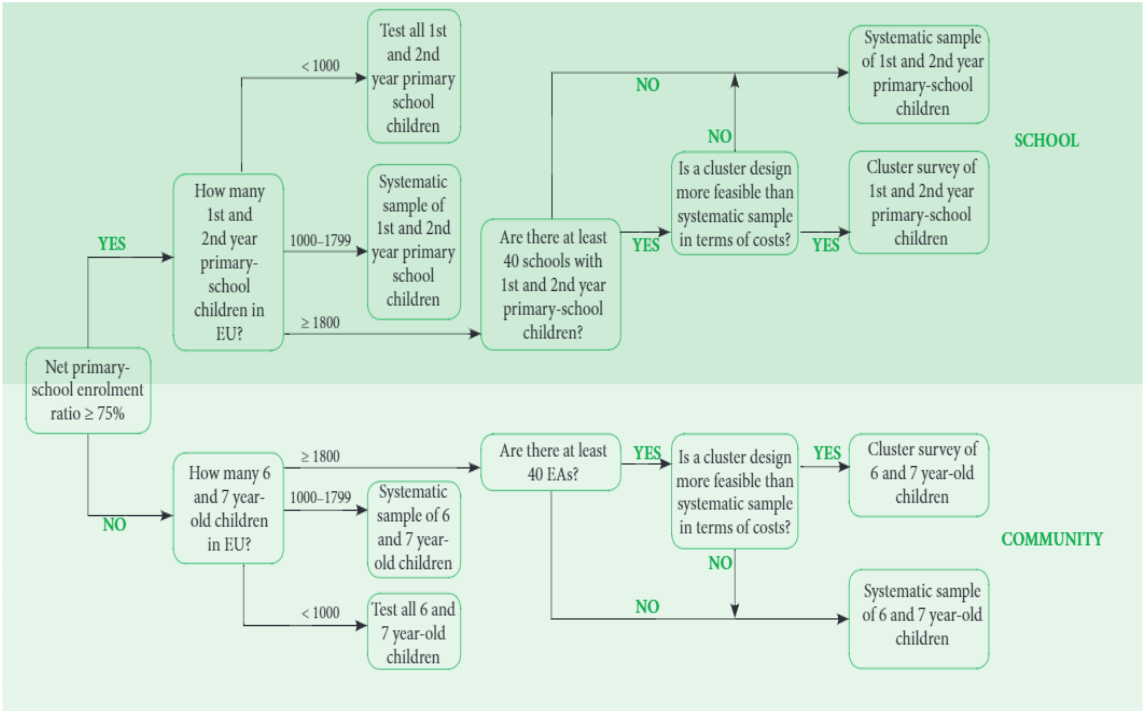
N.B.: Human serum or plasma is added to a sample pad that contains dried polyclonal antifilarial antibodies coupled to colloidal gold (a); Labelled antibody and filarial antigen flow towards the top of the card across a nitrocellulose strip, when the cardboard kit is closed; A monoclonal antifilarial antibody (AD12.1) bound to the nitrocellulose traps free antigen and antigen-antibody complexes and concentrates the gold labelled conjugate to form a visible pink line (b) (Weil et al. 1997)

3.3.2. Target population and survey design

The recommended target population for TAS is 6-7-year-old children, based on the assumption that they should have been protected from LF infection if the previous MDA rounds have been successful in interrupting LF transmission in the area (WHO 2011c). For school-based surveys, the target population would be the first and second-year primary school

children where the school enrolment rate is high (> 75%) (WHO 2011c). The community-based survey should enrol children aged 6-7 years. However, the age range can be expanded where there has been no evidence of LF transmission for many years (WHO 2011c).

Figure 3.7 Algorithm for the choice of TAS design in areas where *Aedes* spp. is the principal vector (WHO 2011c)



The survey designs and sample sizes of the TAS differ based on the type of principal vector and the size of the population in the EU. The original strategy proposed to assess the impact of LF MDA was based on lot quality assurance sampling (LQAS), with a hypothesis to test whether the true prevalence of antigenemia (p) is ≥ 0.1% (Deming and Lee 2009). Specifically, it was the LQAS scheme with a sample of 3,000 school entrants but no antigenemic student, to conclude that further MDA rounds are stopped. There was a practical difficulty of the LQAS design found: a large number of schools need to be visited if school entrants are selected with systematic sampling up to 3,000 (Deming and Lee 2009). Also, the scheme does not perform as well concerning Type II error, the probability of accepting the null hypothesis when in fact the null hypothesis should be rejected (Deming and Lee 2009). There was a substantial risk that areas that have reached the target prevalence range of <0.1% would fail to demonstrate this achievement (Deming and Lee 2009).

Table 3.4 Sampling intervals, sample sizes, and decision values for TAS in *Aedes* spp. areas (WHO 2011c)

Population surveyed	Sampling interval	Systematic sampling size	Systematic sampling decision value	Sample size for cluster design	Number of clusters if cluster-sample survey is		Cluster design decision value
					School-based	household survey	
<1000	1	N	First integer <0.01N	NA	NA	NA	NA
1000	1.4	704	4	Cluster-sampling not recommended. Use systematic sampling and the corresponding values.			
1200	1.6	730	4				
1400	1.6	854	5				
1600	1.8	876	5				
1800	2	896	5	1344			8
2000	1.9	1014	6	1521			9
2400	2.3	1042	6	1563			9
2800	2.3	1172	7	1758			11
3200	2.6	1188	7	1782			11
4000	3.2	1214	7	1821			11
5000	3.7	1350	8	2700			16
6000	4.4	1364	8	2728	**	***	16
7000	5	1376	8	2752			16
8000	5.7	1384	8	2768			16
9000	5.9	1510	9	3020			18
10 000	6.6	1516	9	3032			18
12 000	7.8	1524	9	3048			18
14 000	9.1	1530	9	3060			18
16 000	10.4	1536	9	3072			18
≥18 000	Calculate*	1540	9	3080			18

N.B.: *Divide the size of the survey population by 1,540, rounding down the nearest tenth; **Divide the sample size by the average number of target-year children per school. The minimum number is 30; ***Divide the sample size by the average number of target-year children per enumeration area (EA).

Therefore, in the strategy published in 2011, adjustment for the new antigenemia threshold prevalence of 2.0% rather than 0.1% was made (WHO 2011c). For the areas where *Aedes polynesiensis* is the principal vector, the new LF antigenemia threshold prevalence is 1.0% (WHO 2011c). In this case, the same risks of Type I and Type II error are achieved with smaller sample sizes. The risk of Type II error can be further decreased, while the risk of Type I error is kept low by increasing the sample size (n) and decision value (d), thus a new set of n and d was proposed (Deming and Lee 2009; WHO 2011c). When the population size is recognized as finite (<50,000), sample sizes can be further reduced (Deming and Lee 2009). An algorithm for selecting the survey design is based on the number of 6-7-year-olds children and on the number of schools as well as enumeration areas (EAs) in the surveyed area and is illustrated in Figure 3.7 where *Aedes* spp. are the principal vector.

When the number of children in the target age group is less than 1,000, then the survey will be a census, targeting everybody in the area. If there are more than 40 EAs or schools, then cluster survey can be implemented by selecting EAs or schools and testing all children in the selected EAs or schools (WHO 2011c). If not, then systematic sampling can be applied, by selecting children in all EAs or schools with regular intervals. Sampling intervals, sample sizes and decision values for the areas where *Aedes* is the principal vector are included in Table 3.4. The maximum number of samples required for systematic sampling is 1,540 and 3,080 for cluster design, assuming at least a 75% chance of passing if true prevalence of antigenemia is 0.5% and no more than about 5% chance of incorrectly passing if true prevalence of antigenemia is ≥1% (Deming and Lee 2009; WHO 2011c).

3.3.3. Organization of field work for data collection

Each team is consisted of at least the team leader, a phlebotomist, and a test reader. A multi-day training session covering the survey objectives, survey composition, blood sampling procedure, and test reading would be desirable (WHO 2011c). On the survey date, the team will arrive at a school or a village, and work with teachers or village health workers to gather all the first and second year or 6-7-year-old children in the area. For cluster design, the team will proceed to collect demographic data and blood specimens for all available children. If it is a systematic sampling, the team leader will choose the child according to the list prepared for selection of children. The team then proceed to collect demographic data and blood specimens from the selected children. ICT tests will be conducted and read in the field with capillary tubes at ten minutes (WHO 2011c). Once the test results are available, all positive ICT cases should be treated with albendazole and DEC. Programme managers may also choose to do follow-up

microfilaraemia testing at night time during the hours of peak microfilaraemia circulation (WHO 2011c).

3.3.4. Interpretation of survey results

Critical cut-off values are used to determine if the level of infection has been reduced to such a level that transmission is not sustainable as presented in Table 3.4. If the number of ICT positive cases is below the critical cut-off value, then the surveyed EU passes TAS, and it is assumed that transmission can no longer be sustained. If the number of ICT positive cases is equal to or greater than the critical cut-off value, then the surveyed EU fails and MDA should be resumed for at least 2 additional rounds (WHO 2011c). If a census has been used to conduct the survey, the overall prevalence of LF antigenaemia will be calculated to guide the assessment (WHO 2011c).

Once MDA is stopped based on a passing the first TAS (TAS 1) result, it is recommended to conduct post-MDA surveillance activities at least for 5-6 years: Repeating a TAS is the best option and a series of two post-MDA surveillance surveys (TAS 2 and TAS 3) should be conducted to evaluate whether recrudescence has occurred, approximately within every 2–3 years following the previous TAS (WHO 2011c).

3.3.5. Utilization of TAS as a survey platform

Once the LF endemicity in the area becomes available, programme managers may opt additional spatial analyses or multi-site comparison studies to enrich survey outcomes (WHO 2011c). Benefits of collecting stools samples during TAS have been well established (Chu et al. 2014; Drabo et al. 2016), given that administration of albendazole or mebendazole for the control of STH infections should be determined in the absence of further LF MDA rounds (WHO 2015a). The standardized approach for collecting data on STH infections when a TAS is conducted has been proposed, advocating an integrated approach to the control of NTDs in order to avoid duplication of effort and to reduce costs (WHO 2006). A TAS–STH survey is a good opportunity to collect additional biological specimens (WHO 2015a), and it should be more actively advocated (Chu et al. 2014). As for stool samples, the prevalence and intensity of infection of other parasitological species including intestinal schistosomiasis and *Strongyloides stercoralis* can be also determined. Data on these parasites are rarely collected because special laboratory techniques are required especially for the latter, such as Harada-Mori culture, or the Baermann technique (WHO 2015a). Reporting their presence can help programme managers

to take a decision about establishing or linking control programmes for the parasites. Blood or urine specimens can be also collected at the same time.

Conducting a brief physical examination of the children would be another option in obtaining on the nutritional status, as recording age, height and weight would allow estimates of stunting, wasting and underweight (WHO 2015a). Visits to schools and villages during the survey could provide an opportunity to collect information on water, sanitation and hygiene (WASH), which can help facilitate interventions for achieving the goal of elimination of STH infections, together with preventive chemotherapy (WHO 2015e; WHO 2015a).

3.4. Capacity building process to adapt LF TAS as an assessment tool and its expansion as a platform

3.4.1. Lymphatic filariasis transmission assessment survey training workshop for the Pacific Island and Territory Countries (PICTs)

As its methodology was not yet introduced to the programme managers of the Pacific, a 3-day training workshop was organized for the 16 LF endemic countries (Figure 3.8) in order to develop the capacity on designing and implementing TAS. With the support of the WHO headquarter, the US Centre for Disease Control (CDC), and the Liverpool School of Tropical Medicine Centre for Neglected Tropical Diseases 16 LF programme managers from the respective ministries of health in the Pacific attended the workshop where Fijian Ministry of Health and Medical Services (MHMS) was the host. It started on July 17th, 2012, with the aim of consolidating their experience and skills in working on the LF elimination programme and lasted until 19th July. The writer was a main coordinator of the workshop and facilitated the majority of the modules, together with Dr. Kazuyo Ichimori, the WHO Headquarter LF focal point, and Ms. Kimberly Won, a CDC scientist.

The 3-day period of the workshop was divided into ten pre-designed modules (http://www.who.int/lymphatic_filariasis/resources/TAS_training_modules/en/) with ample time for programme managers to understand the TAS methodology and how to adapt it into the country programme's context, that the national programmes can be benefited by the survey results in their programmatic decision making. It was emphasized that having a sample sizes which are sufficient and applying the pre-designed sampling frame appropriately would be critical to make stop-MDA decisions for the national programmes. The writer had a separate discussion with each of the national programme managers of Wallis and Futuna as well as Fiji,

to review the LF programmes' progress and finally came up with the conclusions of considering TAS for the implementation units (IUs) that had never been considered in both countries.

Figure 3.8 Participants of the LF TAS training workshop in Nadi, Fiji, July 2012

N.B.: The writer is in the 4th from the right in the first row

3.4.2. Creating enabling environment for TAS and its utilization as a platform

3.4.1.1. Wallis and Futuna

As a small island territory, Wallis and Futuna (WAF) had limited resources and man-power to conduct any public health surveillance. Also, the history of conducting annual rounds of LF MDA for several decades had created reluctance to consider any major programmatic decision such as conducting TAS or stopping MDA. In addition, the new programme guideline is different from the previous monitoring and evaluation framework for the LF programme, which required a antigen prevalence survey (previously called C-survey among PacELF countries) among general population before moving into TAS. Nevertheless, from 2011 on, C-survey is not recommended any more as for pre-TAS condition and several spot-check or sentinel site surveillance data showing the CFA prevalence levels < 2% became sufficient to consider TAS among children 1-2 year. This was a new finding for the WAF LF elimination programme which became the major step to move forward, as organizing a general population-based survey was reckoned to be highly challenging in the island setting.

Fortunately, the programme manager of the WAF was able to participate the TAS training workshop where further planning for the first ever TAS was possible. However, the major concern of the programme was the limited human resources and lack of funding to conduct any field work. Thus, the writer had made commitment to provide any necessary technical and financial support in designing, planning, and implementing the survey. The new soon attracted interests of other public health programme within the WHO Western Pacific Regional Office (WHO WPRO), as the access to this island territory had been limited for many years. The Expanded Programme for Immunization under the WHO WPRO contacted the writer and the programme manager on the feasibility of the piggy-packing the assessment of hepatitis vaccination impact in WAF, for which further technical discussions were facilitated to include hepatitis B vaccination coverage survey on top of the hepatitis B surface antigen (HBsAg) prevalence survey. A careful review of the diagnostic tests for the detection of HBsAg revealed that the testing methods in comparison to the CFA rapid test confirmed the feasibility of conducting two tests with one finger prick, which was specifically requested to be possible by the programme manager. The draft of the protocol for the combined survey was prepared by the writer, where further analysis of the survey data and writing up of the report were also facilitated by the writer. For on-site supervision and translation into French was further supported by one of the French-speaking colleagues. The report was adapted by the Public Health Agency of WAF and the territory finally decided to stop MDA from 2013. This collaborative activity was later documented separately and published in one of the peer-review journals.

3.4.2.2. Fiji

When the writer commenced her work at the WHO Division of Pacific Technical Support in September 2011 as a Scientist who was responsible for overall coordination and provision of technical support to the 16 national LF elimination programme in the Pacific, also called as PacELF Coordinator, the writer quickly learnt that there STH infection would be the next priority diseases among NTDs, with the rapid scaling down of the LF MDA in several countries. Also, there had been a knowledge gap in terms of assessing the burden of STH infections in the region, which had not been measured for more than a decade even with the lieu of continuing LF MDA rounds since the inception of the PacELF. Discussions with the key stakeholders and partners brought the conclusion that it was primarily due to that there was no diagnostic capacity across the Pacific for intestinal parasite infections, for which development would require substantial amount of the capital investment as well as technology transfer from the external partners.

Recognizing its needs, the Fijian MHMS showed the interest in taking the lead of developing in-country capacity with the aim to serve as a regional hub for the intestinal parasitic infection diagnosis and control, after a serious of discussion. With the political willingness of the higher management of the MHMS, the writer had contacted several donor agencies and potential collaborators which could provide financial resources as well as technical expertise in establishing the parasitology laboratory at the Fiji Centre for Communicable Diseases Control (FCCDC) and shared concept papers and short proposals on the idea with the potential partners during the period of 2012-2013. Finally, in mid-2013, the Seoul National University College of Medicine in the Republic of Korea confirmed the availability of the required resources for the Fijian MHMS through the JW LEE Centre for Global Medicine. The writer facilitated the series of discussions between the MHMS and the Seoul National University College of Medicine including on-site visits by Professor Sung-Tae Hong and Professor Min-Ho Choi in July 2013. who were the main collaborators from the Department of Parasitology and Tropical Medicine, Seoul National University College of Medicine.

In the attempts of establishing longer term partnership, the concept on strengthening the control programme for STH in Fiji, the scope of the collaboration finally agreed between two parties are: 1) To support the nationwide control programme of STH infections in Fiji; 2) To organize and establish a reference laboratory for parasitology in Fiji to assist in monitoring and evaluation of the STH infection control programme; 3) To build local capacity in parasitology diagnosis and maintain a reference laboratory for the project period; 4) To collaborate efforts in STH infection prevalence and morbidity surveys and surveillance; and 5) To collaborate in organizing the regional initiative for STH infection control in the Pacific. In line with the scope above, in November 2013, the Memorandum of Agreement was signed between the MHMS and the Seoul National University College of Medicine upon the acquisition of the Cabinet approval from the Government of Fiji Island.

From the funding support made available, the writer travelled to Korea in December 2013 to organize the procurement of necessary equipment and consumables to set up the parasitology laboratory, as the writer was the only one who had the knowledge of the conditions of the premise where the laboratory would be set up with the condition of the funding support of which procurement should be made in Korea. Consequently, the items were purchased by the Department of Parasitology and Tropical Medicine of the Seoul National University College of Medicine then were transported to Fiji in January 2014. Refurbishment of the laboratory rooms was financially supported and organized by the MHMS itself, which finally made possible of the early opening of the laboratory at the FCCDC in late January 2014 to meet the

timeline of the TAS-intestinal parasite infection prevalence survey scheduled in February - March 2014. Recruitment of the laboratory technician who would be responsible for stool processing, microscopic examination, and laboratory maintenance was completed also in early January 2014 where the writer participated as one of the panel members together with the Director of the FCCDC. Finally, the project was launched on January 27, 2014 (Figure 3.9).

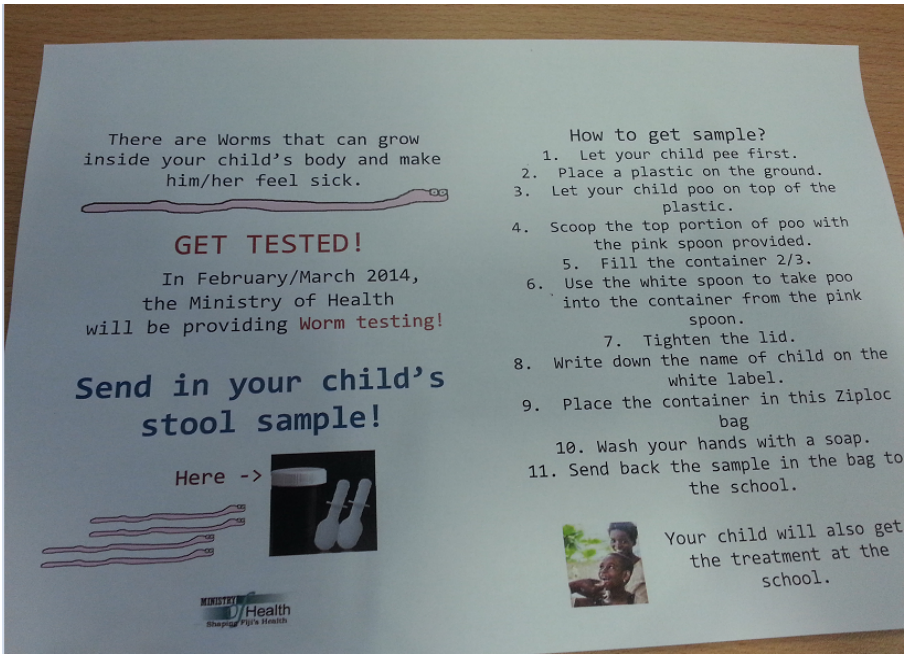
Figure 3.9 Opening ceremony for the parasitology reference laboratory at the FCCDC and launching of the STH control project in Fiji, with the presence of the HE Ambassador Republic of Korea to Fiji, the WHO Representative, and the HE Minister of Health and Medical Services, Fiji .

A group of parasitologists with Professor Min-Ho Choi payed a visit to the FCCDC at the beginning of February 2014, in order to provide on-site hands on training for the laboratory technicians, together with the writer based on the WHO Basic Laboratory Methods in Medical Parasitology (WHO, 1991), prior to the field work being carried out for stool collection. The training modules included stool handling procedures, how to apply the Kato-Katz and formol-ether-acetate concentration techniques with faecal samples and recording and report of the examination results for the period of three days. The workshop included both practical and theoretical training, which provided the laboratory technicians of the FCCDC with a good background knowledge and practical experience in the methods they would use. During the initial two weeks of period for the survey, the group stayed and conducted microscopic examinations together with the technician in order to ensure that the technician could feel confident to make parasitologic diagnosis based on the microscopic examinations. Every day,

a sample of 5% of stool specimen was re-examined by the writer as well as by Professor Min-Ho Choi, as a supportive supervision and quality control activity continued for the consecutive surveys.

Meanwhile, from the request of the Fiji MHMS, the writer initiated preparatory process for the staffs of the FCCDC, including selection of schools for stool collection, development of the survey questionnaire, consultation to the stakeholders such as the Ministry of Education, and finalization of the survey protocol and surveyor training materials for the first-ever TAS-intestinal parasite infection prevalence survey. A workshop was carried out to train the staff in the methods of collecting demographical and WASH related information, blood and stool collection standard operating procedures, and student registration process on site in January 2014 for the first TAS in February. School sensitization and pre-visit was also coordinated by the writer, covering all 30 schools selected for stool sample collection in the Western Division, responding to the specific request from the Ministry of Education for the writer to visit schools as it was the first time that the authority allowed their student to bring and submit stool samples in the school premise. The flyer designed by the writer and distributed during the school pre-visit is included in Figure 3.10.

Figure 3.10 Photo of the flyer provided to schoolchildren



During the field work, the writer also provided supportive supervision to teams from the FCCDC (Figure 3.11). Two teams were alternatively visited day by day, to ensure that survey procedures were strictly observed, the reading of the CFA results were appropriate, and the

collected stool samples were well kept in the cool box (Figure 3.10). Also, if there is any question arose, the writer provided expert opinions to the concerned teachers, students, and sometimes parents. Upon completion of the daily task, the writer also transported the stools samples to the parasitology reference laboratory at the FCCDC for further processing. The other team sent stool samples with the driver who dropped the samples and returned to the field site to join the team members for the next day.

Figure 3.11 Photos of the survey team and participants during the supervisory visit

As most of schools were visited during the morning, the writer joined the laboratory team to support and supervisor stool sample processing in the afternoon. For each day, around 50-100 samples were processed with Kato-Katz and FEC formol-ether-acetate concentration techniques, and the results were recorded in the laboratory registration booklet together with the student identification number, which was later transferred to Microsoft Excel by the writer and matched with the demographical information as well as other data collected during the survey. For the data entry of the TAS as well as responses to the questionnaires, it was mainly facilitated by the staff of the national programme for lymphatic filariasis elimination (NPLF) at the FCCDC, which was later cleaned and analysed by the writer as required in consultation to the colleagues of the MHMS, WHO and Professors of Seoul National University of College of Medicine and Liverpool School of Tropical Medicine.

Chapter 4

Assessing the impact of mass drug administration (MDA) against lymphatic filariasis via transmission assessment survey (TAS) in Wallis and Futuna and Fiji

Chapter 4. Assessing the impact of mass drug administration (MDA) against lymphatic filariasis via transmission assessment survey (TAS) in Wallis and Futuna and Fiji

4.1. Introduction

4.1.1. Lymphatic filariasis elimination programme in Wallis and Futuna under PacELF

Wallis and Futuna (WAF) joined Pacific Programme to Eliminate Lymphatic Filariasis (PacELF) and conducted a new baseline survey at five sentinel sites in 2001, showing circulating filariasis antigen (CFA) prevalence ranging between 0 and 2.3% per site (Table 4.1). This led to the decision to classify WAF as partially endemic for LF (WHO WPRO 2006). Annual mass drug administration (MDA) rounds using the combination of DEC 6mg/kg and albendazole 400mg, targeting the entire territory as one implementation unit (IU) and all age groups, except children below two years old, pregnant women, and severely ill patients, was commenced by the Public Health Agency in 2002 (WHO WPRO 2006).

The first MDA round covered 8,522 persons out of 14,966, reaching 60% epidemiological coverage, and the following annual rounds were conducted with variable coverage ranging from 48% to 66% (Public Health Agency, unpublished data) after that. In 2006, a follow up sentinel-site survey was conducted after five rounds of MDA for 1,539 people showing that CFA prevalence ranged from 0 to 0.8% per site (Table 4.1) (WHO WPRO 2006), implying that the remaining burden of island-wide LF is minimal. However, further MDA rounds have been continued by the Public Health Agency until 2011, where the last round of MDA involved Wallis Island only. With the availability of the new guideline for the epidemiological assessment of the impact of MDA against LF in 2011 (WHO 2011c), a nation-wide TAS was proposed to decide if MDA could be finally stopped in Wallis and Futuna. The survey was conducted by a team of local health staff from the Public Health Agency who were trained by the experts from WHO.

Table 4.1 Results of circulating filariasis antigen prevalence assessment per site, all age groups, Wallis and Futuna, 2001 and 2006. (WHO WPRO 2006)

Area	Site	2001			2006		
		Number tested	Number ICT positives	CFA Prevalence (%)	Number tested	Number ICT positives	CFA Prevalence (%)
Wallis	Hahake	175	4	2.3	317	1	0.3
	Hihifo	196	2	1.0	267	0	0
	Mua	235	0	0	152	0	0
Total Wallis		606	6	1	736	1	0.1
Futuna	Alo	114	0	0	493	4	0.8
	Sigave	72	0	0	310	1	0.3
Total Futuna		186	0	0	803	5	0.6
Total WAF		792	6	0.8	1539	6	0.4

N.B.: Immunochromatographic test (ICT); Circulating filariasis antigen (CFA)

4.1.2. Lymphatic filariasis elimination programme in Fiji

Lymphatic filariasis (LF)was once one of the leading causes of morbidity in the Pacific and also in Fiji (Mataika et al. 1971). Fiji joined the Pacific Program to Eliminate Lymphatic Filariasis (PacELF) in 1999 with the aim of eliminating lymphatic filariasis as a public health problem (WHO WPRO 2006). The primary strategy of PacELF was conducting mass drug administration (MDA) with diethylcarbamazine citrate (DEC) 6mg/kg and albendazole 400mg to reduce the lymphatic filariasis prevalence rate to a level that transmission is disrupted or unsustainable in the population (WHO 2011c).

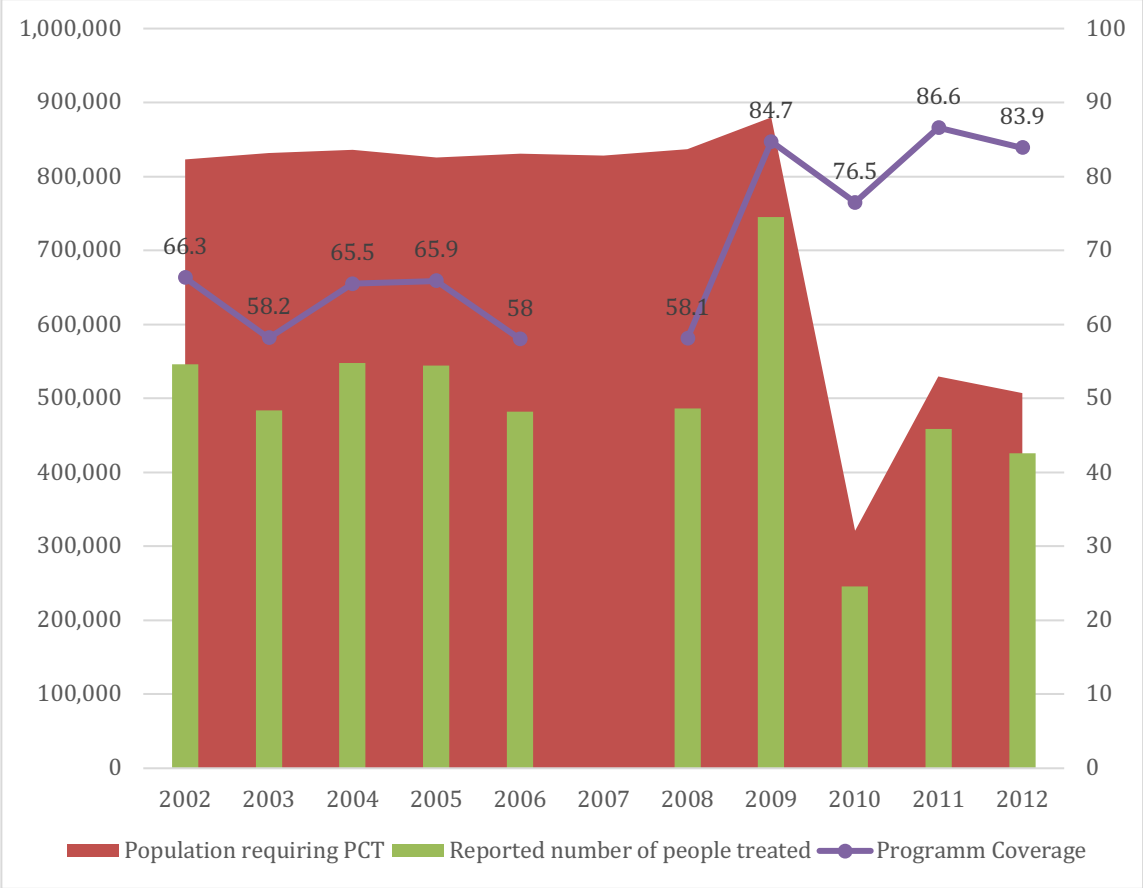
A baseline assessment survey conducted in 2000-2001 on a sample of 5,983 people revealed a prevalence of *Wucheria bancrofti* antigen by immuno-chromatographic testing (ICT) of 16.6% nationwide (WHO WPRO 2006). The recommendation provided by PacELF to the national programme for lymphatic filariasis elimination (NPLF) was to implement at least five annual rounds of MDA with anti-filariasis medications in order to achieve the goal of LF elimination (WHO WPRO 2006; Burkot and Ichimori 2002). This recommendation is based on the model predictions of the number of years required to reach the 0.5% *Wuchereria bancrofti* microfilaria prevalence threshold using combination of DEC plus albendazole mass treatment (Michael et al. 2004).

The first round of annual MDA began in 2002 with the population coverage of 66.3 %, which was further continued until 2006 (Figure 4.1) (WHO WPRO 2006). The overall MDA coverage of the first five rounds was lower than the initially recommended coverage of 80%, which was estimated to reduce the LF prevalence and density of microfilaria in the population below the threshold that would not allow recrudescence without MDA (Michael et al. 2004) in the beginning of PacELF. The LF prevalence survey in 2007 estimated the national circulating antigen prevalence of 9.5%, and this was attributed to the suboptimal MDA coverage levels from 2002 – 2006 (Rinamalo et al. 2014) . Therefore, additional two rounds of annual MDA were conducted in all four Health Divisions in 2008- 2009 (Figure 4.1).

While the Central and Eastern Divisions continued another round of MDA in 2010, the Western and Northern Divisions stopped annual MDA based on the low prevalence measured among all age groups in the 2007 national LF prevalence survey (NPLF, data unpublished) and underwent further evaluation activities. Firstly, the Northern Division conducted another LF prevalence assessment in 2010, based on the LF antigen prevalence of 2.9% in 2007, which revealed an ICT positivity of 1.1% among the general population (NPLF, data unpublished). The country programme decided to conduct two more rounds of MDA in

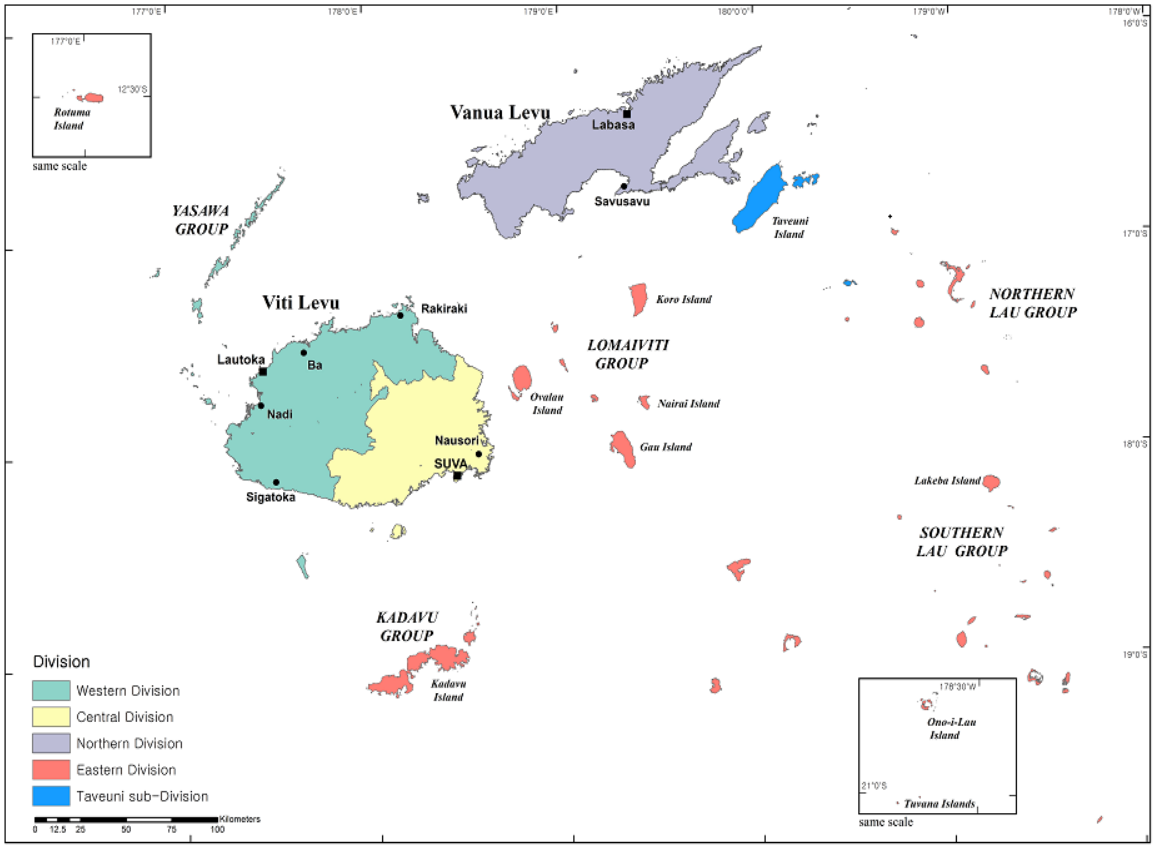
the Northern Division as a final push simultaneously with the Central and Eastern Divisions in 2011 and 2012 (Figure 4.1). Meanwhile, with the LF antigen prevalence 0.9% as assessed in 2007 for the Western Division, NPLF decided to move to conduct TAS 1 in 2011, by adopting the newly published global guideline with the aim of assessing the impact of MDA in lowering the burden of the disease (WHO 2011b). The success of the Western Division was confirmed in a single ICT positive out of 3,245 students selected, and NPLF confirmed its decision of stopping MDA in the respective implementation unit (IU) of the Western Division (NPLF, data unpublished).

Figure 4.1 Lymphatic filariasis MDA at risk population, treated population and its national MDA coverage by years in Fiji, 2002-2012 (NPLF, data unpublished)



N.B.: PCT denotes preventive chemotherapy, where in this case with the regimen of albendazole and DEC combination

Figure 4.2 Sketch map of Fiji showing 3 LF evaluation units for TAS and 2 implementation units for MDA as of 2013



In contrast to two other Divisions, the Central and Eastern Divisions continued the annual rounds of MDA from 2010 to 2012. This is mainly based on the high LF prevalence estimated by the ICT positivity in the 2007 survey, namely 15.4% for the Central and 11.1% for the Eastern Division, and NPLF decided to hold the impact assessment until at least five more annual rounds in these areas were completed (Rinamalo et al. 2014). However, the sampling methodology for the Central and Eastern Divisions in the 2007 survey actually differed from what was used for the Western and the Northern. The Central and Eastern Divisions' samples were very few and purposely selected from historically known hot-spots from sentinel site surveillance, rather than being random, which would have been biased to the direction of over-estimation of the LF antigen prevalence in these two Divisions (Rinamalo et al. 2014). By 2013, there had been altogether ten consecutive annual rounds of MDA conducted in both Divisions (Figure 4.1).

In 2013, reflecting the fact that seven to ten rounds of MDA have been conducted previously, the national programme set the goal and timeline of the country-wide impact assessment of MDA by having each Division as an evaluation unit (EU) in coming months (Figure 4.2 and Table 4.2). Firstly, the Northern Division was targeted for its TAS 1 in 2013, where the Taveuni sub-Division was not included as it failed in its pre-TAS assessment (NPLF, data unpublished). With the passing results, namely three positives out of 2,711 tested, the EU was consequently targeted for its TAS 2 in 2015 after a two-year interval (Table 4.2). NPLF also determined the timeline of the 2nd TAS (TAS 2) in the Western division and the TAS 1 of the Central Division to be in 2014, considering the interval from the TAS 1 of the Western Division. The study presented in this thesis includes activities conducted in 2014 and 2015 by NPLF, namely TAS 2 in the Western and Northern Divisions and TAS 1 in the Central Division, following the establishment of the reference parasitology laboratory for stool examination at the Fiji Centre for Communicable Diseases Control (FCCDC) in early 2014. The objectives of TAS were to a) provide a simple, and robust survey to determine the prevalence of lymphatic filariasis among 6-7-year-old; b) provide the evidence base for the programme managers that MDA can be stopped; and c) assure national government that national programmes have achieved the elimination goals of LF (WHO 2011c).

Table 4.2 Fiji national LF programme's timeline of assessing the impact of MDA (NPLF, data unpublished)

	TAS 1	TAS 2	TAS 3
Western	2011	2014*	2017
Central	2014*	2017	2019
Northern	2013	2015*	2018

N.B.: *: included in the study

4.2. Material and methods

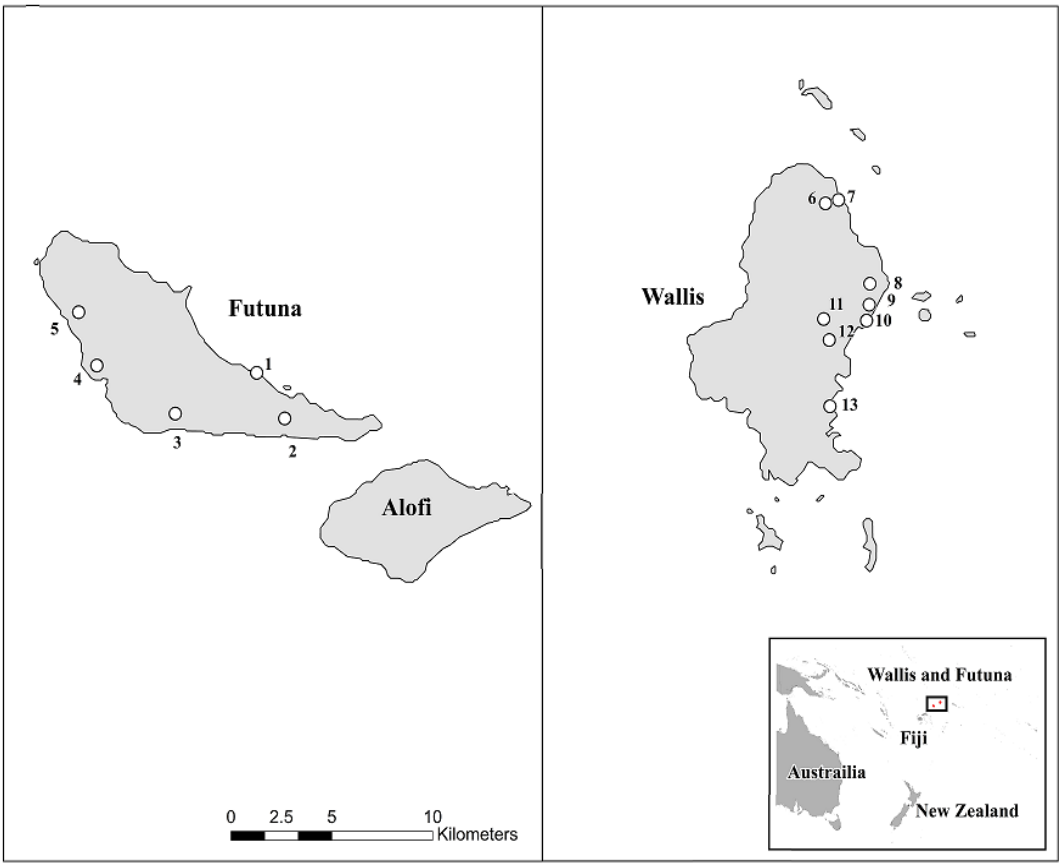
4.2.1 Study settings and target population

4.2.1.1. Wallis and Futuna

The French overseas territory of Wallis and Futuna (WAF) is located at about two-thirds of the way from Hawaii to New Zealand, (west of Samoa and north-east of Fiji) in the South Pacific, and one of 22 Pacific Island Countries and Territories (PICTs). It is made up of three volcanic islands along with 20 islets, which are further divided into two island groups that lie about 260 km apart, namely Wallis Islands and Futuna (Figure 4.2). The territory occupies a land area of 145 km2 and is one of the smallest countries in the world, with a total population of 14,000 (Wallis and Futuna Statistics Department 2000). Most of the residents are inhabitants of the two major islands of Wallis and Futuna proper.

WAF follows the French schooling system, and there are 13 'elementary' schools altogether (Figure 4.3), with five grades, starting from the first grade comprised of children aged between five and six years to the fifth grade with children aged between ten and eleven years. According to the algorithm for the choice of the TAS design in areas where *Aedes* is the principal vector (WHO 2011c), the target population would be all the first and second grade children enrolled in the elementary schools, given that net primary school enrolment ratio ≥ 75% in WAF and 1st and 2nd year primary school children in the evaluation unit (EU) < 1,000.

Figure 4.3 Sketch map of Wallis and Futuna showing the location of all elementary schools



4.2.1.2. Fiji

Fiji is an archipelago comprised of more than 330 islands lying between 12°and 22°S and 175° and 178°E in the South Pacific (FAO 2009). Up to 835,000 Fijians reside on the 100 or so consistently inhabited, among which Viti Levu and Vanua Levu are the two major islands with 87% of the total population (Fiji Bureau of Statistics 2009). Viti Levu is divided into the Western and Central Health Divisions, whereas Vanua Levu forms most of the Northern Division and the other remaining islands do the Eastern Division as presented in Chapter 3 (Figure 3.5), by the health authorities. These 4 Health Divisions are further comprised of 21 Health sub-Divisions, which are further divided into Health Areas and Nursing Zones (WHO WPRO 2011). Health Areas are served by the respective health centres and some of the Nursing Zones have their own nursing posts if the Nursing Zones are remotely located.

There are two main ecological zones in the country: (1) Wet Zone where receives rain greater than 3,000 mm/year; and (2) Dry Zone where receives rain less than 2,000 mm/year (FAO 2009). Most of the Western Division falls under Dry zone which can be further divided into Strong Dry Zone in the western half, and Moderate Dry in the eastern half, while the Central Division primarily does under Wet Zone. The Northern Division has mostly Dry Zone in its north and Wet zone in the south (FAO 2009).

Primary school education in Fiji is compulsory and covers the first eight years, namely from class 1 to class 8. In 1999, the country achieved the net primary enrolment rate of 94 percent, and 98 percent in 2015 (UNESCO 2017). The official school age who should be enrolled in primary schools is 6-14 years old. A school year is consisted of three terms, 14 weeks each, and holidays at the end of each term. Term 1 and 2 usually commence around mid-January and early May, whereas Term 3 starts in late August. There are 731 registered primary schools in Fiji, attended by around 140,000 students as of 2014 (MOE 2017). According to the algorithm for the choice of the TAS design in areas where *Aedes* is the principal vector (WHO 2011c), the target population would be all class 1 and 2 students enrolled in the selected primary schools, given that net primary school enrolment ratio ≥ 75%.

4.2.2. Study design and sampling strategy

The objective of the study for TAS 1 was to confirm whether CFA prevalence levels had been lowered to the threshold that recrudescence of the infection would not occur, among a sufficient number of children in the EU who went through at least 4-6 rounds of MDA. This is critical to enable the health authority to make stop-MDA decisions for the assessed EU, even

if sporadic data had hinted that the CFA prevalence levels via sentinel or spot-check sites surveys were close to the threshold as evidenced in pre-TAS studies. As for TAS 2, the study was designed to confirm that the lower level of CFA prevalence achieved in the last TAS 1 had been continued, in order to detect any possible remaining infection foci or recrudescence among the sufficient number of children in the EU, noting that TAS 2 was built on the previous of success of passing TAS 1 results in the EU.

In either survey, the hypothesis was to test whether CFA prevalence is higher than 1% among children who were borne after at least 4-6 rounds of MDA were implemented, and the sample size would be calculated following lot quality assurance sampling as the principal vector for LF was *Aedes* spp. (WHO 2011b).

4.2.2.1. Wallis and Futuna

Table 4.3 Number of grade 2-5 students registered in Wallis and Futuna (Education Department, Wallis and Futuna)

School identification numbers	Number of registered students
Futuna schools	
1	28
2	54
3	108
4	100
5	52
Sum	342
Wallis schools	
6	120
7	69
8	87
9	40
10	104
11	72
12	76
13	104
Sum	672
Total	1014

We designed a survey following the WHO TAS guideline, having the whole territory as one evaluation unit (EU), where 1,014 second to fifth-grade students at 13 elementary schools were registered (Table 4.3). The objective of the study was to assess whether a sufficient number of children who went through at least 4-6 rounds of MDA showed low levels of CFA prevalence across the country, in order to enable the health authority to make stop-MDA decisions for the country. Given that the majority of the children in the first grade are five years old and the TAS guideline recommends 6 to 7-year-old children to be tested (WHO 2011c), we did not include the first grade in the samples. Instead, we firstly selected the children in the second and third grades whose age groups overlap mostly 6 to 7 years old. To be more conservative, we have expanded our target age groups up to ten to eleven-year-old by including fourth and fifth grades as well, yielding the total estimated sample size of 1,000. Children in fourth and fifth grades were also selected for the hepatitis B surface antigen prevalence survey and their hepatitis B vaccination coverage surveys, of which results are separately reported in Chapter 5.

4.2.2.2. Fiji

Following the existing NPLF's monitoring and evaluation framework, for the second TAS in the Western Division, which passed its first TAS in 2011, 77 schools were selected as clusters out of 249 registered schools in the Division by the Survey Sample Builder (The Task Force for Global Health, Georgia, USA). The estimated sample size (n_cluster), i.e. students in class 1 and 2 in selected schools, was 3,695 out of all 11,652 students in these two classes of the Western Division (Table 4.4), with the critical cut-off (d-cluster) of 18 (Table 3.4) (WHO 2011b). As for the Central TAS, initially, the Evaluation Unit (EU) was set at the Divisional level. However, NPLF decided to separate the Rewa sub-Division, which has shown the historically high LF endemicity, thus two independent TAS were designed, i.e. having the Rewa sub-Division as one EU and other four sub-Divisions of the Central Division as another EU (Table 4.4). This yielded the sample sizes of 2,379 in 34 schools and 3,531 in 48 schools respectively for each survey. For the Northern, as the Taveuni sub-Division formed a separate EU since 2013, 50 schools were selected yielding a sample size of 1,930 out of 141 schools. To be more conservative, we have set the cut-off values which were matched to the nearest sample sizes for cluster-sample surveys in Table 3.4 (WHO 2011c).

Table 4.4 Number of the selected schools and estimated sample sizes for LF TAS in 3 Divisions of Fiji, 2014-2015 (MOE, 2014)

	Western Division	Central Division		Northern Division**	Total
		Rewa sub-Division	Others*		
Number of total registered schools	249	54	149	141	593
Number of schools selected by Survey Sample Builder	77	34	48	50	209
Total number of students registered in class 1 and 2	11,652	3,473	11,149	5,398	31,672
Estimated sample size	3,695	2,379	3,531	1,930	11,535
Critical cut-off	18	11	18	11	N/A

N.B.: *: Others include Naitasiri, Navua, Suva, and Tailevu sub-Divisions; **: The Northern Division here excludes Tavenui sub-Division

4.2.3. Data collection procedure and specimen examination

4.2.3.1. Wallis and Futuna

We contacted school principals of the 13 elementary schools through the Department of Education to explain the purpose of the survey. We also requested the lists of students from each school in advance, which included the students' name, gender, age, and grade. As participation was voluntary, we organized a letter to parents explaining the purpose of the survey in French, requesting their written consent and asking them to have their children bring their vaccination cards to schools on the survey date.

A total of 150 µl of blood was collected from each participant by finger prick on the school site for the fourth and fifth-grade students, while 100 µl for the second and third graders. We used rapid tests on-site in the field, which did not require any special equipment, apart from a capillary tube measuring 100 µl for the circulating CFA immunochromatographic test (ICT). Before the implementation of the survey, investigators were trained on blood sampling procedures and on how to use and interpret the tests. For CFA, 100 µl of the blood collected

was inoculated to a *W. bancrofti* antigen rapid test known as a filariasis ICT card (Binax, Portland, ME, USA). The results were interpreted immediately in the field at ten minutes as per instructions of the manufacturer. The test was considered positive when a red bar appeared in both the case and the control windows; negative, when a red bar appeared only in the control window; and invalid, when no red bar appeared, or it appeared only in the case window.

4.2.3.2. Fiji

The selected schools were informed via the Ministry of Education (MOE) and the head teachers were requested to send the consent form and information sheets to the parents of class 1 and 2 students, so that they could express that they would be happy for their children to participate in the survey A day before the survey visit, the field teams from FCCDC made a pre-visit to the school, and teachers of class 1 and 2 students participated in a briefing meeting to be informed about the aims of the survey and how it would be carried out. When the field team arrived at the school on the survey day, they conducted a short health education class, with the teachers and the students about LF, followed by collecting the consent form if any parent/legal guardian decided that they would like to have their child involved in the survey.

A total of 100 µl of blood was collected from each child by finger prick with a lancet and a capillary tube for filariasis antigen test. The blood was placed directly onto an ICT card (Binax, Scarborough, ME), which is proven to be a useful and sensitive tool for the detection of circulating *W. bancrofti* antigen. The surveyors were trained to read the cards at 10 minutes and marked the result as either positive or negative, according to manufacturer's instructions, in the data collection form. The cards were kept for the duration of the survey in a secure place at FCCDC for quality control purposes and disposed of at the end of the survey.

4.2.4. Data management and interpretation of the survey results

4.2.4.1 Wallis and Futuna

Data were recorded on the paper-based questionnaires by the trained investigators. The questionnaire form included basic demographic information and test results. The investigators reviewed the questionnaires before leaving the school to ensure completeness. Data were entered in a database programmed with EPIDATA version 3.1 (EpiData Association, Odense, Denmark) and analyzed using STATA version 12 (StataCorp LP, College Station, TX, USA).

The TAS analysis is based on the lot quality assurance sampling (LQAS) methodology that classifies EUs as above or below a specified cut-off based on the number of failures (i.e., the number of CFA positive individuals) found in a sample. However, if the population size of

the targeted age group is 1,000 or less in a territory where *Aedes* spp. is the main vector for LF transmission, it is recommended to conduct a census survey instead of sampling. In this case, the critical cut-off (1%) was calculated as the first integer smaller than 0.01 times the total number of individuals tested (WHO 2011c).

4.2.4.2. Fiji

All data were initially hand recorded by survey team members and then entered into an electronic data management system using a Microsoft Office Excel spreadsheet 2007 (Microsoft). All survey participants were assigned a unique identification number. Data analysis was carried out by sex and sub-geographical areas to detect any remaining specific focus of LF endemicity. All personal identification information was kept securely at FCCDC by the MHMS officers and also archived, to allow for easy retrieval if needed at later stages of the programme.

As all TAS were constructed following school-based cluster sampling designs, the critical cut-off (d_cluster) were set based on the critical values for TAS and post-MDA Surveillance Surveys in *Aedes* areas suggested in the WHO guideline (Table 3.4) (WHO 2011b). The interpretation of the survey results is: If the number of ICT positive cases is below the critical cut-off as defined in Table 4.4, then we interpret the EU passes the TAS; the number of ICT positive cases is equal to or greater than the critical cut-off, then the surveyed EU fails in the TAS.

4.2.5. Ethical consideration

A written informed consent in French in WAF and in English, Fijian, and Hindi in Fiji was provided to the parents or guardians of participating children. The field team also provided the opportunities for students to opt out if they would not like to participate in the study: Students were orally ought to say that they would not like to take part in the study, especially for blood collection, in an environment where they felt free to express their wishes, even if their parents sent the signed consent form. If they did not express orally that they did not want to take part in the study and there was a consent form from their parents, then it was assumed that they were eager to provide blood samples.

This study was reviewed and approved by the Public Health Agency of WAF, the Fiji National Health Research Ethics Review Committee, and the WHO Western Pacific Regional Office Ethics Review Committee.

4.3. Results

4.3.1. TAS 1 in Wallis and Futuna

4.3.1.1. Demographic characteristics of the study population

The survey was conducted between 15th and 26th November 2012. Out of 1,014 children targeted for LF TAS, 950 (94%) were present at schools, but 11 refused the test (Table 4.5). Overall participation was 93%. Out of the 939 children tested, 477 (51%) were male. They were born between December 1999 and January 2006 and their mean age was 9.5 years.

4.3.1.2. Geographical distribution of the circulating filariasis antigen prevalence

The result was invalid for four, so we excluded them from further analysis. Three children out of 935 were found to be CFA positive. This was below the critical cut-off of nine cases (i.e., first integer <0.01x935), and we concluded that WAF passed the TAS. The positive cases were a ten-year-old girl and an eight-year-old boy, both residents in Wallis, and a seven-year-old girl in Futuna (Figure 4.4 and Table 4.5).

Figure 4.4 Sketch map of Wallis and Futuna showing locations of surveyed schools and schools with positive ICT test case(s)

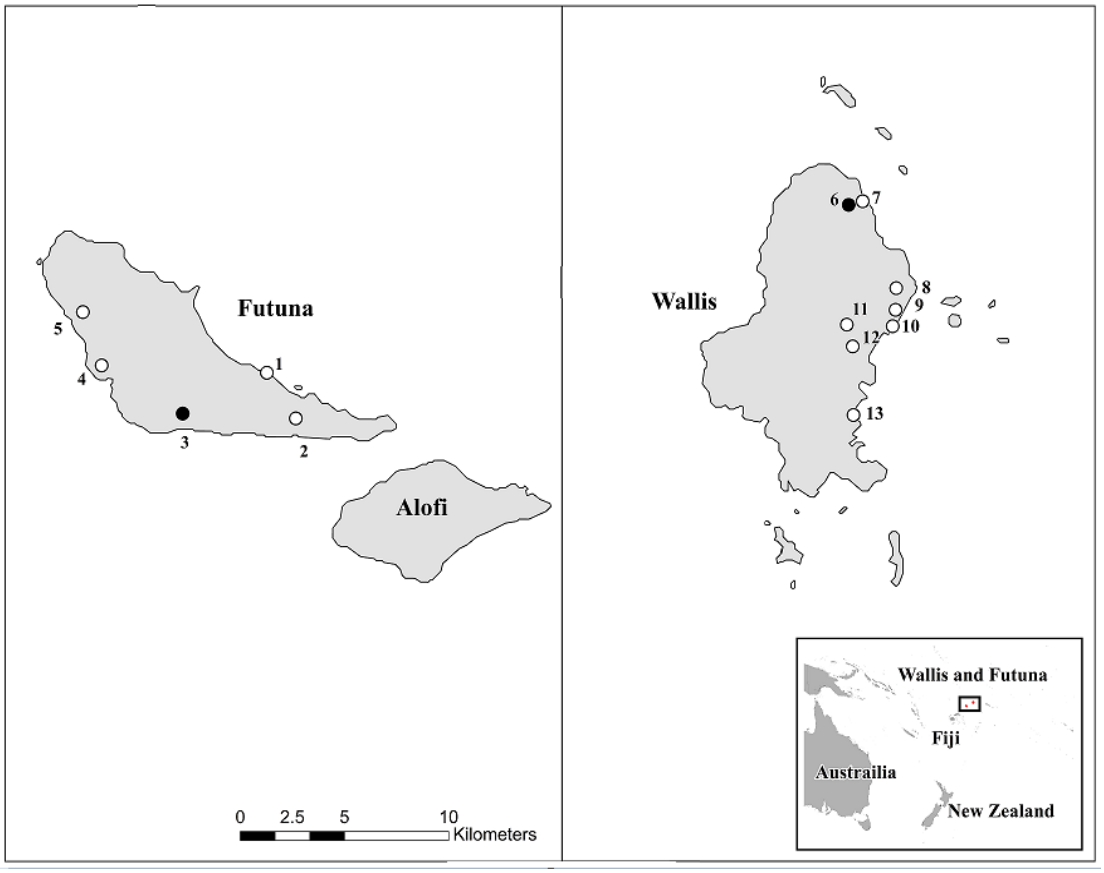


Table 4.5 Survey coverage and CFA prevalence of second and fifth-grade students at school level in Wallis and Futuna, November 2012

School Number	Number of students who attended	Number of students who refused the test	Number of students tested	Survey Coverage * (%)	Invalid**	Number of CFA positive, (%)
Futuna schools						
1	27	0	27	96	0	0 (0)
2	52	0	52	96	0	0 (0)
3	105	0	105	97	0	1 (1.0)
4	95	0	95	95	0	0 (0)
5	52	0	52	100	0	0 (0)
Sum	331	0	331	97	0	1 (0.3)
Wallis schools						
6	121	4	117	98	1	2 (1.7)
7	61	2	59	86	0	0 (0)
8	64	1	63	72	0	0 (0)
9	34	0	34	85	0	0 (0)
10	90	0	90	87	0	0 (0)
11	71	1	70	97	1	0 (0)
12	76	0	76	100	0	0 (0)
13	102	3	99	95	2	0 (0)
Sum	619	11	608	90	4	2 (0.3)
Total	950	11	939	93	4	3 (0.3)

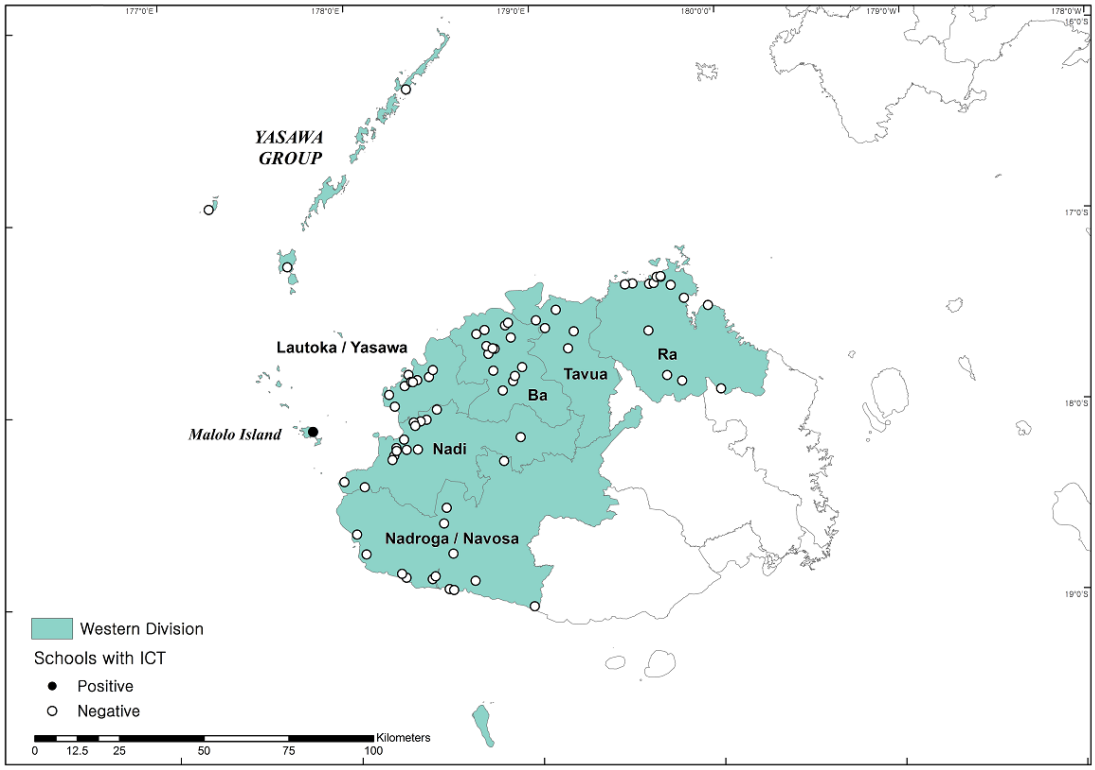
NB: *: Coverage is defined as the proportion of students who are tested divided by a total number of students registered as given in Table 4.3; **: Invalid means when ICT cards failed to yield the result.

4.3.2. TAS in Fiji

4.3.2.1. TAS 2 in the Western Division

The survey was conducted between February and March 2014, when the Term 1 commenced. Out of 77 schools targeted in six sub-Divisions, 76 schools were surveyed except one international school in Nadi (Figure 4.5). The estimated sample size was 3,695 class 1 and 2 children, but there were in fact 4,199 students registered for the school year of 2014. Altogether 3,279 (78%) were enrolled at schools where five refused the blood test, and 32 were absent at the time of testing.

Figure 4.5 Sketch map of the Western Division of Fiji showing locations of surveyed schools and the school with positive ICT test case(s)



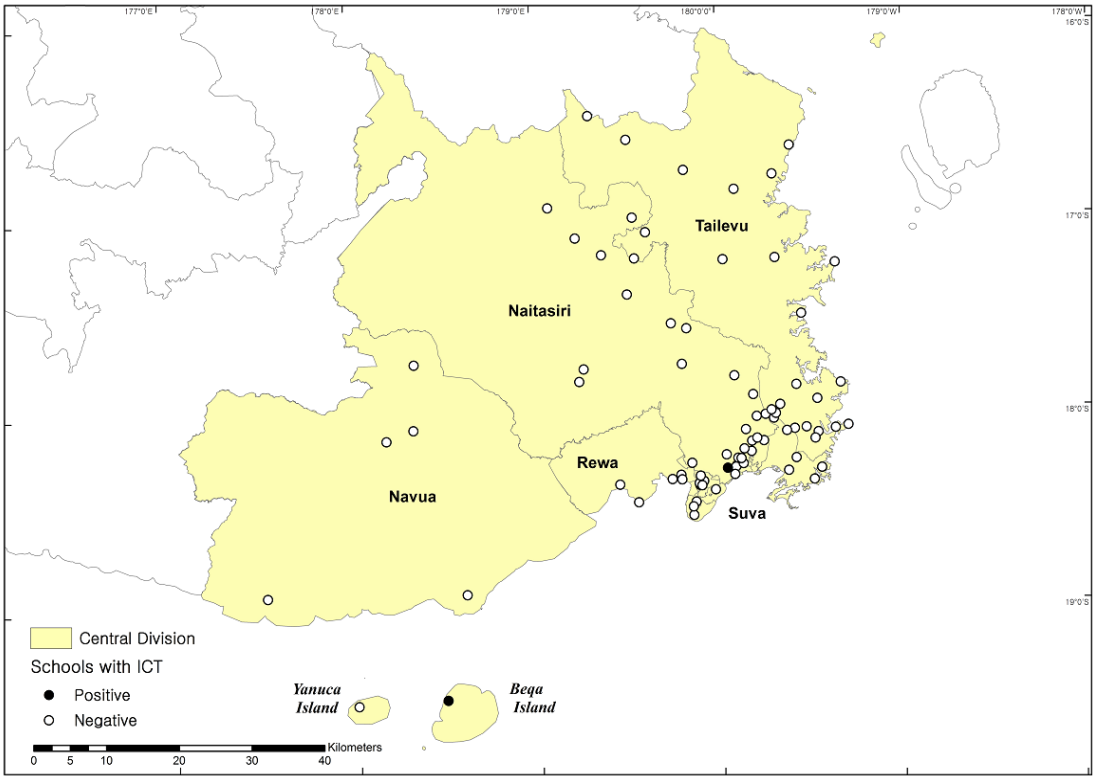
Out of the 3,242 children tested, 1,600 (49%) were male. The youngest child enrolled was four years old, while the oldest was nine. Most of them (96%) were either six or seven years old. There was no invalid test but seven children, all from the same school located on the Malolo Island (a part of Mamanuca Island group and 30 km away from Viti Levu) (Figure 4.5), were found to be CFA positive among those tested with the ICT test. Estimated school level LF prevalence for the school was up to 18%, and the review of the previous data confirmed that the school was not surveyed in 2011 during the TAS 1. Among CFA positive cases, there were four girls and three boys, who are either six or seven years old. Excluding the Malolo

Island Medical Area from the EU, there was no CFA positive case, thus NPLF concluded that the Western Division except the Malolo Island Medical Area passed TAS 2. Considering the high number of positive cases from Malolo Island, an independent IU, the Malolo Island Medical area, was formed to resume MDA in coming years.

4.3.2.2. TAS 1 in the Central Division of Fiji

The survey was conducted between July and August 2014, when the Term 2 commenced. Out of 34 and 48 schools initially chosen for Rewa sub-Divisions and other four Subdivisions, 33 and 47 schools were surveyed, except one special school for the children with disabilities in Rewa and a public school in Suva sub-Division respectively, which were not available for survey participation (Figure 4.6). The initially estimated sample sizes were 2,379 for Rewa and 3,531 for others, but there were 2,516 registered students in Rewa and 3,579 in others. In total 1,340 students were enrolled for TAS 1 in Rewa and 2,075 for four other sub-Divisions, yielding the overall survey participation rate of 53% and 58%.

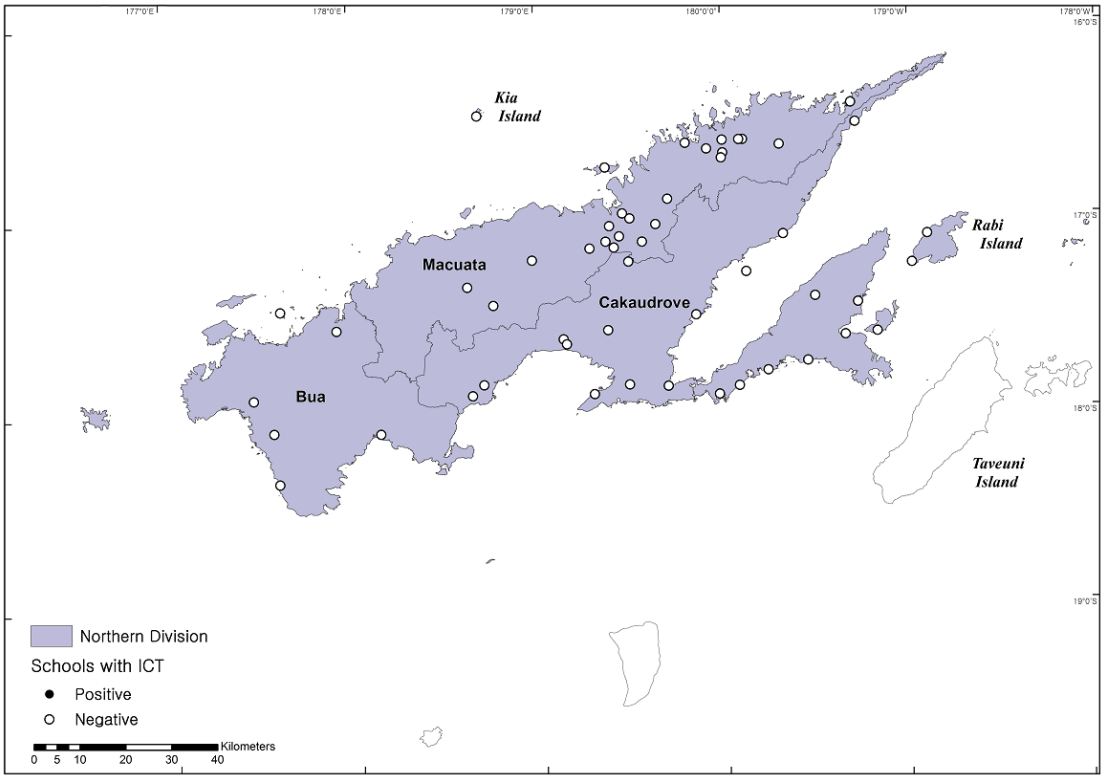
Figure 4.6 Sketch map of the Central Division of Fiji showing locations of surveyed schools and the school with positive ICT test case(s)



The youngest age enrolled was five years old, while the oldest was ten. Most of them (96%) were either six or seven years old, and 49% were male. Out of 1,340 students who underwent ICT testing in Rewa, there was neither invalid test nor CFA positive (Figure 4.6). In other four sub-Divisions, there were two CFA positive cases, a seven-year-old girl in Suva sub-Division and the other six-year-old girl in Navua sub-Division. Altogether, the number of positive CFA cases, two, was below the pre-set critical threshold of 11 for four sub-Divisions (Table 3.4). Thus, NPLF concluded that the Rewa sub-Division and the other four sub-Divisions passed the TAS 1 and MDA can be stopped.

4.3.2.3. TAS 2 in the Northern Division of Fiji

Figure 4.7 Sketch map of the Northern Division, except the Taveuni sub-Division of Fiji showing locations of surveyed schools and the school with positive ICT test case(s)



The survey was conducted between February and March 2015, when the Term 1 of 2015 School-year started. Out of 50 schools initially chosen, 50 schools were surveyed, and four additional schools were also included from the list of additional five schools identified by the Survey Sample Builder, to meet the pre-defined sample size of 1,930 class 1 and 2 students. In total, 1,826 students were registered in the survey, where 174 students were absent on the survey date, yielding the survey coverage of 86%. Altogether 28 refused the blood test, and 1,624 children underwent ICT testing, so the overall survey participation rate was 84%.

The youngest child enrolled was five years old, while the oldest was 11. Most of them (97%) were either six or seven years old, and 50% were male. Out of 1,624 tested with ICT, there was neither invalid nor CFA positive (Figure 4.7). NPLF concluded that the Northern Division passed the TAS 2.

4.3.2.4. Changes in EU and IU boundaries following TAS and its implications to the national programme

Table 4.6 Summary of 3 TAS outcomes in Fiji, 2014-2015

	Western Division	Central Division		Northern Division**	Total
		Rewa sub-Division	Others*		
Total number of surveyed schools	77	33	47	50	207
Number of enrolled students for TAS	3,279	1,340	2,075	1,826	8,528
Number of students tested for CFA	3,242	1,340	2,075	1,624	8,371
Number of students with CFA positive	7	0	2	0	9
TAS result	Pass***	Pass	Pass	Pass	

N.B. *: Others include Naitasiri, Navua, Suva, and Tailevu sub-Divisions; **: The Northern Division here excludes Tavenui sub-Division; and ***: Except Malolo Medical area which was separated from the EU

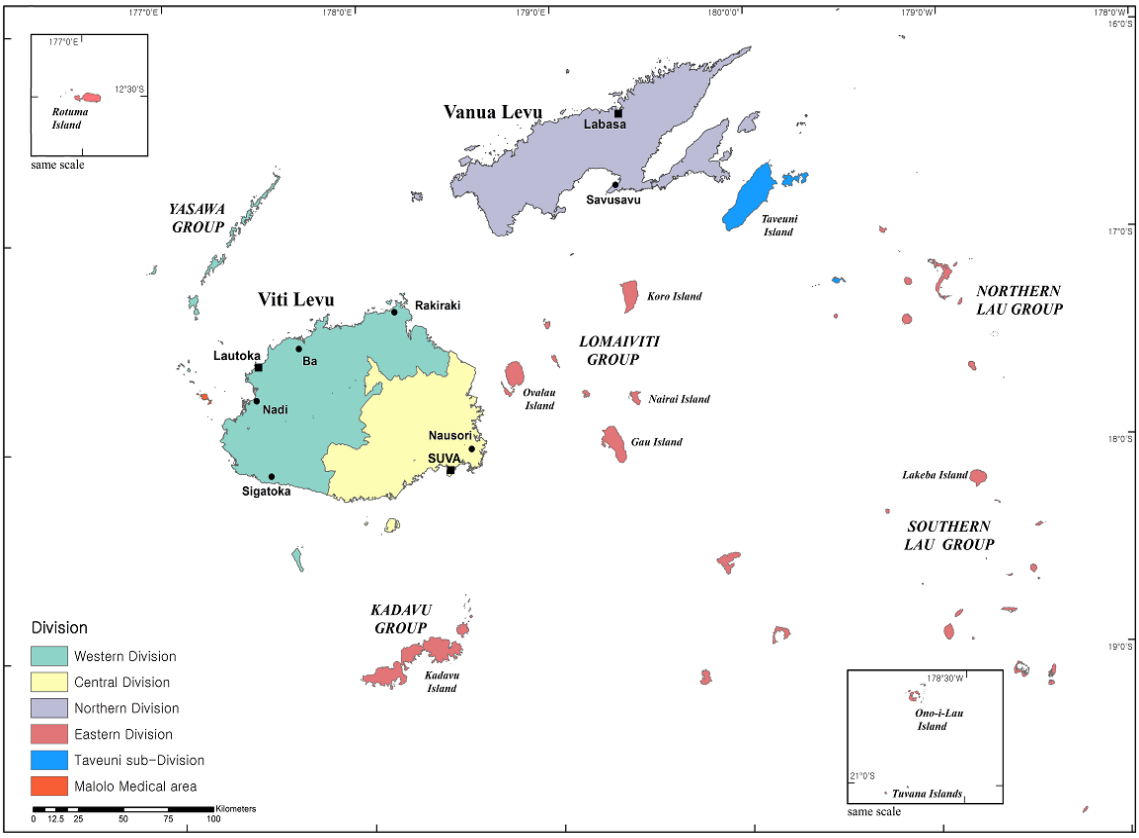
Reflecting all TAS results as summarized in Table 4.6, as of 2015, there were three EUs, including one newly defined (the Western Division excluding the Malolo Island Medical Area) and three IUs for the national programme to implement the activities (Figure 4.8). A new IU, the Malolo Island Medical Area, was added, compared to the scheme of 2013 (Figure 4.2) prior these TAS. Decisions were made to the remaining part of the Western Division, and Northern except Taveuni sub-Division, as to not to re-commence MDA, while for the Central Division the decision was to stop MDA based on the TAS results. For the Malolo Island Medical Area, it was decided to join the Eastern Division and Taveuni sub-Division, by recommencing MDA for at least two more rounds. The results of further MDA rounds following these TAS are summarized in Table 4.7.

Table 4.7 Lymphatic filariasis MDA coverage in Fiji, 2014-2015, following prior this study (NPLF, data unpublished)

Year	Population requiring PCT	Participating IUs	Total population in IUs	Reported number of people treated	Programme coverage (%)
2014	56,880	E, Taveuni SD, and Malolo Island MA	59,115	43,011	72.8
2015	55,340	E, Taveuni SD, and Malolo Island MA	55,340	51,032	92.2

N.B: E: Eastern Division; MA; Medical Area and SD: sub-Division

Figure 4.8 Sketch map of Fiji showing 3 EUs and 3 IUs as of 2015



4.4. Discussion and ways forward

4.4.1. Impact of mass drug administration against lymphatic filariasis in Wallis and Futuna

This survey targeting all students in grade 2 to grade 5 of elementary schools in Wallis and Futuna provides evidence of interrupted LF transmission in the territory, by showing the CFA prevalence less than 1% (WHO 2011c), which satisfies the global target of below 1% antigen prevalence in *Aedes*-endemic areas for LF elimination. Since vector management against LF has not been an ongoing activity in the territory during the last decade, it is likely that continued rounds of MDA (the last one conducted in 2011) have contributed to the interruption of LF transmission. However, this is difficult to conclude since we are lacking recent coverage data and there have not been any post-MDA coverage surveys conducted in WAF. Despite the lack of coverage data, it is unlikely that LF interruption would have occurred without 4-6 effective MDA rounds (WHO 2011c).

It is also possible that LF endemicity in the territory had been already close to the level that would not allow any LF recrudescence, several years prior to this survey. As it was observed in the survey conducted in 2006, there were no sentinel sites showing LF antigen prevalence higher than 1% (Table 4.1). Though its statistical power is questionable as a sentinel site surveillance with unknown ways of selecting participants, the 2006 survey results were, in fact, sufficient to move forward to a TAS as per the current guideline of monitoring and epidemiological assessment of MDA (WHO 2011c). In this regard, it may have been possible that the programme had passed the TAS if it had organized one right after the 2006 survey, namely around 2007. This shows the importance of continued supports provided in a coordinated manner for the country programme in a highly remote setting, as some of the other endemic countries under PacELF were able to move to the post MDA surveillance around that time (Chu et al. 2014; Allen et al. 2017). All in all, this first TAS in WAF provides critical evidence for the national programme in making a programmatic decision of stopping MDA.

4.4.2. Impact of mass drug administration against lymphatic filariasis in Fiji

This study presents the results from the nation-wide post-MDA surveillance activities that were recently conducted by the national programme, which have shown a significant reduction in LF antigen prevalence since the commencement of MDA in 2002. The results confirmed that more than 95 % of people across Fiji are now considered to be at very low or no risk of LF infection (Table 4.7). This is a major step towards the national programmatic goal,

specifically the interruption of transmission, as well as the projected goal of eliminating LF by 2020 set by the Global Programme to Eliminate Lymphatic Filariasis (GPELF) (WHO 2010b). Furthermore, the study presents a detailed narrative of the WHO TAS implementation (WHO 2011c) process in an island setting. Its potential use in assessing the impact of key communicable diseases as a survey platform, which may be of interest to other countries in similar epidemiological situations will be described in the following Chapter 5-8.

Though it is early to conclude, Fiji is likely to have achieved the overall success of the LF programme from implementing MDA, reducing the LF prevalence and scaling up TAS activities as an endgame surveillance strategy in two main islands, where 95% of the population out of total 837,271 resides (Fiji Bureau of Statistics 2009). The success could be attributed to many key determinants, as discussed in the review by Kyelem et al: Biological/epidemiological/therapeutic; economic/political/social; and programmatic operational effectiveness (Kyelem et al. 2008; Shamsuzzaman et al. 2017). The most notable factors identified that relate to Fiji include; i) two drug MDA regimen, ii) sustained political commitment, and iii) population compliance. Prior to commencing two-drug regimen MDA under PacELF, there were foci in Fiji with high transmission, i.e. the majority of sentinel sites' ICT prevalence levels > 15%, even after a long history of implementing MDA with a single dose regimen (WHO WPRO 2006). Since the beginning of MDA using DEC and albendazole in 2002, the programme was able to achieve MDA coverage at least around 60% across the country for more than a decade (Figure 4.1). The programme also managed to re-commence another five rounds of MDA right after when the remaining burden of LF endemicity in the two evaluation units, namely the Central and Eastern Divisions, was far from the pre-set target of 1% (Rinamalo et al. 2014), which would not have been possible without the strong willingness of the government of Fiji to tackle the infection and acceptance of the general public. In addition, as the long-time host for the PacELF office, the national programme has established and maintained an excellent collaboration network with partner organizations, which have been able to provide key financial and technical support. It has helped the national programme to overcome challenges and to continue the momentum towards achieving the elimination goal.

Nevertheless, NPLF has faced several challenges, particularly when the programme had to commence another five rounds in the Central and Eastern Divisions, even if there had been already five MDA rounds across the country following the GPELF's initial recommendation of 4-6 effective MDA rounds (Ottesen 2006). The reason for the high number of MDA rounds could be related to two main factors. Firstly, though the Central and Eastern Division had

historically shown high levels of LF endemicity (WHO WPRO 2006), the 2007 survey applied a different approach in these two EUs compared to other EUs, by surveying a few historic sentinel sites to collect CFA prevalence rather than a systematic sampling of the survey participants (Rinamalo et al. 2014). This could eventually over-estimate the actual burden of LF in the surveyed area, which was further confirmed in a follow-up LF prevalence survey in 2013 (NPLF, data unpublished). Another possible reason would be differences for the Central and Eastern Division in the localized demographical or environmental characteristics such as poverty, peri-urbanization, an abundance of *Aedes* spp. mosquito breeding sites (Shamsuzzaman et al. 2017), as well as lower effective coverage than reported administrative coverage. Therefore, NPLF had to address the latter in the next batch of the MDA rounds by; i) extensive community awareness campaigns on the importance of swallowing the tablets on site with general public as well as re-training of drug distributors on proper recording and reporting (i.e. only those who were observed their swallowing of tablets were marked), and ii) mapping of coverage at health centre levels and implementing the coverage surveys which helped to identify and target problem areas, in order to ensure effective epidemiological coverage >65%.

There are also difficulties faced in implementing post-MDA surveillance activities by the national programme, especially in designing and modifying the monitoring and evaluation framework with the availability of new epidemiological information. Since the 2007 LF prevalence survey, it was widely agreed to set the LF evaluation units at the Divisional levels which are the major implementation structure for all public health programmes. When the Taveuni sub-Division failed in its pre-TAS assessment prior to the Northern Division's TAS 1, unlike other sub-Divisions, there were concerns whether the whole Northern Division should not proceed to TAS and resume MDA. NPLF consulted extensively to the stakeholders and concluded to separate the Taveuni-sub-Division from the Northern Division EU which eventually had passing result in its TAS 1, given that islands of the Taveuni sub-Divisions are geographically separated by the sea from the other part of the Northern Division and local factors could differ (Figure 4.8). Similarly, when the TAS 2 results from the Western Division became available, there were questions how to re-define the EU for this area: Whether the whole sub-Division enclosing the island or the Medical Area for the island only should be excluded. Following the example of the Taveuni sub-Division, it was decided to redefine the original EU without the Malolo Island Medical Area, given that it is geographically separated from the main island of Viti Levu and the responsible health centre's ability to implement MDA on its own. In the early phase of the monitoring and evaluation framework development by

NPLF, all IUs and EUs were defined following Divisional boundaries. However, as the programme advanced there were modifications on the IU and EU boundaries: As of 2015, IUs were set either at Divisional, sub-Divisional, or Medical Area level (Figure 4.8), which shows the national programme's advanced capacity in dealing with new epidemiological features of the infection, overall in a low endemicity setting with remaining high endemic foci that are focalized.

Addressing low endemic areas or re-evaluating endemicity using a decision making survey based on probability sampling is becoming increasingly important as countries need to provide nationwide evidence that LF is no longer a public health problem (WHO 2015c; Shamsuzzaman et al. 2017). One thing which should be highlighted in this study is that it is important to understand the magnitude of non-response and the impact on the prevalence estimates: The survey participation should be high enough to back up the conclusion of 'low prevalence', which was not always the case in our study. As it was seen in TAS 2 in the Central Division, unlike two other surveys, the overall participation did not reach 60%. This non-response bias can yield mistakes in estimating a population prevalence based on the sample of survey data in which, due to non-response, certain types of survey respondents, namely CF positives, are under-represented (Cheung et al. 2017). Based on our observation from the field, most of the schools with low response rate were located in urban or peri-urban areas around the capital city, Suva, and students were not enrolled as parents/guardians did not send the signed consent form on time. Discussions with the teachers of the concerned schools and the Ministry of Education found that more active awareness campaigns using mass-media would help to increase the number of parents/guardians who would agree with their child to take CFA testing, rather than relying on the information on the survey conveyed to homes from schools. The challenges in implementing successful MDA rounds in the urban areas has been described (Nandha et al. 2007; Njomo et al. 2014) and the necessity to acquire specific parental consent for drug administration was considered as one of the potential factors (Njomo et al. 2014). In order to achieve the optimum level of coverage in the next surveys, it is imperative to assess what actually caused non-response in the previous survey and develop innovative communication strategies to target the groups of parents and guardians.

4.4.3. Next steps for the national programmes

4.4.3.1. Wallis and Futuna LF Elimination programme

In light of these results, we recommended stopping MDA and moving to the post-MDA surveillance phase to detect any LF recrudescence in Wallis and Futuna. The final TAS is recommended in five years of time (2016-2017) from the moment that the last round of MDA conducted in 2011, in order to confirm that the disease has been eliminated as a public health problem in the territory. Like in other LF-endemic countries in the Pacific, the LF control programme has been in operation for more than decades, and there may be challenges to shift to the surveillance phase upon stopping the intervention all at once. Thus, advocacy about the programme progress and its strategic direction should be well maintained with the stakeholders and as well as the public regarding the next steps for LF elimination in the territory.

4.4.3.2. Fiji LF Elimination programme

It will be possible for the national programme to follow the predetermined monitoring and evaluation timeline in 2013 (Table 4.2): The last TAS (TAS 3) for the Western Division except for the Malolo Island Medical Area and the second TAS (TAS 2) in the Central Division are scheduled in 2017, and TAS 3 for the Northern is set to be in 2018. It is recommended that the LF programme continues to maintain post-MDA surveillance efforts, firstly TAS as the main strategy and other pre-TAS requirements, and also to explore the application of TAS as a survey platform for other key communicable diseases of public health importance. Measures to ensure that sufficient proportions of the targeted children in urban and peri-urban settings to participate in TAS should be actively sought and applied. As for the Malolo Island Medical Area IU, it is recommended to join the Eastern Division and the Taveuni sub-Division for the continuing MDA rounds.

4.4.3.3. Post-validation surveillance

The WHO recommends conducting ongoing or periodic surveillance activities even after the WHO acknowledged the elimination of LF as a public health problem (WHO 2017a). One of the objectives is to detect and respond to any recrudescence or reintroduction of LF in the country. In the island setting with limited resources, it would be important to explore the opportunities to integrate LF surveillance with the ongoing programmes or existing public health initiatives, so that the country programme should be able to take necessary actions. The span of TAS, from TAS 1 to TAS 3, is usually around 5-6 years, and in the long run, it would be helpful to continue the surveillance efforts in more efficient ways, especially for another 5-

6 years, as well as to maintain the country programme's advanced capacity in conducting surveillance activities.

4.4.4. Conclusion

Since it's joining the PacELF, the National LF Programme of Wallis and Futuna as well as Fiji has successfully implemented MDA rounds with the two-drug regimen, reduced LF prevalence, and been able to implement the WHO standard Transmission Assessment Survey (TAS) methodology across all endemic areas as part of LF endgame surveillance strategy (WHO 2010b). This Chapter presents TAS results of two country programmes, highlighting the evidence of a reduction in the risk of LF across the whole territory in Wallis and Futuna and two main islands of Fiji, and its contribution to the global elimination target of 2020. The entire population in Wallis and Futuna and 95% of them in Fiji is now considered to have very low or no risk of LF infection after 15 years of programmatic activities and is on track to meet elimination targets. The studies presented here will also serve as a backbone of other studies which will be described in the following Chapters.

Chapter 5

Expanded communicable disease surveillance: Assessing the impact of hepatitis B virus vaccination in Wallis and Futuna

Chapter 5. Expanded communicable disease surveillance: Assessing the impact of hepatitis B virus vaccination in Wallis and Futuna

This chapter has been published in a modified form in the American Journal of Tropical Medicine and Hygiene, 96(3), 2017, pp. 715–719. The published article is enclosed in the Appendix.

5.1. Introduction

Hepatitis B virus (HBV) infections lead to a wide spectrum of liver diseases ranging from acute hepatitis, including fulminant hepatic failure, to chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma (Liang 2009). Currently, an estimated 257 million people are living with hepatitis B virus infections (defined as hepatitis B surface antigen positive) worldwide (WHO 2016). Fortunately, a vaccine against HBV has been available since 1982 (WHO 2016), and it is 95% effective in preventing the infection and also in the development of chronic diseases such as hepatocellular carcinoma from chronic hepatitis B infection (WHO 2010a). In 1992, the World Health Organization (WHO) made a recommendation that countries introduce three doses of hepatitis B virus vaccine (HepB), to be administered with diphtheria, pertussis, and tetanus vaccine (DPT), to their national immunization schedules (WHO 2010a). In 2009, the WHO's recommendation was further specified to administer the first dose as soon as possible after birth, preferably within 24 hours, emphasizing the importance of preventing mother-to-child HBV transmission in controlling HBV infection (WHO 2010a), given that it is a most common mode of viral transmission in high-endemic settings (WHO 2016).

5.1.1. Epidemiology of hepatitis B virus infection in Oceania

Prior to introducing HBV vaccination, nearly all countries in the WHO's Western Pacific Region including those in Oceania showed the HBV infection prevalence levels higher than 6% (WHO WPRO 2013). These elevated levels of HBV infection have caused the highest rates of liver diseases in the world among six regions of WHO (WHO 2003). Every country and area in the region responded to control HBV infection by including HBV vaccination as a part of their national immunization policy by 2005, and a timely birth dose vaccination by 2007

(WHO 2003; Rani 2009), except Japan and New Zealand, where screening of pregnant women and selective immunization was practiced. The regional goal for the control of HBV infection was further set to reduce the prevalence levels of HBV infection to below 2% among children by 2012, and below 1% by 2017 (WHO WPRO 2013).

5.1.2. Epidemiology and ongoing interventions on hepatitis B virus infections in Wallis and Futuna

Overall HBV infection prevalence has been historically high in the Pacific (Wong et al. 1979; Wilson et al. 2000; Bialek et al. 2010) and Wallis and Futuna (WAF) was not an exception: the HBV prevalence levels in the pre-vaccination era was estimated up to 8% by WHO (WHO WPRO 1999). Like other territories in the region, a survey conducted in WAF targeting the general population in 1988-1989 found a high chronic HBV infection rate measured by the serologic presence of hepatitis B surface antigen (HBsAg) which was positive among 39% of survey participants (Louis et al. 1992). The more recent data from 2006 showed that the French Pacific territories continued to be highly endemic for hepatitis B virus infection (Denis and Levy-Bruhl 2006).

Following the WHO recommendations, WAF introduced the hepatitis B virus vaccine (HepB) in their routine immunization programme in 1992. The initial vaccination schedule was administering 1st, 2nd and 3rd dose of HepB at 2, 3, and 8 months. This is rather unique, considering that most of other developing countries adopted a schedule of three doses given at birth, 2 months, and 6 months of age (Posuwan et al. 2016). According to the WHO/UNICEF Joint Reporting, the country programme reported the immunization programme coverage only in 2000 and 2003 for the period of 2000-2016, and the reported coverage of HepB in both years was 100% (WHO 2017b). Considering the importance of preventing mother-to-child HBV transmission in controlling HBV infection, a birth dose of HepB was introduced in 2006. The timing for 2nd and 3rd dose was also changed from 3 and 8 months to 1 and 6 months. All vaccinations administered by the Public Health Agency of WAF have been recorded on the individual immunization cards given to mothers and also in the immunization registers, which were kept by the national immunization programme.

5.1.3. Justification of the study

The impact of hepatitis B vaccination programmes can be assessed by conducting an HBsAg prevalence survey among children born after the introduction of the vaccine (Zanetti et al. 2008). Most of other other countries in the region had made extensive efforts to assess the prevalence of HBV infection after the introduction of the vaccine (Zanetti et al. 2008) (Ni and Chen 2010; Bialek et al. 2010; Luo et al. 2012), but no assessments of the impact of HepB vaccination introduction had been conducted in WAF. When there was an opportunity to access school-aged children via a lymphatic filariasis (LF) transmission assessment survey (TAS) in 2012, the Public Health Agency of Wallis and Futuna decided to monitor the impact of the HepB immunization after 20 years from its introduction, as LF TAS would provide a unique opportunity to access all elementary school children. As this was the first time to conduct a combined TAS and HBsAg prevalence survey, the objective of the present study was not only to assess HBsAg prevalence levels and HepB vaccination status among school-aged children together with LF circulating antigen prevalence (CFA) levles but also to explore the feasibility of implementing two surveys at the same time.

5.2. Methods

5.2.1. Study area

Wallis and Futuna is composed of 23 islands, and located approximately 600 km North-East of Fiji, with a population of 14,900 in a land area of 145 square kilometres (WHO WPRO 2006) as described in detail in Chapter 2. Following the French school system, there are 13 elementary schools in Wallis and Futuna (Figure 4.2) which are comprised of five grades, from first grade starting at five years of age. Here we conducted an LF TAS combined with the HBsAg prevalence and HBV vaccination coverage surveys as a school-based approach, which was facilitated by a team of local health staff from the Public Health Agency who were trained by the experts from WHO.

5.2.2. Study design and participants

We designed a cross-sectional study to measure HBsAg prevalence and HepB immunization status among schoolchildren, in order to assess the impact of the introduction of HBV vaccine including the birth dose for infants by nesting it to an LF TAS, assuming that the children who had developed protective antibodies from HBV vaccination would not have

HBsAg presence in their blood. This had not been tested in WAF among children who were born after the introduction of the HepB immunization in the territory's immunization programme. Also, apart from the sporadic report on the immunization administrative coverage, national HepB vaccination coverage and its timeliness was never assessed,

For LF TAS, grade 2-5 students were selected to meet the recommended sample size of approximately 1,000 students in an area where the principal vector is *Aedes* spp. and for the selection of the participants, given that majority of the first graders are five years old and the TAS guideline recommends 6 to 7-year-old children to be tested (WHO 2011c). More details on the TAS design are described in Chapter 4. For the concurrent HBsAg prevalence and HepB vaccination coverage assessment, it would have been sufficient to select a random sample of 165 children out of one birth cohort, who were born after the introduction of HepB vaccination, to estimate HBsAg prevalence, where the size of the birth cohort in WAF was estimated to be 178 (2009 census), alpha=0.05, HBsAg prevalence (p)=2%, design effect=1, and using a correction factor for finite populations and one-sided 95% confidence interval (CI). However, for logistic reasons and being more conservative, we chose all children enrolled in grade 4 and 5, which is approximately a half of the sample size for the LF TAS, based on the assumption that HBV prevalence would be higher in the elder population (Alter 2003).

5.2.3. Data collection procedure and specimen examination

Before the survey, we contacted school principals of the 13 elementary schools through the Department of Education to explain the purpose of the survey. We also requested the lists of students from each school that included the students' name, gender, age, and grade. The field staff from the Public Health Agency were trained on blood sampling procedures and on how to use and interpret the HBsAg and LF antigen rapid diagnostic tests, as described in the WHO monitoring and impact assessment manual (WHO 2011c) and following the manufacturer's instruction (Alere 2017). The blood samples were collected from each participant by finger prick and tested on site without any special equipment, apart from buffer solutions for the HBsAg and a capillary tube for LF antigen. A total of 50 µl whole blood for the presence of HBsAg by Determine HBsAg (Alere Inc., Waltham, MA, USA) was needed and the test was to be recorded at least 15 minutes after the specimen was placed on the test strip, together with 100 µl for the presence of circulating filariasis antigen by BinoxNow (Alere Inc., Waltham, MA, USA). The team was composed of a member skilled on specimen collection and conduction of the rapid tests, and a second member who was in charge of collecting the consent forms and recording data.

The questionnaire included basic demographic information, test results, and HepB vaccination dates copied from the child vaccination cards if the child brought the card on the survey date. If vaccination cards were not available, investigators obtained information from the Public Health Agency immunization registers which were kept by the national immunization programme. The investigators reviewed the questionnaires before leaving the school to ensure the completeness of data.

5.2.4. Statistical analysis

Data were entered in a database programmed with EPIDATA version 3.1 (EpiData Association, Odense, Denmark) and analyzed using STATA version 12 (StataCorp LP, College Station, TX, USA).

5.2.4.1. Hepatitis B virus infection prevalence

We estimated the prevalence of HBV infection by calculating HBsAg positivity in the blood among children tested. Prevalence levels were also estimated by categories, such as sex, the location of schools, grade, completeness, and timeliness of HepB vaccination coverage (as defined later in 5.2.4.2), where its 95% Wilson confidence intervals (CI) at the $P<0.05$ significance level were calculated considering that it has good properties even for a small number of trials and/or an extreme probability (Lott and Reiter 2018). In order to test whether being in any category was associated with being HBsAg positive or not, STATA tabi command was used to calculate Fisher's exact *p*, given that the expected values in several of the cells of a contingency table are below 5.

5.2.4.2. Immunization coverage and its timeliness

We estimated both completeness and timeliness of hepatitis B vaccination coverage. Completeness was calculated as to whether a child received HepB vaccine regardless of the timing; the denominator was all children surveyed for HBsAg testing. Timeliness was calculated based on whether a child was vaccinated within the specified time window. As the children tested for HBsAg in the survey were born during a time when the recommended schedule to administer the HepB vaccine was at months 2, 3 and 8 rather than 0, 1, and 6, we defined timeliness as HepB vaccination by 3, 4 and 12 months for each dose, allowing four weeks of window. For the purposes of comparison, we applied a 2006 measure of timeliness to this cohort (even though they were born before 2006), and we defined it as HepB vaccination within 24 hours after birth, 2 and 7 months.

To describe timeliness, we calculated the cumulative probability of being vaccinated at age t, by inverse Kaplan-Meier survival function, or 1-SKM(t) in the vaccinated cohort (Dayan et al. 2006). An event was defined as having been vaccinated before the upper limit of age range in months for each specific HepB dose schedule. A child was censored if the scheduled HepB dose was received within the recommended time range. Person-months of observation were estimated as time spent during the eligibility period up to when the dose was received either; within the recommended time range or earlier (thus censoring) (Kimura 2011). If a child missed a preceding dose, then this child was not eligible to the follow-up of the subsequent Hep B vaccination dose. Only children with previous vaccination would be followed up for the subsequent vaccination and remained in this analysis. We also checked how many children received it timely following that schedule with the same method, to have an idea of the actual practice that could have prevented perinatal HBV infection, even though the HepB birth dose was not included in the recommended immunization schedule when the 4th and 5th graders were born.

5.3. Results

5.3.1. Demographic characteristics of the study population

Out of 476 children registered for the 4th and 5th grade in 13 elementary schools of WAF, 447 (94%) were present at the school on the survey date. A total of 19 refused to be tested yielding an overall participation rate of 90%. The 427 children had their demographic data available thus included in the analysis. They were born between December 1999 and June 2004, their mean age was 10.5 years, and 235 (55%) were male.

5.3.2. Geographical distribution of hepatitis B virus infection in Wallis and Futuna

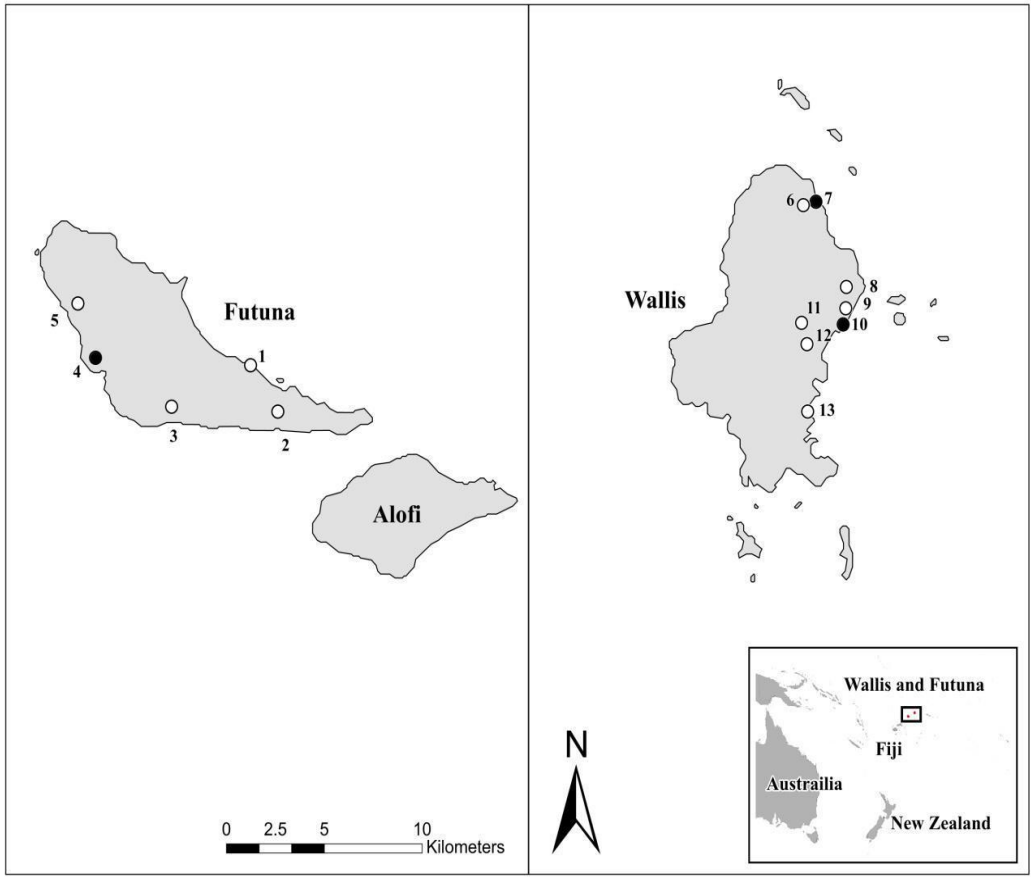
Overall HBsAg prevalence was 0.9% (4/428) (Table 5.1). Prevalence was higher in Wallis but without any statistical significance and this tendency was similarly observed in the circulating filariasis antigen prevalence as described in Chapter 3. There was no difference in HBsAg prevalence between grade 4 and 5 students. Those who were vaccinated 'untimely' showed higher HBsAg prevalence but without statistical significance, either. In testing the association between the HBsAg positivity and the abovementioned catetories, none of them showed the statistically significant association (Table 5.1). As for four HBsAg positive children, their ages ranged from 9 to 11 years with 3 males and 1 female, and all of them had 'untimely' vaccination. The location of schools with any HBsAg positive case is shown in Figure 5.1.

Table 5.1 Demographic characteristics and HBsAg prevalence among grade 4 - 5 schoolchildren in Wallis and Futuna, November 2012

	Number of students tested	Number of students positive for HBsAg	HBsAg prevalence (%), 95% Wilson CI		*P*-value
Sex					
- Female	193	1	0.5	0.1-2.9	0.631
- Male	235	3	1.3	0.9-2.9	
Location of schools					
- Wallis	267	3	1.1	0.4-3.3	1.000
- Futuna	161	1	0.6	0.1-3.4	
Grade					
- 4	217	2	0.9	0.3-3.3	1.000
- 5	211	2	0.9	0.3-3.4	
HepB vaccination status*					
- Unvaccinated	5	0	0	0-43.4	
- Partially vaccinated	14	0	0	0-21.5	0.911
- Fully vaccinated	409	4	1.0	0.4-2.5	
HepB vaccination timeliness 1992-2005 schedule					
- timely	211	0	0	0-17.9	0.124
- untimely	217	4	1.8	0.7-4.6	
Overall	428	4	0.9	0.4-2.4	

N.B.: *: Unvaccinated: 0 dose of HepB; partially-vaccinated: 1-2 doses; fully vaccinated: 3 doses: **_P_ value is from Fisher's exact.

Figure 5.1 Sketch map of Wallis and Futuna showing locations of surveyed schools and schools with positive hepatitis B virus surface antigen (HBsAg) case(s)



5.3.3. Hepatitis B virus vaccination coverage and its timeliness in Wallis and Futuna

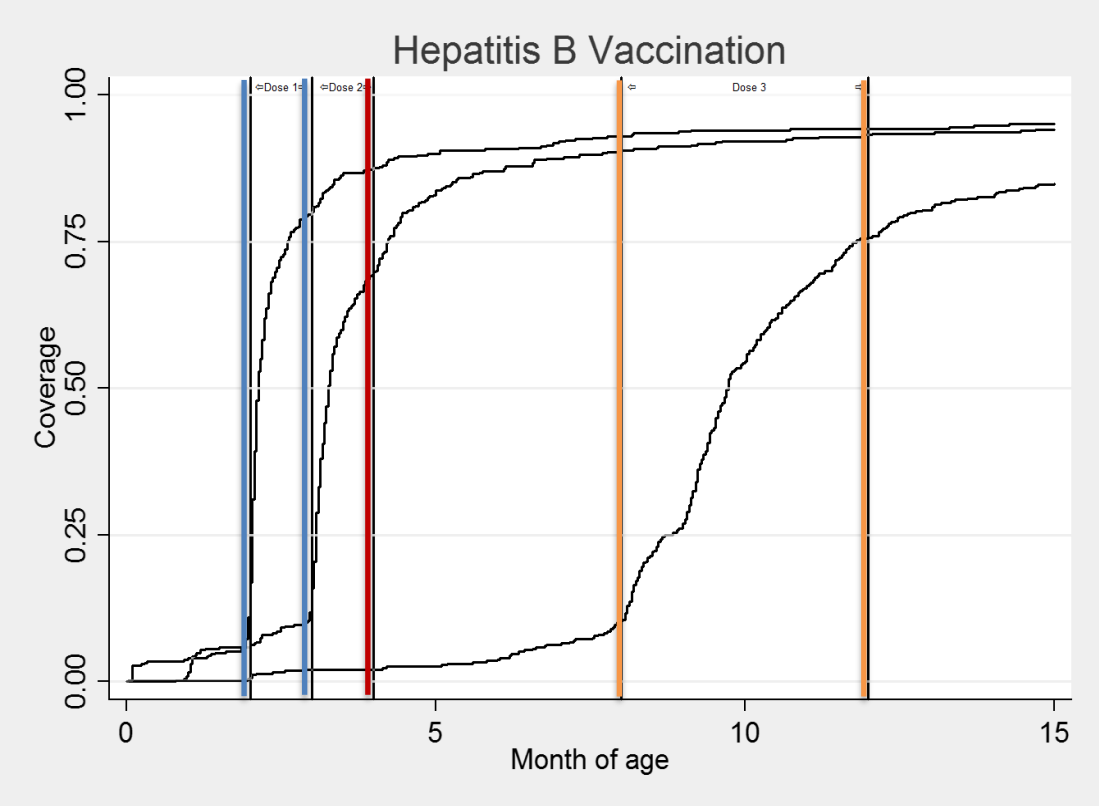
Among 427 children included in the analysis, the coverage for the three doses of hepatitis B vaccine was 97% for the first two doses, and 96% for the third dose (Table 5.2). According to the schedule in place when the children were born, the proportion of timely vaccination was 80%, 56%, 65% for doses one, two, and three, respectively. A total of 49% of children received timely vaccination for all three doses, before one year of age. When the current schedule was applied to measure the proportion of children who had the timely vaccination, the proportion was 6%, 5%, 72% for doses one, two, and three, respectively. Figure 5.2 shows the inverse Kaplan-Meier curves of vaccination coverage by month of age. As for four children who were HBsAg positive, they received all three doses of hepatitis B vaccine. One child received the first dose within 24 hours of birth while the other three received their first dose on the dates ranged from 65 to 322 days. As for the 2nd and 3rd doses, the vaccination dates ranged from 31-392 days and 60-441 days, respectively.

Table 5.2 Hepatitis B virus vaccine (HepB) coverage and its timeliness among grade 4-5th schoolchildren in Wallis and Futuna

Dose	Vaccination regardless of timeliness (%)	No of students who were vaccinated N=427	
		By 1992-2005 schedule* N (%)	By 2006-2012 schedule** N (%)
HepB 1st or HepB birth dose (BD)	97	344 (80)	24 (6)
HepB 2nd dose	97	239 (56)	21 (5)
HepB 3rd dose	96	280 (65)	307 (72)
All 3 doses	96	211 (49)	0 (0)

N.B.: *: HepB 1st, 2nd, and 3rd vaccination schedules were at 2, 3, and 8 months; timeliness was defined as vaccination by 3, 4 and 12 months respectively; **: HepB BD, 2nd, and 3rd schedules are at birth; 1, and 6 months; and timeliness was defined as vaccination within 24 hours of birth, by 2, and 7 months respectively.

Figure 5.2 Coverage of hepatitis B virus vaccination per each dose by inverse Kaplan-Meier survival function up to 15 months among surveyed students of Wallis and Futuna



N.B.: Two vertical lines in blue marked the time interval for the 1st dose, and the right blue line and the red line for the 2nd doses of HepB vaccination according to the 1992-2005 schedule (1st dose: by 2 months and 2nd dose: by 3 months), while right 2 vertical lines in orange mark the recommended timing for the 3rd doses, namely by 12 months.

5.4. Discussion

5.4.1. Low levels of hepatitis B virus infection prevalence in Wallis and Futuna

Our results of HBsAg prevalence <1% among grade 4 and 5 children show that Wallis and Futuna has likely greatly reduced early childhood hepatitis B virus transmission, being close to the WHO's Western Pacific Regional HBV infection control goal of reducing chronic HBV infection prevalence to <1% among children (WHO WPRO 2013). As these surveyed children were born even before Wallis and Futuna adopted the HBV birth dose vaccination policy, it is likely that prevalence in younger cohorts is even lower.

5.4.2. Control impact assessment, program monitoring, and evaluation

We believe that this low level of HBV infection is the result of a successful HBV vaccination which was introduced in 1992, not only given that pre-vaccine prevalence was high but also reported 3-dose HBV vaccination coverage has been at least 90% according to the national immunization coverage data collected by WHO/UNICEF and other partners (Asia and Pacific Alliance to Eliminate Viral Hepatitis 2013). This is further verified by our findings that the coverage with three doses of HBV vaccine is about 96% among the survey participants.

The impact of the HBV vaccination program could have been greater, given that less than half of children received all three doses within the recommended time frame using Kaplan-Meier method. It is well known that even in settings with high coverage, delay in receipt of vaccines results in an unnecessary risk of vaccine-preventable infectious diseases (Suárez-Castaneda et al. 2014). Timeliness of HBV vaccination, too, is critical not only for preventing mother-to-child transmission but also for protecting infants and young children against horizontal HBV transmission (Tharmaphornpilas et al. 2009), since children under five have a higher risk of acquiring chronic infection once exposed to the virus (Ni and Chen 2010).

Among the four children who were found positive for HBsAg in our study, all had received three doses of hepatitis B vaccine; however, only one child received HBV vaccination birth dose. None of the children who did not receive the birth dose had the timely administration of all 3 doses, so they were therefore at risk for both perinatal and horizontal infection (Lankarani 2011). Therefore, we encourage that the public health authorities pay continued attention not only to HBV vaccination coverage but also to timeliness for each dose of HBV vaccines.

5.4.3. Feasibility of combining LF TAS and hepatitis B virus prevalence and immunization coverage survey

In this study, we have also explored the feasibility of implementing two surveys at the same time and factors for the success are as follows; Firstly, the target population and required sampling frames for LF and HBsAg surveys were similar, but the TAS design is more stringent than the recommended HBsAg survey requirements, meaning that adhering to the TAS guidance would meet the requirements of the HBsAg survey by default. By relying on LQAS designs to test whether prevalence is below 1 or 2%, the current guidelines for LF TAS generally require a larger sample size among primary school students than other prevalence surveys with point estimates (WHO 2011c). However, simply mirroring our survey with the TAS would have required much larger resources, thus we selected a subset of the target population for the HBsAg prevalence and coverage assessments and nested the survey in LF TAS, which improved the logistic feasibility of the survey.

Secondly, the similarity of sample collection and testing methods were highly advantageous in conducting two surveys simultaneously. In our study, HBsAg and LF antigen detection tests both use a finger prick for collecting whole blood, of which necessary amounts were aliquoted on the separate test strips. The team composition and skill set required for both surveys were identical and there was no need to have extra human resources for fieldwork. This is in contrast to several soil-transmitted helminths prevalence surveys undertaken using TAS as an access platform, which included on-site stool smear examination and accompanied microscopist (Chu et al. 2014; Drabo et al. 2016) or additional teams for soil-transmitted helminths associated components such as stool sample collections in other age groups.

Lastly, there was a detailed planning, including several training workshops for the field staff on the use of the test kits and data collection, in collaboration with the Public Health Agency and the technical partner. For instance, the timing for reading results was different for two tests, namely after 15 minutes for HBsAg and at 10 for LF antigen, but this was well managed by explaining the benefit of allowing the teams to read the LF test first and then read the HBsAg strip 5 minutes later during the training workshop. Also, it was emphasized that while the time window to read LF results was critical, the HBsAg test required waiting for 15 minutes, but after that it could be read or verified anytime on that day.

In addition, the staffs were able to adopt a unique approach to have a robust measure of vaccination coverage among the survey participants, by obtaining immunization data from the health authority's registry when there was a missing immunization card of the child. This

kind of completeness is not feasible in many other settings but was in WAF, because of the completeness of records and staff's willingness. Through these comprehensive process and external technical support, a team of two staff was able to complete the surveys in two weeks of time in a setting with a limited human resource of health (WHO 2011b). A review of the literature reveals that this is the first time that an LF TAS was implemented together with the HBsAg prevalence survey or vaccine coverage assessments, and we believe that this approach could be easily adapted for other similar surveys in a subset of or together with the TAS target population.

5.4.4. Limitation of the study

This survey is subject to a number of limitations. Firstly, there is a possibility that actual burden of chronic hepatitis B infection is slightly higher than our estimates given that; 1) though the rapid point of care test used in our study has sensitivity of approximately 98.2% and specificity of almost 100% (Njai et al. 2015), it may have been possible that an HBsAg level less than 1 IU/ml would not have been detected by the test, and 2) HBsAg negativity is not sufficient to exclude Hepatitis B virus infections completely, as there are reports of occult hepatitis B virus infections, i.e. having HBV DNA presence in the absence of HBsAg with children in hypo endemic areas who were born to HBsAg positive mothers (Shahmoradi et al. 2012). Therefore, it may be worthwhile to consider the use or combination of preferably more sensitive tests such as ELISA or real-time PCR on top of the POC test, adopting archived blood stool samples such as dried blood spots (Mössner et al. 2016).

As for the HBV vaccination coverage, we may have underestimated it, as ad hoc queries revealed that several children whose vaccination records were missed in fact emigrated from France and may not have updated their immunization records in WAF. Furthermore, though we have observed a high proportion of untimely immunization, we did not have opportunities to explore the factors associated with it. In retrospect, adding place of birth and reasons for late vaccinations to the questionnaire would have provided more insight on the reasons for missing HBV vaccination or not being vaccinated on time.

Lastly, the results of this survey may not be entirely representative of the target population. It may be possible that the 10% of children who did not participate in the survey have different characteristics from the other 90% in terms of the HBV infection and HepB immunization status. For instance, school absence could have been linked to a lower likelihood of vaccination in the past (Esposito et al. 2014).

5.5. Conclusion and next steps

This HBsAg prevalence and HBV vaccination coverage assessment among school children in Wallis and Futuna showed that HBsAg prevalence was nearing the regional hepatitis B control target of below 1%. Furthermore, it showed that the vaccination coverage was high, although not always timely. Combining the hepatitis B prevalence survey with the TAS was a feasible and efficient in a remote and resource-limited setting.

5.5.1. Next steps

We recommend: 1) increasing the efforts for timely HepB vaccination in WAF; and 2) utilizing TAS as access platforms to school-aged children for other public health programmes where finger-prick blood samples could be useful, in order to maximize the efficient use of resources.

Chapter 6

Exploring geographical distribution of intestinal parasite infections on Fiji

Chapter 6. Exploring geographical distribution of intestinal parasite infections on Fiji

Part of this chapter has been published in a modified form in the American Journal of Tropical Medicine and Hygiene, 98(4), 2018, pp 1179-1185. The published article is enclosed in the Appendix.

6.1. Introduction

Intestinal parasite infections, caused by intestinal helminths and protozoan parasites, remain as a major public health problem in tropical and subtropical areas of the world, where poor sanitary conditions and limited and/or poor quality water supply have contributed their transmission (Sterling and Adam 2004). Soil-transmitted helminth (STH) infections are among the most common infections worldwide (WHO 2017h), and transmitted by eggs present in human faeces, which in turn contaminate soil (Muller 2002). The main species of nematodes are the roundworm (*Ascaris lumbricoides*), the whipworm (*Trichuris trichiura*) and hookworms (*Necator americanus* and *Ancylostoma duodenale*). As for intestinal protozoan infections, several species are associated with acute and chronic diarrheal diseases, such as *Giardia duodenalis*, *Cyclospora cayetanenensis*, and *Cryptosporidium* spp. (Table 2.5). *Entamoeba histolytica* can also cause dysentery and liver abscess (Sterling and Adam 2004). These protozoan infections usually occur through ingestion of cysts in water (including both unfiltered drinking-water and recreational waters) or food contaminated by the faeces of infected humans or animals (Fletcher et al. 2012).

An integrated approach to the control of neglected tropical diseases (NTDs) has been strongly advocated (WHO 2015a) and one of the examples is the control of STH infections by the implementation of preventive chemotherapy against lymphatic filariasis (LF), given that the drug used such as albendazole or ivermectin also has impacts upon STH infections ((WHO 2011a). Provision of the safe water, sanitation, and hygiene (WASH) interventions for the control of NTDs has been also highly encouraged as a one of the key interventions within the global NTD roadmap, which would be effective not only for the control of STH infections but also for intestinal parasite infections. In this context, it is important to have up-to-date information about the epidemiological situation of intestinal parasite infections in the areas that are or were formerly endemic for LF (WHO 2015a), to make a programmatic decision such as whether to continue albendazole distribution or not, with the down-scaling of LF MDA in

these areas and to complement required WASH interventions. Having the LF TAS described in Chapter 4 as a backbone, there is a need to assess the geographical distribution of intestinal parasite infections on two main islands of Fiji to shed light on future control requirements.

6.1.1. Epidemiology of intestinal parasite infections in Oceania

Oceania is a region of tropical and subtropical islands in the Pacific Ocean where one-quarter of the population is living in poverty (Kline et al. 2013). Intestinal parasite infections remain as a major health problem in this part of the world, with uneven distribution of safe water supply and improved sanitation (Kline et al. 2013), given that LF elimination programmes in many of the Pacific Programme to Eliminate Lymphatic Filariasis (PacELF) countries have achieved elimination or near-elimination (WPRO 2015). Among those, hookworm infection is considered as the most prevalent in the region, with an estimated 5.5 million cases (Kline et al. 2013), and significant numbers of ascariasis and trichuriasis cases are also present (Bethony et al. 2006). Nevertheless, data on intestinal parasite infections are scarce throughout the region, equally in the past and present (WPRO 2008).

The only available study on STH infections covering most of the Pacific island countries and territories (PICTs) of Oceania was a multi-country study conducted from 2001-2002. Typically, one urban and one rural school were selected by the government of the 13 Pacific island countries (Table 2.3) and surveyed by a team comprised of a parasitologist, an environmental health specialist, and a nutritionist (WPRO 2008). There was only one school in Niue, so the total number of schools in the survey was 25 (Hughes et al. 2004). The results were variable regarding levels of endemicity, showing the range of any STH infection prevalence at school level 0 to 100% (Hughes et al. 2004), which are summarized in Table 2.3. As of 2003, out of the 22 Pacific island countries, 17 were classified as endemic in the WHO Preventive Chemotherapy (PCT) Databank including Fiji; one country had no available data, and four countries were not included in the database (Table 2.4). These figures reflected the epidemiological profile of STH infections before the commencement of the PacELF strategy. Most of the PICs initially received anti-helminthic in the context of the LF elimination programmes, which was later switched to the school-based mass deworming upon stopping LF MDA rounds in some countries, but not all (WHO 2017f). As of 2015, ten countries including Fiji were classified as endemic and required preventive chemotherapy against STH infections (WHO 2017f). There are seven countries which implemented the intervention as needed, but only two countries, Kiribati and Tuvalu, had achieved the global target of 75% coverage among school-aged children (WHO 2017f). Preventive chemotherapy either from LF MDA or

deworming has probably reduced the prevalence of STH infections in the region, but data on the updated epidemiological profile are scarce (Kline et al. 2013).

With regard to the gastrointestinal protozoan infections, such as giardiasis, their epidemiology is not well-known (Rodriguez-Morales 2012; Kline et al. 2013), outside Australia (Sinclair et al. 2005; Stark et al. 2007), New Caledonia (Guittet et al. 2004), New Zealand (Lake et al. 2009; Snel et al. 2009) and Papua New Guinea (Owen 2005), even if the infection could be associated with acute and self-limiting illnesses but also chronic diseases such as persistent diarrhoea and malabsorption (Guandalini et al. 2016). Gastrointestinal protozoan infections may persist in Oceania, especially in rural areas (Sarkari et al. 2016), but the evidence base for targeted interventions for the prevention and control of the infection is not sufficient in the region.

6.1.2. Epidemiology and control of intestinal parasite infections in Fiji

Of the five NTDs eligible for preventive chemotherapy (PCT) according to WHO guidelines (WHO 2012a), Fiji is endemic both for STH infections and LF (WHO 2017f). Only a few studies have been available on the prevalence and control of STH infections in Fiji, especially before the initiation of mass drug administration (MDA) against LF (Jansen et al. 1991; Bethani et al. 1998; Hughes et al. 2004; Thomas et al. 2005; Mathai et al. 2008). A study in 1998 showed prevalence levels of 11% for *Ascaris*, 50% for hookworm, and 2% for *Trichuris* among villagers (Bethani et al. 1998). In the multi-country survey conducted for 13 Pacific island countries (PICs) in 2001 (Hughes et al. 2004), results of stool examinations revealed 10% of 58 children at the urban test site were infected with any helminths, and 9% of the 176 children at the rural site.

Initial efforts to control STH infections have been implemented since 2002, when annual rounds of MDA with diethylcarbamazine citrate (DEC, 6mg/kg) and albendazole (400mg per person) was commenced for those older than 2 years by the national programme to eliminate LF (NPLF) (WHO WPRO 2006). Most of the consecutive annual MDA rounds against LF were successful, with around 65% of population coverage for treatment (Table 3.2), and leading to the stopping MDA decisions in the most part of the country (Rinamalo et al. 2014): The final MDA for the Western Division round took place in 2009, and the Central and Northern Divisions (except Taveuni sub-Division) in 2012. The Eastern Division islands and Taveuni sub-Division together with the Malolo Island Medical Area of the Western Division continued additional rounds in 2014 and 2015 (Rinamalo et al. 2014).

Table 6.1 National iron and micro-nutrient supplementation programme scheme and coverage by target group, 2010-2014 (Vasu 2015)

	Pre-school children* (6 months-23 months)	Primary school children (6-14 years)	Women with child-bearing age** (15-44 years)	Lactating women*
Target population	20,970	140,368	209,956	20,970
Supplements administered	Pyrantel pamoate or albendazole Ferrous sulphate Vitamin A	Albendazole Ferrous sulphate	Ferrous sulphate Folic acid	Vitamin A
Year	15.0%	89.0%	-	-
	40.0%	75.0%	5.0%	25.0%
	35.0%	27.0%	4.0%	0.0%
	23.0%	32.0%	2.4%	2.2%
	1.3%	14.1%	0.6%	0.6%

*N.B.: Those who visit Maternal and Child Health Clinics only

The Fijian Ministry of Health and Medical Services (MHMS) also launched the National Iron and Micro-nutrient Supplementation (NIMS) programme with the objective of reducing the prevalence of anaemia and vitamin deficiency in school-age children (SAC), pre-SAC, women of childbearing age (WCBA), and lactating women. As a project, it initiated six monthly rounds of albendazole distributions together with iron supplementation to SAC. For pre-SAC, vitamin A was also added to albendazole and ferrous sulphate (Table 6.1) at maternal and child health clinic. It was a five-year long pilot project initiated in 2010, based on the findings of highly prevalent anaemia discovered by the National Nutritional Survey conducted in 2007 (Vasu 2015). Adding albendazole was based on the assumption that hookworm infection could be one of the major risk factors for anaemia among women and children (Chami et al. 2015).

The decision was further supported as a response strategy in the post LF-MDA setting where the annual distribution of DEC and albendazole had been stopped, such as the Western Division. However, except its first and second year, overall coverage among pre-SAC during a 5-year period and that of SAC after 2011 was poor and did not achieve the global target above 75% (Table 6.1), leaving the question whether the drug pressure at national level had been sufficient to control STH infections.

When it comes to gastrointestinal protozoan infections, their occurrence has been only scantly documented, even with the inadequate sanitation and safe water coverage at national level (UNICEF 2013). Few previous surveillance efforts have attempted to determine the burden of the gastrointestinal protozoan infections in the country (MacMillan and Hawley 1969; Jansen et al. 1991): In 1968, a survey conducted in a rural village reported *Giardia* spp. infections with prevalence levels of 5.4%, while another survey near Sigatoka Valley in 1982 reported *Giardia* spp. infections among children less than 15 years with prevalence levels of 1 to 5%. However, it is likely that these attempts underestimated its true prevalence, as solely insensitive microscopic methods such as direct faecal smear were used (Stanley 2003). Several cases of liver abscess from amoebiasis had been reported at the national reference hospital, but there was no population-based assessment on the burden of the infections (Ram 2014).

6.1.3. Hotspot analysis for infectious diseases

Spatial analytical methods have been used increasingly in public health fields and epidemiological research (Stopka et al. 2014), mainly due to the advent of geographic information systems (GIS)-based software (Columbia University Mailman School of Public Health 2017). Hotspot analysis is a spatial analysis and mapping technique interested in the identification of clustering of spatial phenomena events where a hotspot is defined as an area that has a higher concentration of events compared to the expected numbers given a random distribution of (Columbia University Mailman School of Public Health 2017). There are different methods for detecting hotspots, including spatial autocorrelation and cluster analysis (Stanton 2017). Measures of spatial autocorrelation can be categorized as global or local indicators of spatial association: Moran's I is an example of global spatial autocorrelation statistics, while Getis-Ord Gi* statistic is an example of a local indicator (Ord and Getis 1995; Columbia University Mailman School of Public Health 2017).

Different mapping techniques are used to visualize hotspots (Stanton 2017). Kernel density estimation is a popular mapping method due to its visual impact, as it creates a smooth,

continuous surface map showing gradients of the variation in the intensity of events across the study areas (Columbia University Mailman School of Public Health 2017) .

6.1.4. Justification of the study

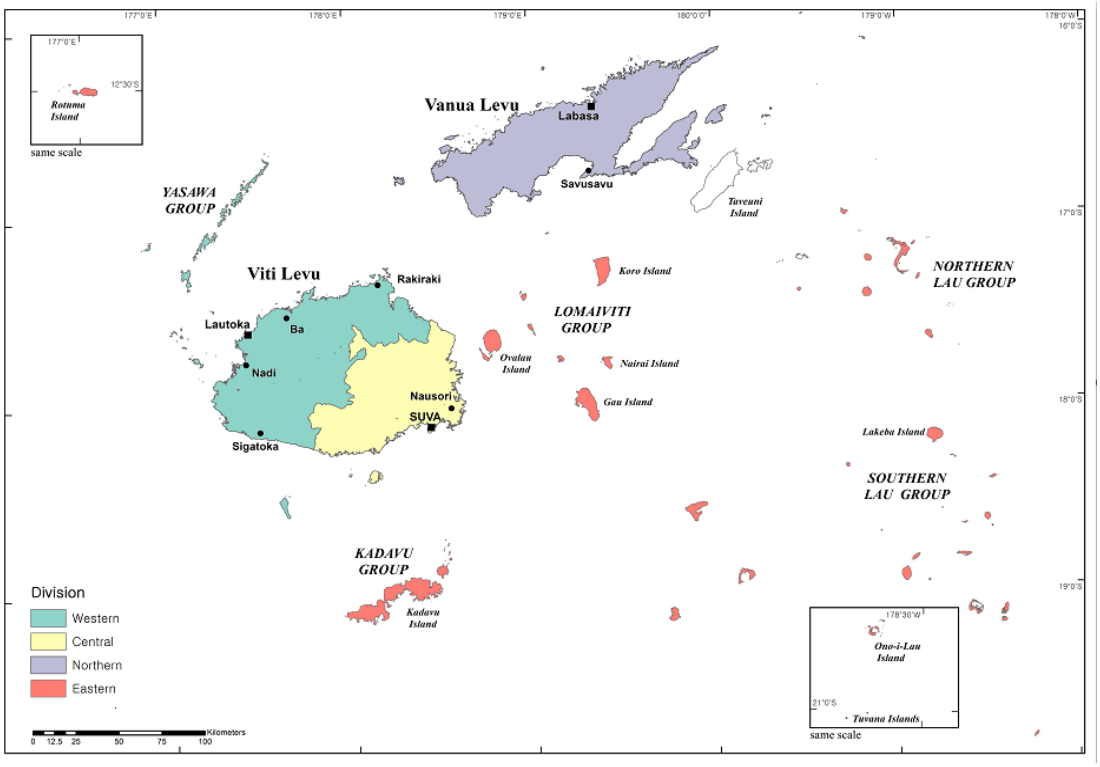
Despite intestinal parasite infections are considered as diseases of public health importance by the MHMS, there is no effort made to obtain nationally representative data with a focus on geographical distribution of the infections so far. Existing data show that there had been spots with the focal endemicity, but these are not sufficient for the health authorities to move forward, as it is well known that developing, implementing and evaluating the control strategy for infectious diseases would need the evidence base for targeted interventions (Fletcher et al. 2014). Also, the routine diseases surveillance system does not capture data on intestinal parasite infections, apart from sporadic severe morbidity cases of *Ascaris* infections such as intestinal bowel obstruction due to worm infestation or liver abscesses caused by *E. histolytica*, which will not be helpful to estimate the true population burden of the infections in the country.

In this regard, a nation-wide cross-sectional prevalence assessment for intestinal parasite infections was planned in the three Divisions of Fiji out of four (Figure 6.1) in conjunction with the lymphatic filariasis (LF) programme evaluation activities via transmission assessment surveys (TAS) (WHO 2011c). This is to take the maximum benefit of limited resources secured for the public health surveillance in an island country setting, as the opportunity to access the representative population is constrained. For the areas where LF TAS is yet to be conducted, namely the Eastern Division, sentinel site surveys were organized in randomly selected primary schools in integration with LF MDA microplanning exercises.

The objective of the study was to obtain the mid-term epidemiological profile of STH infections as a preliminary step for developing a national strategy for STH infection control. As per gastrointestinal protozoan infection, we aimed to provide cthe omprehensive epidemiological information on the extent and distribution of the infections on Fiji via locally available coproscopic techniques. As a pilot, we also assessed the applicability of LF TAS, considering the pre-determined survey scope in the aspect of the study design and implementation in conducting especially STH infection prevalence surveys in the view of the newly released global guideline from the WHO (WHO 2015a). Implementation of the assessment became possible only after the national reference parasitology laboratory at the Fiji Centre for Communicable Disease Control (FCCDC) was established via collaborative arrangement with the Seoul National University College of Medicine (SNUCM), as a part of

the Project for Control of Soil-transmitted helminthiases in Fiji (details are provided in Chapter 3). The project also provided technical and financial support to the MHMS on the other aspects of STH infection control and elimination.

Figure 6.1 Sketch map of Fiji showing areas* targeted for the intestinal parasite infection prevalence assessment via LF TAS



N.B.: *The Northern Division excluding the Taveuni sub-Division and the STH sentinel site surveillance only for the Eastern Division

6.2. Methods

6.2.1. Study area

A detailed description of the study area is included in Chapter 4. Specifically, intestinal parasite infection prevalence assessment through LF TAS was conducted in three different phases for the three Divisions (Western, Central, and Northern) of two main islands, Viti Levu and Vanua Levu. The Taveuni sub-Division of the Northern Division was excluded from the assessment, as it formed a separate evaluation unit (EU) for LF since 2013 due to the remaining pockets of LF endemicity (>1%) discovered during spot check surveys (NPLF, data unpublished). Consequently, the infection prevalence assessment in the Northern Division only covered other three sub-Divisions as the TAS did. For the Eastern Division, we have separately

organized sentinel site surveys covering all five sub-Divisions by randomly selecting schools, rather than awaiting the first TAS to be planned in the area, of which timeline was yet to be defined. Areas and their boundaries included in the intestinal parasite infection prevalence assessment are shown in Figure 6.1.

6.2.2. Timeframe

The first phase of the intestinal parasite infection prevalence assessment through LF TAS was built upon the second TAS (TAS 2) in February-March 2014 for the Western Division which was consequential from the passing results of the first TAS (TAS 1) in 2011 in the area with the conclusion of stopping LF MDA. The second and third phases of the assessment through LF TAS were embedded in conjunction with the TAS 1 for the Central Division in July-August 2014, and the TAS 2 for the Northern Division in February-March 2015. The latter was based on the passing results of the TAS 1 in 2012 with the conclusion of stopping LF MDA in the Northern Division except Taveuni sub-Division. Sentinel site surveillance in the Eastern Division took place in August 2015, when the NPLF field team visited the islands for the LF MDA microplanning workshops.

6.2.3. Selection of schools

As described in Chapter 4, the NPLF decided the LF TAS in Fiji to be school-based rather than community-based considering its high primary school enrolment rate up to 98% (UNESCO 2017) following the sampling frame of the WHO TAS guideline (WHO 2011c). Once schools for each LF TAS were selected using Survey Sample Builder (The Task Force for Global Health, Georgia, USA), the intestinal parasite infection prevalence assessment was nested within the LF TAS sampling framework by sub-sampling of schools.

For the first phase of the assessment in the Western Division, 30 schools (10 rural and 10 urban schools in Strong Dry zone, and 10 in Moderate Dry zone) were sub-sampled out of 77 schools selected for the TAS 2 of the Western Division, following the principle of "5-10 schools per ecological zone" recommended by the WHO (WHO 2011a). The estimated sample size for the assessment was 1,692 class one and two students out of 3,695 registered, in the schools chosen for LF TAS (Table 6.2). As all schools existing in the Moderate Dry Zone are rural, selection of urban schools was made only for the Strong Dry Zone.

By applying similar principles, for the second phase of the survey in the Central Division, ten urban and ten rural schools were selected out of 82 schools chosen for TAS, which mostly falls under the Wet Zone. For the third phase in the Northern Division except

Taveuni sub-Division, ten rural and ten urban schools were randomly chosen for the intestinal parasite infection prevalence assessment out of 50 schools selected for the LF TAS in three sub-Divisions of the Northern Division.

In the Eastern Division, 21 schools were randomly selected from at least one school of all five sub-Divisions (Kadavu, Lomaiviti, Lakeba, Lotuma, and Lomaloma) of the Division were represented in the list of schools. In the Rotuma sub-Division, students in three other primary schools were also included, as the team had to stay at least a week until they returned to Suva due to the limited flight schedule. This yielded 24 schools altogether out of all 115 primary schools registered in the Eastern Division. All schools in the Eastern Division were classified as rural according to the Ministry of Education (MOE).

Table 6.2 Number of selected schools and estimated sample sizes for the intestinal parasite infection prevalence assessment through LF TAS in 3 Divisions of Fiji (Ministry of Education)

		Western	Central	Northern*	Total
Total number of registered schools		249	203	141	593
LF TAS	Number of schools selected	77	82	50	209
Intestinal parasite infection prevalence assessment	Number of schools sub-sampled	30	20	20	70
	Estimated sample size	1,692	1,479	724	3,895

N.B.: * Schools in the Tavenui sub-Division were not included.

6.2.4. Selection of target age group

The targeted age group for the epidemiological assessment of intestinal parasite infection through LF TAS was all class 1 and 2 students registered in the selected schools, which was identical to that of the LF TAS. This is to minimize any additional logistic burden from having two different target-populations by adding the intestinal parasite infection component. However, for sentinel site surveillance in the Eastern Division, all class 1 to class 8 students of the selected schools were targeted, as long as their parents consented. Since most of the schools are in the remote islands where the access to health services is limited, we believed that it would be beneficial for children to have opportunities that their stools could be

tested. This aimed to enable health authorities to explore if there is any difference in intestinal parasite infection prevalence among different age groups, given that the assessment through LF TAS targeted class 1 and 2 students only.

6.2.5. Survey organization and data collection

For the intestinal parasite infection prevalence assessment through LF TAS, the details of survey organization and data collection for TAS were described in Chapter 4. Briefly, the field team of the Fiji Centre for Communicable Diseases Control (FCCDC) firstly contacted the District Education Offices and local health authorities to explain on the objectives and procedures of the survey. Upon their concurrence, the schools and community members were then informed of the background of the survey through a series of workshops during the pre-visits. Parents were sensitized through school meetings, about the purpose and objectives of the study, and procedures for stool sample collection. For all students in the selected schools, a flyer (Figure 3.9) explaining stool sample collection procedures together with the written consent form including questionnaires and mouth screw-capped stool containers were sent to their home. Parents/guardians were asked to fill the form and to help to collect the stool sample of their child on the designated survey date. Students were instructed to bring the signed consent forms and their stool containers half-filled with their fresh morning stool.

Two teams visited the selected schools for the period of 2-3 weeks in each Division and performed stool sample collection in conjunction with TAS procedures. At schools, the survey team member collected the consent forms and the labelled stool containers with the unique identification number. After completion of the stool samples, all children were offered with albendazole 400mg tablet (GSK, Uxbridge, UK) following the WHO guideline for school deworming (WHO 2011a) by the field team members. Questionnaires were checked for completeness by the field supervisor, and the students were recalled retrieving missing information if there was any. Collected stool samples were transported to the national parasitology reference laboratory of the FCCDC located in Suva by the car, the boat, or the plane, stored in cool boxes daily or on the next available date.

For the sentinel site surveillance in the Eastern Division, consent forms and information flyers were sent before the NPLF field teams' visits by post or the drug delivery channels of the MHMS in advance. This was due to geographical accessibility issues given that most of the selected schools were in very remote locations (Figure 6.1). Then the NPLF team simply collected consent forms and stool samples from the schools during their visits for LF

activities and organized the delivery of stool samples via boats or plains to the national parasitology reference laboratory at the FCCDC for microscopic examination and data entry.

6.2.6. Microscopic examination of specimens

Parasitological diagnosis of intestinal parasite infections was performed using a single Kato-Katz (KK) thick smear prepared from a single day stool sample (41.7 mg of stool per smear) as well as the formol-ether-acetate concentration technique (FEC) for all available samples. If there were not enough stool samples for both tests, then the FEC method was favoured, and the KK smear was waivered. Intestinal parasite infection indicated being positive with the presence of helminths ova either in the KK or FEC examination of each stool examined or with the presence of protozoan cysts via FEC. For the stool samples with positive gastrointestinal protozoan cysts, direct iodine wet preparation was used to enhance the detail of the cysts. Results of the KK examination were expressed as eggs per gram of faeces (epg), and infection intensities were categorized following the WHO guidelines (Table 6.3) (WHO 2011a). Microscopic examination was conducted primarily by the FCCDC laboratory technicians and supervised by technical experts for the quality control purpose by re-examining 5% of slides. For the samples collected in the Eastern Division, FEC was not performed due to human resources shortages around the survey period.

Table 6.3 Classes of intensity for soil-transmitted helminth infections (WHO 2011a)

Parasite	Light-intensity infection	Moderate-intensity infection	Heavy-intensity infection
As. lumbricoides	1–4 999 epg	5 000–49 999 epg	>50 000 epg
T. trichiura	1–999 epg	1 000–9 999 epg	>10 000 epg
Hookworm	1–1 999 epg	2 000–3 999 epg	>4 000 epg

N.B.: epg = eggs per gram of faeces

6.2.7. Data management and statistical analysis

The demographic data from the questionnaires and parasitological examinations were entered into a Microsoft Office Excel spreadsheet 2007 (Microsoft) and double checked by the project officers of the FCCDC for errors or missing values. The dataset from each survey was merged to form one master database to allow analysis across three Divisions for the intestinal parasite infection prevalence assessment through LF TAS. Statistical analyses were conducted with the STATA Release 12 (College Station, TX: StataCorp LP), and STATA svyset command

was used to specify primary sampling unit variables and sampling weight, in reflecting the clustering nature of the data set. Samples were weighted according to the proportion of the sub-Divisional population per each Division (Levy and Lemeshow 2008).

Point prevalence with 95% CI of intestinal parasite infections was calculated for mono (*i.e.* each STH or protozoan species) and dual infections (eggs of two STH or cysts of two protozoan species in one sample), as well as any infection (being positive with ova of any STH species or a cyst of at least one intestinal protozoan species). Point prevalence estimation and its confidence intervals were calculated using a logit transform so that the endpoints lie between 0 and 1, with STATA svy: proportion command. To have 95% CI by the category with respect to each locality, sex, or age groups at the $P<0.05$ significance level, the over option was used in combination with svy: proportion (Richardson 1994), where the regress command was applied and F ratio was obtained via adjusted Wald test to explore whether point prevalence estimates were the same across different categories.

For infection intensity values of STH infections, the geometric mean of Williams was chosen as the measure of central tendency (Alexander 2012). Point prevalence with 95% CI was estimated separately for light intensity infections and moderate or heavy infections of each STH species. Comparison and computation of 95% CIs of the geometric mean of epg were conducted on the logs of the epg values with the eform () option of Stata regress command.

The coordinates of surveyed schools were collected using a handheld GPS device, and the location was estimated on the Google Earth where there was an error. All data were imported into the geographical information systems software ArcGIS version 10.2 (ESRI, Redlands, CA) for mapping and spatial analysis. Firstly, the different prevalence distribution of the infections across the surveyed area via LF TAS was mapped. Secondly, hotspot analysis of the infection prevalence was conducted using ArcGIS 10.2 Spatial Statistics tools (ESRI, Redland, CA). The Getis-Ord Gi* statistic was used to identify the specific locations where high and low prevalence were clustered (Z scores, 95% confidence levels (CI) + 1.96 and - 1.96 standard deviations) rather than being a random. In addition, the kernel density estimation (KDE) method, a nonparametric way of estimating a probability surface using a Gaussian probability density function, was used to create a continuous surface representing the high to low prevalence distributions of intestinal parasite infections.

6.3. Results

From the period of February 2014 to August 2015, a nation-wide cross-sectional intestinal parasite infection prevalence assessment was carried out in four Divisions of Fiji.

6.3.1. Demographic characteristic of the study population

6.3.1.1. Intestinal parasite infection prevalence assessment through LF TAS

In total, 53.2 % (1,890/3,551) of class 1 and 2 students who were enrolled in 69 schools (30 Western, 19 Central, and 20 Northern) out of 70 targeted, excluding one special school for children with disabilities in the Central Division, participated in the assessment through LF TAS. Altogether 1,839 stool samples were available out of 1,890 students for microscopic examination using KK and/or FEC. The age distribution of the participants was between 4 to 10 years old, and most of them (93.4%) were either 6 or 7 years old. Table 6.4 summarizes the demographic characteristics of the study participants. The results of molecular analysis on the archived stool samples from the Western Division are presented separately in Chapter 8.

Table 6.4 Demographic characteristics of the study participants in 3 Divisions of Fiji, intestinal parasite infection prevalence assessment through LF TAS in 2014-2015

Characteristics	Western Division (n=932)	Central Division (n=553)	Northern Division** (n=405)	All* (n=1,890)
Response rate (%)	74.5	64.5	61.6	68.3
% Distribution by the current age (years)				
4 or 5	4.5	0.4	0.5	2.6
6	50.0	45.2	46.0	47.9
7	42.0	49.0	50.1	45.5
8, 9, or 10	3.5	5.4	3.4	4.0
% Class 1	50.5	49.1	50.8	50.1
% Female	49.5	48.4	50.3	49.3

N.B.: * Weighted based on the proportion of sub-Divisional per Divisional population sizes; and **: Excluding Taveuni sub-Division.

6.3.1.2. Sentinel site surveillance in the Eastern Division

In total, the stool samples of 927 primary school students (430 females and 497 males) in 24 schools were available for microscopic examination via KK, whose median age was ten years (Interquartile range: 8-12 years). Table 6.5 summarizes the demographic characteristics of the study participants in the Eastern Division.

Table 6.5 Demographic characteristics of the study participants in the Eastern Division of Fiji, sentinel site surveillance in 2015

Characteristics		All* (n=927)
Response rate (%)		49.1
% Distribution by current age (years)		
	6 or 7	21.7
	8 or 9	27.3
	10 or 11	22.1
	12 and more	28.9
% Female		46.4

N.B.: *Weighted based on the proportion of sub-Divisional per Divisional population sizes

6.3.2. Geographical distribution of intestinal parasite infections in Fiji

6.3.2.1. Intestinal parasite infection prevalence assessment through LF TAS

By coproscopy, overall 12.4% of children were found to be infected either with *Ascaris* (8.7%, 95% CI 4.3-16.6%) or hookworm (3.8%, 95% CI 2.2-6.5%), while no *Trichuris* infection was found (Table 6.6a). Infections with *Ascaris* showed the highest prevalence up to 18.2% among children in the Central Division, while hookworm infections were most prevalent (12.8%) among children in the Northern Division. Dual STH infections with *Ascaris* and hookworm were found in 0.6% of children based on microscopic findings and did not exist in the Central Division (Table 6.6a).

Table 6.6a Prevalence and intensity of STH infections by species in the Western, Central, and Northern Divisions of Fiji, 2014-2015.

	Western Division (n=914)	Central Division (n=526)	Northern Division (n=399)	*P*-value**	All* (n=1,839)
Any STH infection					
Prevalence (%) (95% CI)	5.9 (3.7-9.3)	20.7 (8.7-41.4)	16.8 (10.4-25.8)	**0.0172**	12.1 (7.3-19.4)
Mono STH infection					
Ascaris					
Prevalence (%) (95% CI)	4.3 (2.8-6.7)	18.2 (6.7-40.6)	5.1 (3.3-7.9)	**0.0061**	8.7 (4.3-16.6)
Light intensity infection (%) (95% CI)	2.5 (1.5-4.3)	9.6 (3.7-23.0)	3.1 (1.6-6.0)	**0.0014**	4.8 (2.4-9.2)
Moderate/heavy intensity infection (%) (95% CI)	1.3 (0.6-2.7)	4.2 (0.9-17.1)	1.7 (0.7-3.9)	0.1787	2.2 0.9-5.5)
Geometric mean eggs per gram (95% CI)	1382.2 (611.1-3126.3)	3528.3 (1929.6-6451.6)	832.3 (302.2-2292.1)	0.410	1480.4 (633.2-3456.8)
Range of school level prevalence (%)	0.0-66.7	0.0-63.0	0.0-16.7	-	0.0-66.7
Hookworm					
Prevalence (%) (95% CI)	2.1 (0.7-6.1)	2.5 (1.1-5.8)	12.8 (7.3-21.7)	**0.0004**	3.9 (2.3-6.6)
Light intensity infection (%) (95% CI)	1.3 (0.4-4.9)	1.1 (0.3-3.7)	1.9 (0.8-4.5)	0.8060	1.3 0.6-3.0)
Moderate/heavy intensity infection (%) (95% CI)	0.0	0.0	0.0	-	0.0
Geometric mean eggs per gram (95% CI)	145.5 (19.1-1109.2)	-	24	-	104.6 (65.1-168.1)
Range of school level prevalence (%)	0.0-25.0	0.0-22.2	0.0-26.3	-	0.0-26.3
Dual STH infection					
Ascaris and hookworm					
Prevalence (%) (95% CI)	0.7 (0.2-2.1)	0.0	1.2 (0.6-2.5)	0.1666	0.6 (0.2-1.3)

N.B.: ***** Weighted based on the proportion of sub-Divisional per Divisional population sizes; ** *P* value was for the null hypothesis whether the point prevalence estimates are the same across three division

For the stool samples whose egg counts were available, light intensity *Ascaris* infections were more prevalent (4.8%, 95% CI 2.4-9.2%) than moderate or high-intensity infections (2.2%, 95% CI 0.9-5.5%). As for hookworm infections, there were no moderate or high-intensity infections but light intensity infections (1.3%, 95% CI 0.6-3.0%) only for the cases whose egg counts of *Ascaris* were available. The divisional geometric mean of eggs per gram was below 5,000 for *Ascaris* infections and 2,000 for hookworm infections in all three Divisions (Table 6.6a).

Table 6.6b Prevalence of intestinal protozoan infections* by species in the Western, Central, and Northern Divisions of Fiji, 2014-2015.

	Western Division (n=915)	Central Division (n=491)	Northern Division (n=394)	*P*-value	All* (n=1,839)
Any protozoa					
Prevalence (%)	4.8	3.8	5.7	0.7699	4.7
(95% CI)	(2.6-8.6)	(1.4-9.9)	(3.1-10.3)		(3.0-7.1)
Mono protozoa					
Giardia spp.					
Prevalence (%)	2.0	0.7	1.2	0.2671	1.5
(95% CI)	(1.0-4.2)	(0.1-3.6)	(0.5-2.8)		(0.8-2.8)
Entamoeba histolytica/dispar					
Prevalence (%)	1.0	0.0	0.0	0.6794	0.6
(95% CI)	(0.4-3.0)				(0.2-1.7)
Entamoeba coli					
Prevalence (%)	1.6	3.8	4.7	0.1230	2.7
(95% CI)	(0.8-3.0)	(1.4-9.9)	(2.5-8.8)		(1.6-4.4)
Others**					
Prevalence (%)	0.4	0.0	0.0	0.8083	0.2
(95% CI)	(0.1-1.6)				(0.1-0.9)
Dual protozoa					
Giardia spp. and *E. coli*					
Prevalence (%)	0.1	0.6	0.1	**0.0311**	0.2
(95% CI)	(0.0-0.4)	(0.1-3.8)	(0.0-0.9)		(0.0-1.1)
E. coli and others					
Prevalence (%)	0.3	0.0	0.0	0.8510	0.1
(95% CI)	(0.0-1.7)				(0.0-1.0)

N.B: *: Diagnosis was based on FEC technique; **: Other include *Iodamoeba buetschlii* (0.1%) and *Blastocystis hominis*

Based on FEC, overall 4.7% of the examined stool samples were positive for any protozoan cyst and 2.0 % of the stool samples examined were identified as having *Giardia* spp. cysts (Table 6.6b). Other protozoa species discovered were *Entamoeba coli*, (2.7%), *Entamoeba histolytica/dispar* (0.6%), *Iodamoeba buetschlii* (0.1%) and *Blastocystis hominis* (0.1%), singly or in combination with other species. Out of 915 children whose stool samples were examined in the Western Division, the overall prevalence of any protozoan infection was 4.8% (95% CI 2.6-8.6%) while it was 3.8% (95% CI 1.4-9.9%) and 5.7% (95% CI 3.1-10.3%) in the Central and Northern Division. As per *Giardia* spp. infection prevalence, it was 2.0% (95% CI: 1.0-4.2%) in the Western, while it was lower to 0.7% (95% CI: 0.1-3.6%) and 1.2% (95% CI: 0.5-2.8%) in the Central/Northern. No significant statistical difference regarding any protozoan or *Giardia* spp. infection prevalence levels across Divisions was observed (Table 6.6b).

Regarding any STH infection prevalence at the sub-Divisional level, in more than half (8/14) of sub-Divisions estimated any STH infection prevalence levels were less than 10%, whereas in another 5 sub-Divisions the estimates were within 10-20% and in only one within 20-50% ranges (Table 6.7). Any STH infection prevalence levels were different across 14 sub-Divisions with statistical significance (P=0.0014) with the highest in the Nataisiri sub-Division of the Central Division up to 38.2%, followed by Cakaudrove (19.1%) of the Northern and Suva in the Central (Figure 6.2 and 6.3).

As for any moderate or heavy STH infection intensities at sub-Divisional level, more than half (9/14) of sub-Divisions had point estimates lower than 1% (Table 6.7), which is the elimination goal of STH infections as a public health problem (WHO 2012a). The Suva sub-Division (rank 12 out of 14) of the Central Division (Figure 6.4) had the highest point prevalence of any moderate or heavy intensity STH infection, followed by the Naitasiri in the Central (rank 14) and the Caukadrove (rank 13) in the Northern, but estimated point prevalence levels were not statistically significant across 14 sub-Divisions (P=0.8759) (Table 6.7).

Table 6.7 Estimated sub-Divisional any STH infection prevalence and moderate/heavy intensity infection prevalence in the Western, Central and Northern Divisions, excluding the Taveuni sub-Division, of Fiji, 2014-2015

Rank	Sub-Division	Any STH infection			Any STH moderate/heavy intensity infection	
		Prevalence (%)	95% CI	School prevalence range	Prevalence (%)	95% CI
1	Ba	0.0	-	-	0.0	-
2	Nadi	1.8	0.7-4.6	0.0-6.7	0.0	-
3	Rewa	3.8	2.1-6.5	0.0-14.3	0.4	0.0-3.1
4	Tavua	4.6	-	4.6	0.0	-
5	Ra	4.6	2.9-7.4	0.0-12.5	0.7	0.1-4.2
6	Lautoka/ Yasawa	6.7	2.3-17.5	0.0-66.7	3.5	0.9-12.3
7	Macuata	9.0	5.4-14.6	0.0-15.4	0.0	-
8	Tailevu	9.1	-	9.1	0.0	-
9	Nadroga/ Navosa	11.1	5.5-21.1	2.2-25.0	2.0	0.8-4.8
10	Navua	14.3	2.4-53.6	0.0-33.3	0.0	-
11	Bua	16.8	7.4-33.7	0.0-30.0	0.7	0.1-5.3
12	Suva	17.2	6.5-38.3	0.0-32.3	8.6	2.5-25.6
13	Cakaudrove	19.1	13.3-26.8	10.3-29.0	3.7	1.7-7.8
14	Naitasiri	38.2	17.8-63.8	5.3-63.0	7.9	1.4-33.6
	All*	12.1	7.3-19.4	0.0-66.7	2.2	0.9-5.5

N.B.: *Weighted based on the proportion of sub-Divisional per Divisional population sizes

Figure 6.2 Distribution of sub-Divisional any STH infection prevalence by its rank in the Western, Central and Northern Divisions except the Taveuni sub-Division, of Fiji, 2014-2015

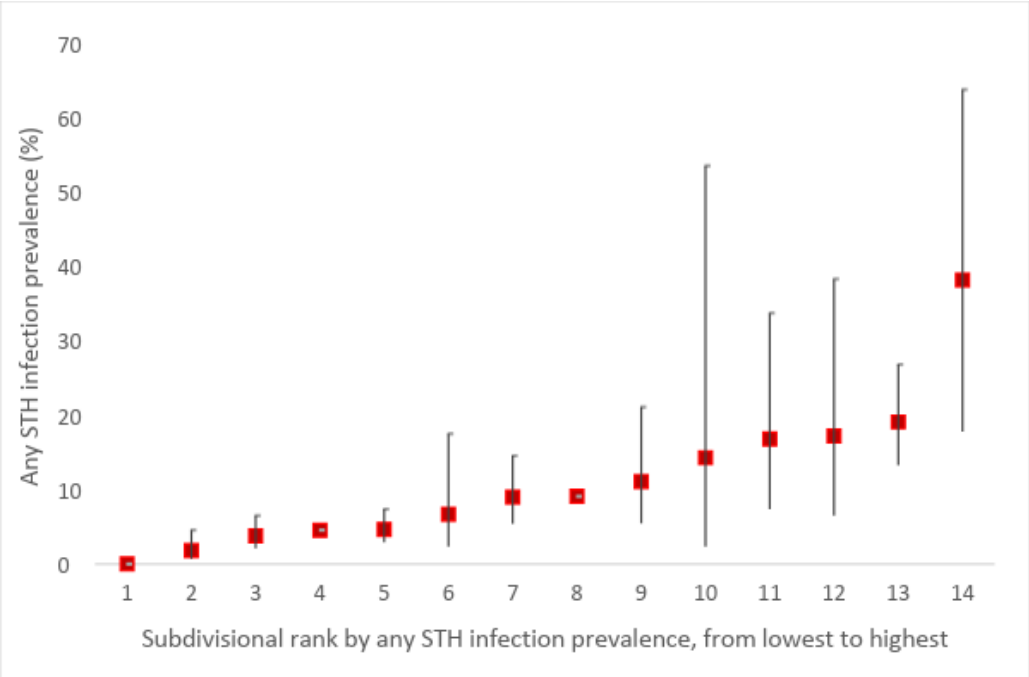


Figure 6.3 Sketch map of sub-Divisional any STH infection prevalence in the Western, Central and Northern Divisions except the Taveuni sub-Division of Fiji, 2014-2015

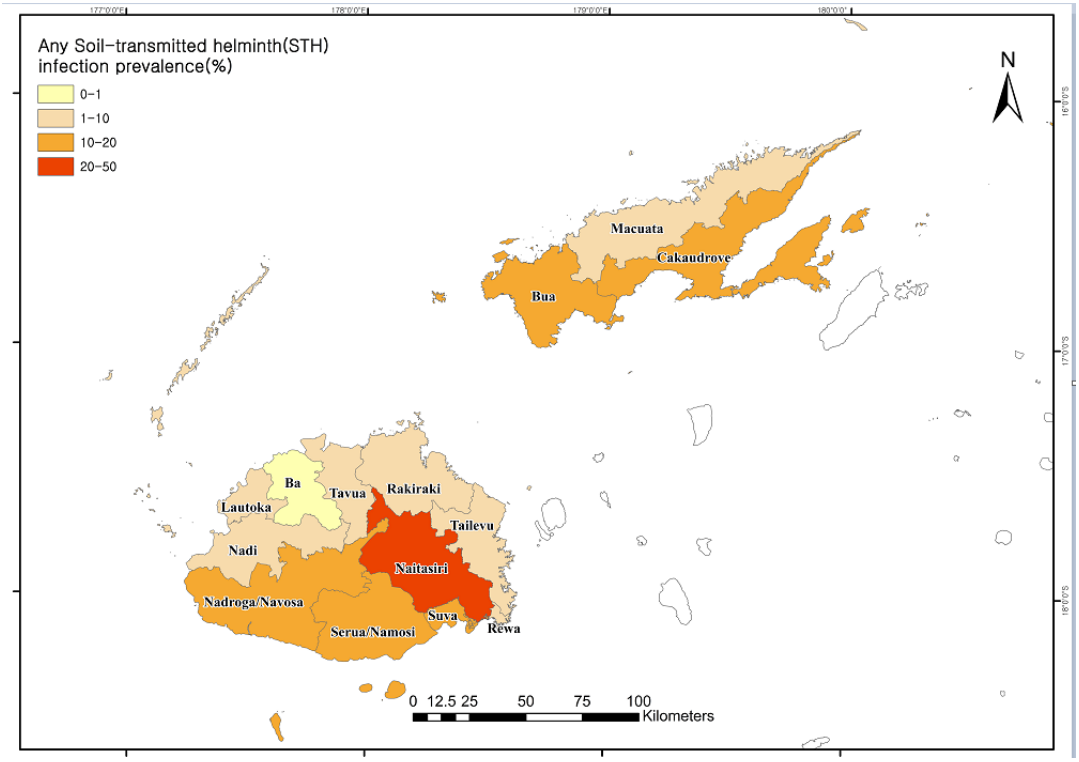
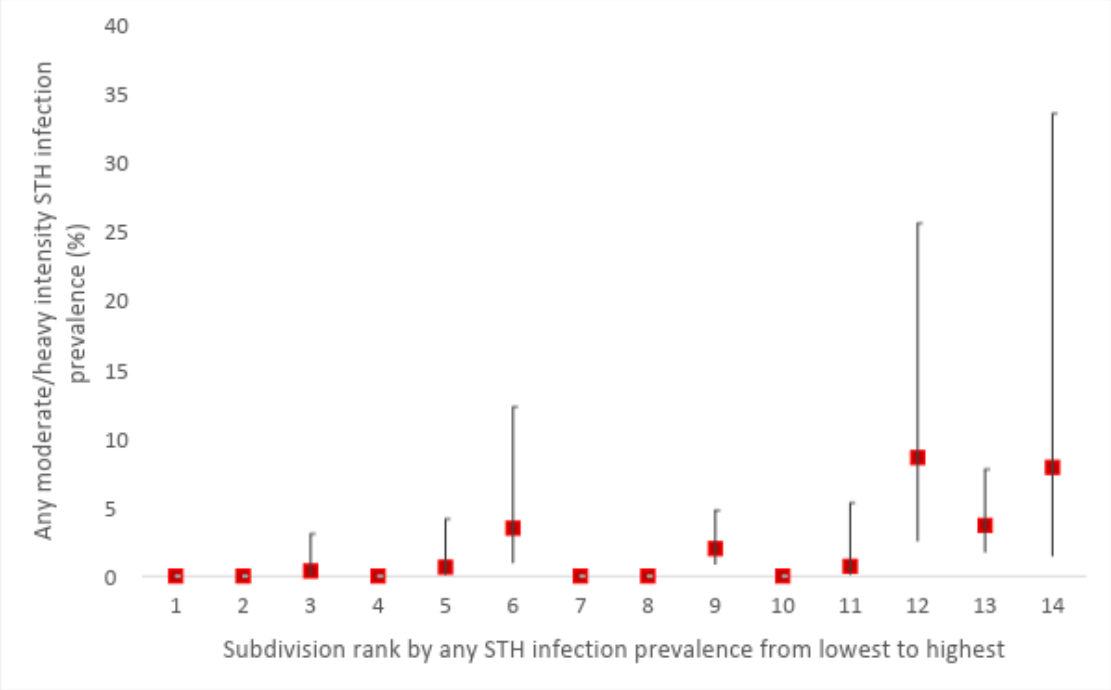


Figure 6.4 Distribution of sub-Divisional any moderate/heavy STH infection prevalence by its rank in the Western, Central and Northern Divisions except the Taveuni sub-Division, of Fiji, 2014-2015



Those three sub-Divisions which had the highest ranking for any STH infection prevalence (rank 12, 13, and 14) also showed top3 high any moderate or heavy STH infection prevalence (Figure 6.4). Also, most of the sub-Divisions with any STH infection prevalence lower than 10% (rank 1-8) showed any moderate or heavy intensity STH infection prevalence less than 1% (Figure 6.4). However, there was some exception, such as the Lautoka/Yasawa sub-Division, which had 3.5% of any moderate or heavy intensity STH infection prevalence (95% CI: 0.9-12.3), while its any STH infection prevalence was less than 10%. Similarly, the Bua sub-Division's any STH infection prevalence was fourth highest (rank 11), but its estimated moderate or heavy intensity infection point prevalence was less than 1% (Table 6.7).

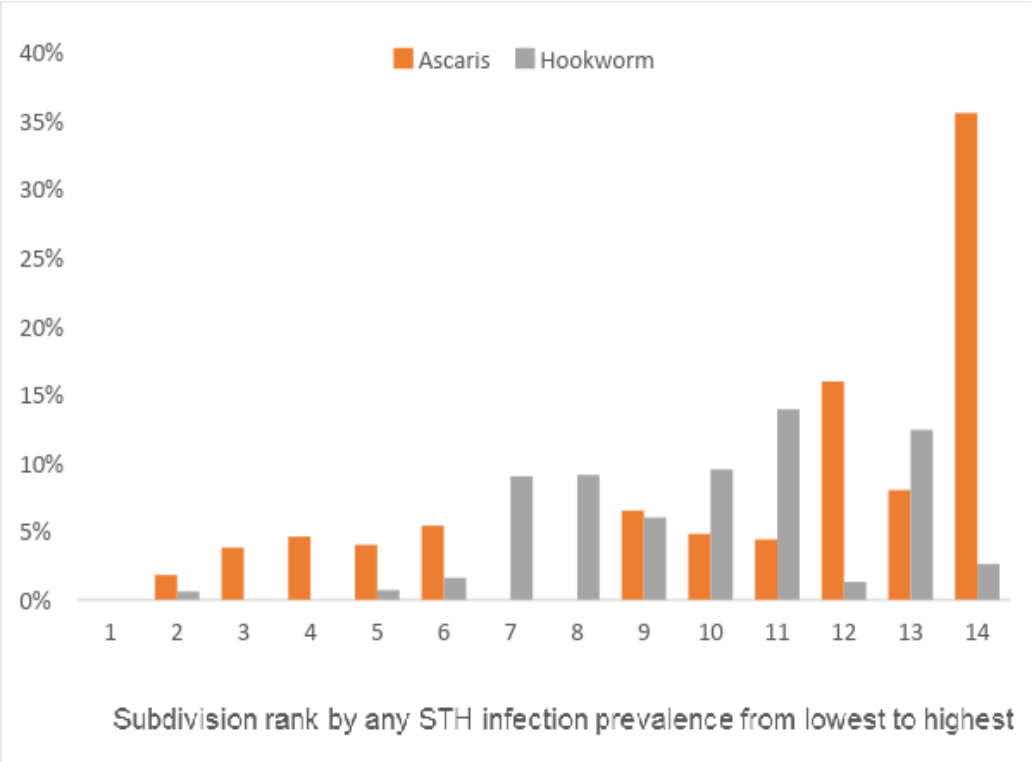
As for distribution of STH infections by the species, *Ascaris* infection prevalence was the highest in the Naitasiri sub-Division, followed by Suva (Table 6.8), and was different across the sub-Divisions with statistical significance (P=0.0093). Hookworm infection prevalence was the highest in the Bua sub-Division and followed by the Cakaudrove, but there was no statistical significance by sub-Divisions (P=0.4328). Distribution of *Ascaris* and hookworm infection prevalence in each sub-Division by its any STH infection prevalence rank, from lowest to highest, is presented in Figure 6.5.

Table 6.8 Sub-Divisional *Ascaris* and hookworm infection prevalence in the Western, Central and Northern Divisions except the Taveuni sub-Division of Fiji, 2014-2015

Rank	Sub-Divisions	*Ascaris* infection		Hookworm infection	
		Prevalence (%)	95% CI	Prevalence (%)	95% CI
1	Ba	0.0	-	0.0	-
2	Nadi	1.8	0.7-4.6	0.6	0.1-2.6
3	Rewa	3.8	2.1-6.5	0.0	-
4	Tavua	4.6	-	0.0	-
5	Ra	4.0	2.5-6.2	0.7	0.1-3.9
6	Lautoka/Yasawa	5.4	1.7-15.5	1.6	0.5-5.1
7	Macuata	0.0	-	9.0	5.4-14.6
8	Tailevu	0.0	-	9.1	-
9	Nad/Nav	6.5	2.9-14.3	6.0	1.6-20.8
10	Navua	4.8	0.9-22.2	9.5	1.7-39.7
11	Bua	4.4	2.3-8.3	13.9	5.8-29.7
12	Suva	15.9	6.3-34.8	1.3	0.3-5.0
13	Cakaudrove	8.0	4.7-13.4	12.4	6.6-22.1
14	Naitasiri	35.5	14.4-64.3	2.6	1.0-6.5
	All*	8.7	4.3-16.6	3.9	2.3-6.6

N.B.: * Weighted based on the proportion of sub-Divisional per Divisional population sizes

Figure 6.5 Distribution of sub-Divisional *Ascaris*/hookworm infection prevalence by its any STH infection prevalence rank in the Western, Central and Northern Divisions except Taveuni sub-Division of Fiji, 2014-2015



6.3.2.2. STH sentinel site surveillance in the Eastern Division

Overall Divisional *Ascaris* infection prevalence was 8.7% (95% CI: 3.6-19.6%) in the Eastern Division (Table 6.9). There was no statistically significant difference in *Ascaris* infection prevalence between male (9.2%, 95% CI: 3.9-20.4%) and female students (8.1%, 95% CI: 3.0-19.9%). When the age groups were considered, *Ascaris* infection prevalence was not the same across the age quartiles, having 13.5% in the first quartile (95% CI 6.2-26.8%) and 5.7% in the fourth (95% CI 1.7-17.4%) with statistical significance (P=0.0228) (Table 6.9 and Figure 6.6).

Table 6.9 Prevalence and infection intensity of STH infections by sex and age group in the Eastern Divisions of Fiji, 2015

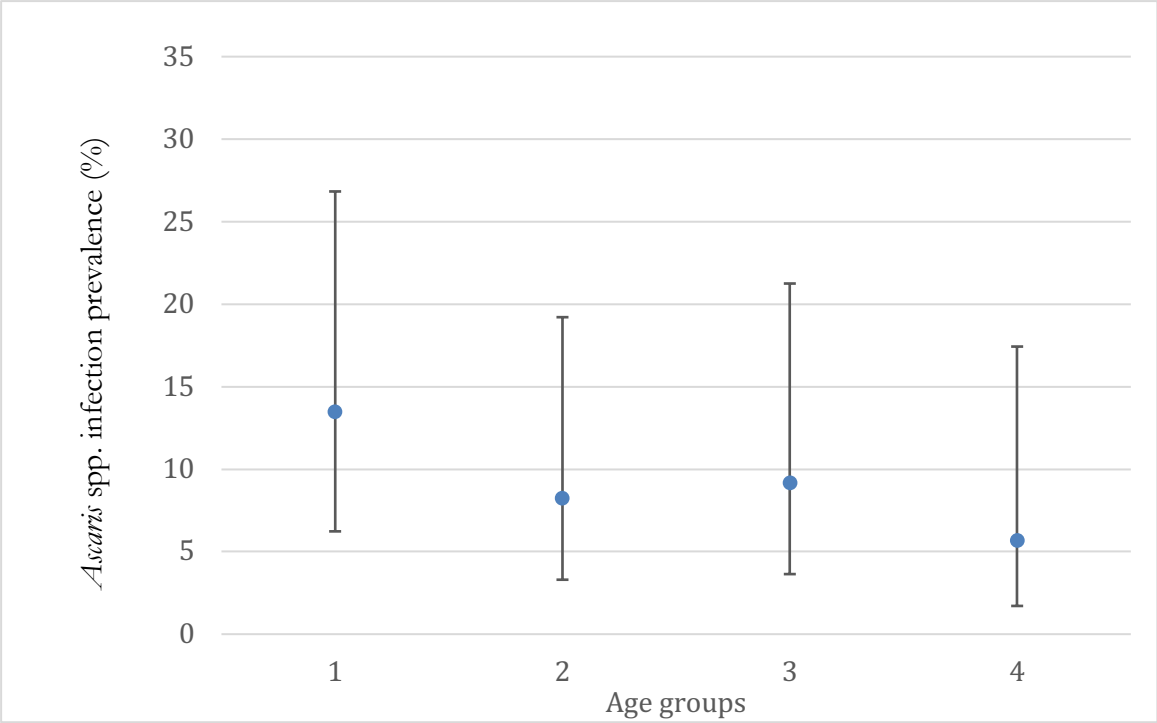
	All*	Sex			Age quantiles				
		Male	Female	*P*	Age group 1 6-7 years	Age group 2 8-9 years	Age group 3 10-11 years	Age group 4 ≥12 years	*P*
	(n=927)	(n=497)	(n=430)		(n=201)	(n=253)	(n=205)	(n=268)	
Ascaris infection									
Prevalence (%) (95% CI)	8.7 (3.6-19.6)	9.2 (3.9-20.4)	8.1 (3.0-19.9)	0.5471	13.5 (6.2-26.8)	8.3 (3.3-19.2)	9.2 (3.6-21.3)	5.7 (1.7-17.4)	**0.0228**
Light intensity infection (%) (95% CI)	5.3 (2.3-11.7)	5.7 (2.5-12.6)	4.9 (1.9-11.9)	0.5816	8.1 (3.8-16.6)	5.0 (2.1-11.4)	5.8 (2.5-13.0)	3.6 (1.0-11.5)	0.1278
Moderate/heavy intensity infection (%) (95% CI)	3.3 (1.0-10.5)	3.4 (1.1-9.5)	3.2 (0.8-12.1)	0.8491	5.4 (2.1-13.2)	3.0 (0.0-11.3)	3.4 (1.0-10.8)	2.1 (0.4-11.0)	0.1388
Geometric mean epg	2155 (913.2-5030.3)	1997.9 (1007.3-3962.6)	2377.3 (811.7-6962.9)	0.575	2586 (1589.5-4207.4)	1697.1 (564.0-5107.2)	1749.4 (451.9-6772.6)	2749.1 (930.6-8121.8)	0.997
Hookworm infection									
Prevalence (%) (95% CI)	0.5 (0.2-1.4)	0.4 (0.1-1.3)	0.5 (0.1-1.9)	0.6244	0.8 (0.2-3.0)	0.3 (0.0.-2.2)	0.8 (0.2 -2.9)	0.2 (0.0-1.4)	0.194

Continued

	All*	Sex			Age quantiles				
		Male	Female	*P*	Age group 1 6-7 years	Age group 2 8-9 years	Age group 3 10-11 years	Age group 4 ≥12 years	*P*
	(n=927)	(n=497)	(n=927)		(n=201)	(n=253)	(n=205)	(n=268)	
Light infection (%) (95% C	0.5 (0.2-1.4)	0.4 (0.1-1.3)	0.5 (0.1-1.9)	0.624 4	0.8 (0.2-3.0)	0.3 (0.0.-2.2)	0.8 (0.2 -2.9)	0.2 (0.0-1.4)	0.194
Geometric mean epg	64.5 (36.1-92.8)	73.7 (54.2-0.2)	47.8 (28.0-81.6)	0.262	40.2 (22.2-72.7)	72	83.4 (63.5-109.5)	48	0.385
Trichuris infection									
Prevalence (%) (95% CI)	0.1 (0.0-0.4)	0.0 (0.0-0.8)	-	-	-	-	0.2 (0.0-2.0)	-	-
Light infection (%) (95% CI)	0.1 (0.0-0.4)	0.0 (0.0-0.8)	-	-	-	-	0.2 (0.0-2.0)	-	-
Geometric mean epg	24	24	-	-	-	-	24	-	-

N.B.: *Weighted based on the proportion of sub-Divisional per Divisional population sizes

Figure 6.6 *Ascaris* spp. infection prevalence by age groups in the Eastern Division of Fiji, 2015



N.B.: Age groups 1, 2, 3, and 4 follows the description of Table 6.9.

As per the infection intensity, more than half of *Ascaris* infected cases were light-intensity infections, and both of low-intensity or moderate to high-intensity *Ascaris* infection prevalence was higher in the first age quantile (Table 6.9). However, neither point prevalence levels of low-intensity nor moderate to high-intensity *Ascaris* infection statistically differ across gender or age quantiles. The geometric mean of *Ascaris* eggs per gram was 2155.0 (95% CI: 913.2-5030.3%), which was not different across gender or age quantiles categories.

Every identified hookworm infection case was also positive for *Ascaris* eggs, yielding Divisional hookworm infection prevalence of 0.5% (95% CI: 0.2-1.4%) (Table 6.9). A single case of *Trichuris* infection was discovered in one of the eighth hookworm cases with a Divisional prevalence of 0.1% (95% CI: 0.0-0.4%). There was no statistically significant difference in estimated hookworm infection point prevalence across gender. When the age groups were considered, hookworm infection prevalence was higher in the first and third quartiles than in the second and fourth quartiles, but prevalence levels were not different across the age quartile with no statistical significance (P=0.194). All of hookworm and *Trichuris* infections were low-intensity infections, and there was no moderate or heavy-intensity infection found. The geometric mean of epg was 64.5 (95% CI: 36.1-92.8%) for hookworm and 24.0 for *Trichuris*,

respectively (Table 6.9). There was no statistically significant difference for geometric mean of hookworm epg across gender or age quantiles.

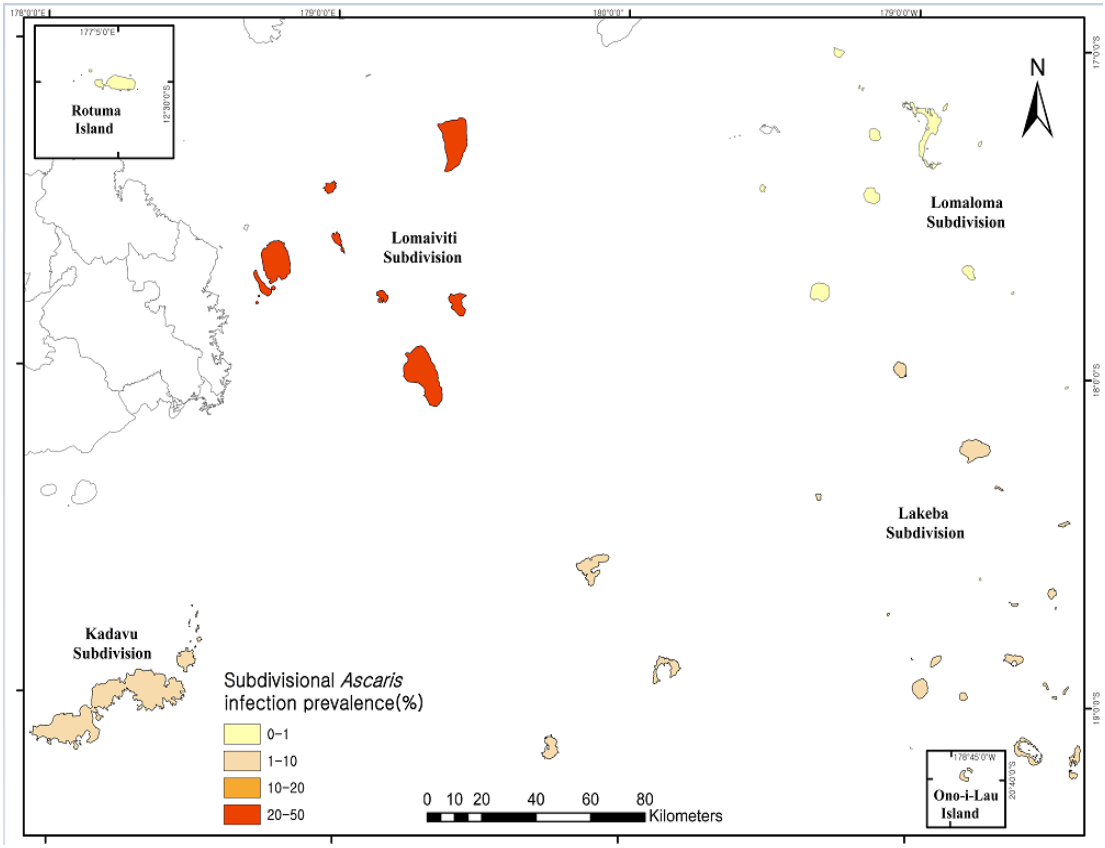
Table 6.10 Estimated sub-Divisional any STH infection prevalence and moderate/heavy intensity infection prevalence in the Eastern Division of Fiji, 2015

Rank	Sub-Division	Any STH infection			Any STH moderate/heavy intensity infection	
		Prevalence	95% CI	School level prevalence range	Prevalence	95% CI
1	Lomaiviti	27.0%	13.2-47.2	0.0-94.9	11.7%	3.9-30.0
2	Kadavu	8.2%	4.9-13.5	5.3-13.6	0.0%	-
3	Lakeba	7.9%	7.4-8.8	7.5-8.2	2.3%	0.6-8.3
4	Lomaloma	0.0%	-	-	0.0%	-
5	Rotuma	0.0%	-	-	0.0%	-
	All*	8.7%	3.6-19.6	0-94.9	3.3%	1.0-10.5

N.B.: *Weighted based on the proportion of sub-Divisional per Divisional population sizes

Regarding any STH infection prevalence at sub-Divisional level of the Eastern Division, in the majority (4/5) of sub-Divisions, the estimated any STH infection prevalence levels were less than 10%, whereas in remaining one sub-Division, the estimate was within 20-50% ranges (Table 6.10). Any STH infection prevalence levels were different across sub-Divisions with statistical significance (P=0.0019) with the highest in the Lomaiviti sub-Division up to 27.0%, followed by Kadavu (8.2%) and Lakeba (7.9%) (Figure 6.7). As for any moderate or heavy STH infection intensities at sub-Divisional level, more than half (3/5) of sub-Divisions had point estimates equal to 0% (Table 6.10), achieving the elimination goal of STH infections as a public health problem (WHO 2012a). The Lomaiviti sub-Division (rank 1 out of 5) had the highest point prevalence of any moderate or heavy intensity STH infection, followed by the Lakeba, but estimated prevalence levels were not different across sub-Divisions (P=0.2948).

Figure 6.7 Sketch map of sub-Divisional any STH infection prevalence in the Eastern Division of Fiji, 2015



6.3.3. School-level intestinal parasite infections epidemiology on Fiji and their spatial analysis

6.3.3.1. Divisions surveyed via TAS

Geographical distribution of STH infections by the species showed that both *Ascaris* (Figure 6.8a) and hookworm infections (Figure 6.8b) were widespread in all three Divisions surveyed via LF TAS. The range of *Ascaris* infection prevalence at school level varied more widely in the two Divisions of Viti Levu (0.0-66.7% and 0.0-63.0% respectively) than that of Vanua Levu (0.0-16.7%). The proportion of schools with a low *Ascaris* prevalence (<10%) was 86.7 % and 85% for the Western and Northern Division respectively, while it was 63.2% in the Central Division. For hookworm infections, the range of school-level prevalence (0.0-26.3%) did not have much difference per Division. However, more than half of the schools (15/29) with hookworm-infected cases were clustered in the Northern Division (Figure 6.8b). Also, out of 11 schools with higher than 10% of hookworm infection prevalence, eight schools were in the Northern Division (Figure 6.8b). Both of the infection prevalence levels were over-dispersed with frequent zeros.

Figure 6.8a Sketch map of 69 schools and their school level *Ascaris* infection prevalence in the Western, Central, and Northern Divisions, excluding the Taveuni sub-Division of Fiji, 2014 and 2015

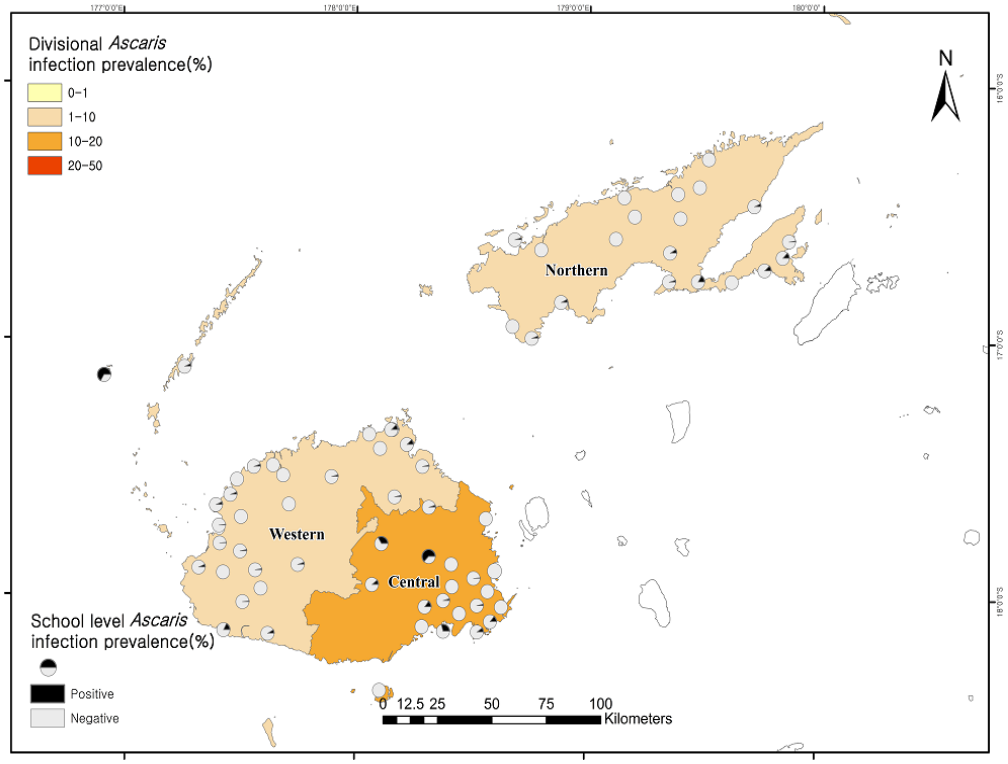


Figure 6.8b Sketch map of 69 schools and their school level hookworm infection prevalence in the Western, Central, and Northern Divisions, excluding the Taveuni sub-Division of Fiji, 2014 and 2015

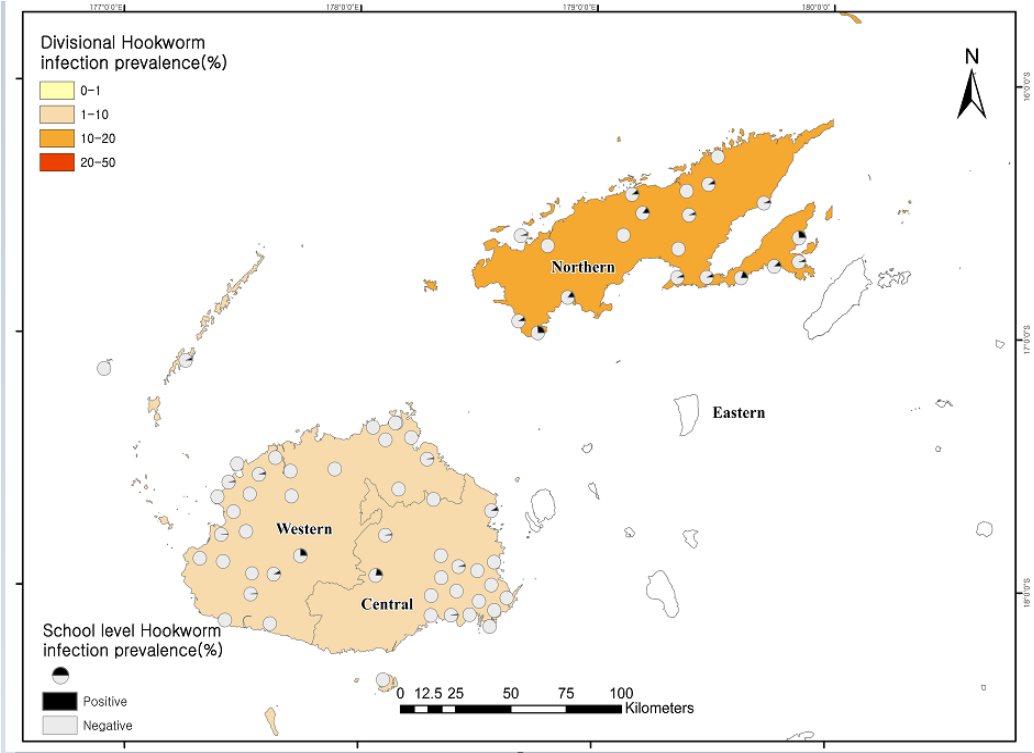


Figure 6.9a Distribution of school level any STH infection and any moderate to heavy intensity STH infection prevalence, by any STH infection prevalence rank from the lowest to highest, in the Western, Central and Northern Divisions of Fiji, 2014-2015

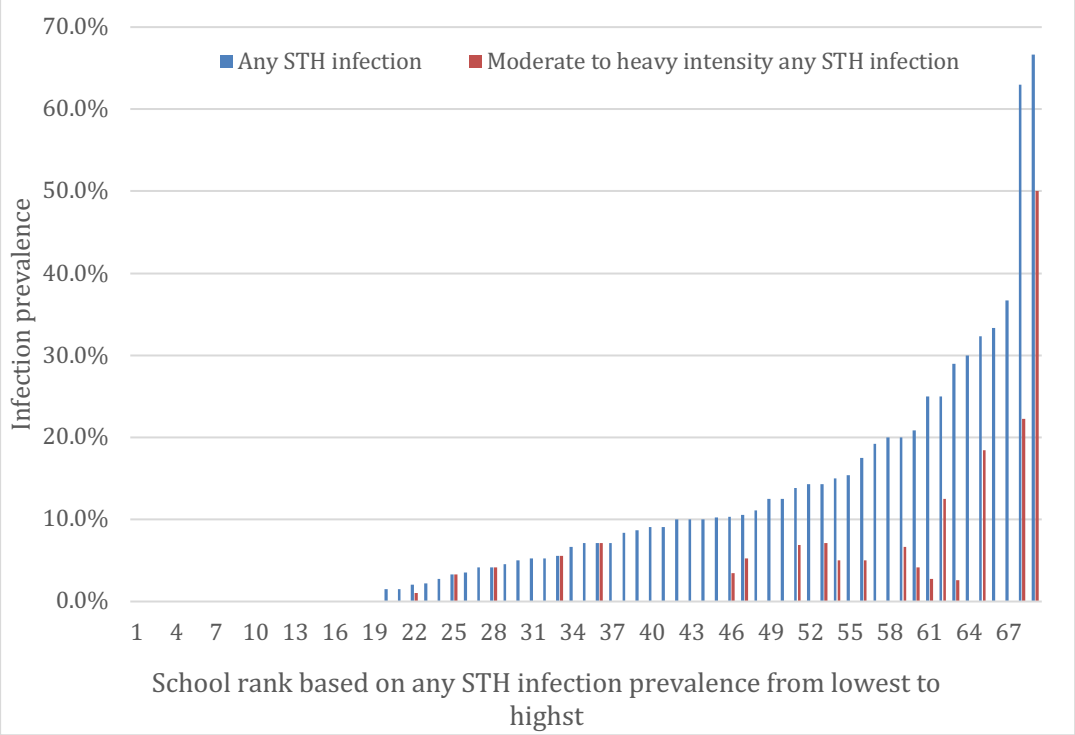
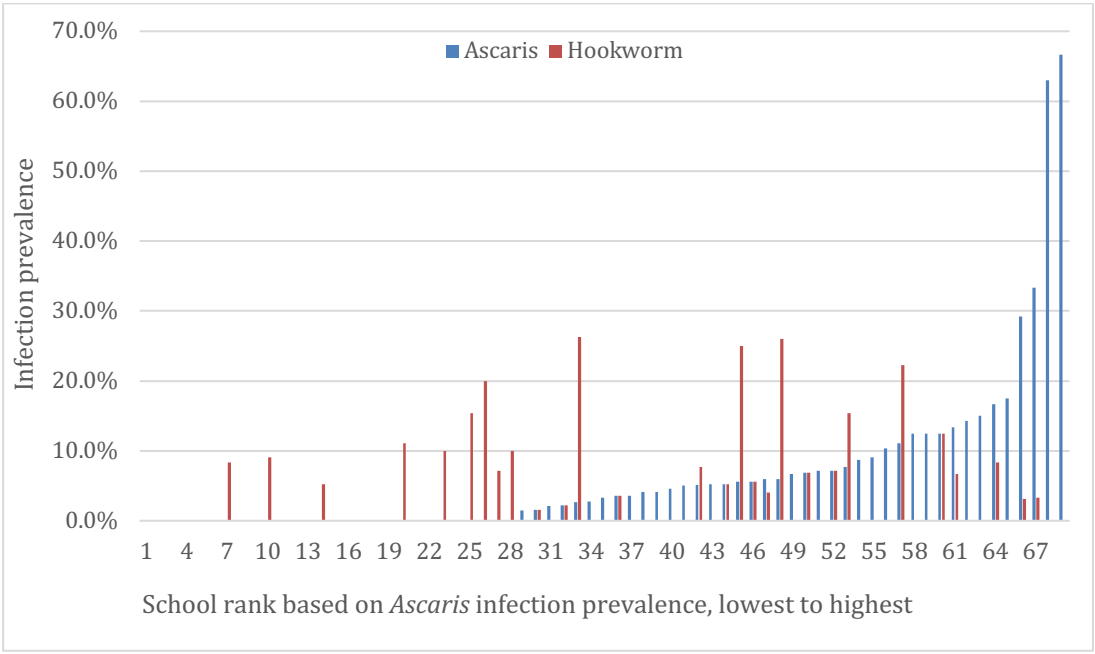


Figure 6.9b Distribution of school-level *Ascaris* and hookworm infection prevalence, by *Ascaris* infection prevalence rank from the lowest to highest, in the Western, Central and Northern Divisions of Fiji, 2014-2015.



As per school level any STH infection prevalence, large variations were observed across the surveyed schools, showing 0-10% in 41 schools, 10-20% in 16 schools, 20-50% in 10 schools, and >50% in 2 schools (Figure 6.9a). Considering infection intensities, the majority (50/69) of schools had achieved the elimination goal of any moderate to heavy intensity STH infection prevalence less than 1% (Figure 6.9b). However, there were two schools with any moderate to heavy intensity STH infection prevalence between 20% and 50%, and even among 41 schools with lower than 10% any STH infection prevalence, there were schools with any moderate and heavy intensity STH infection prevalence higher than 1%.

School level prevalence of *Giardia* spp. infections ranged from 0 to 15.6%, and up to half (46.7%) out of 30 schools in the Western Division had *Giardia*-infected cases (Figure 6.10).

Figure 6.10 Sketch map of 69 schools and their school level *Giardia* spp. infection prevalence in the Western, Central, and Northern Divisions, excluding the Taveuni sub-Division of Fiji, 2014 and 2015

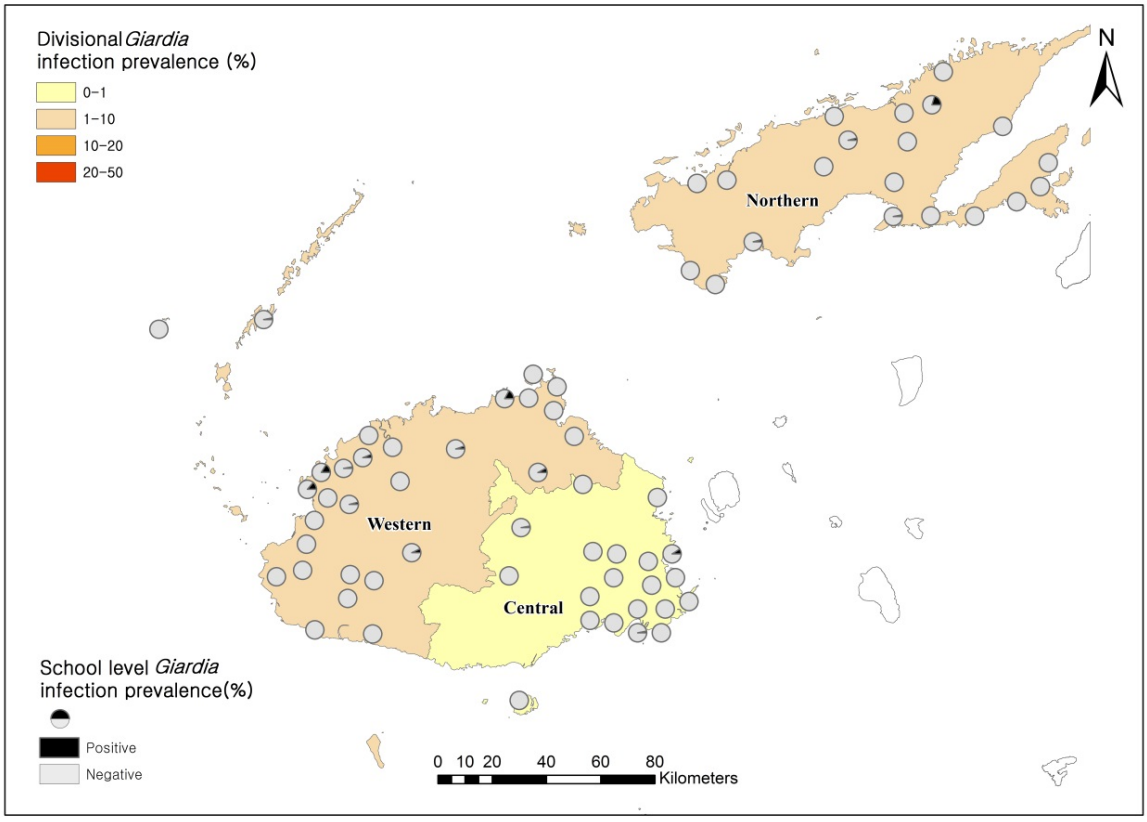
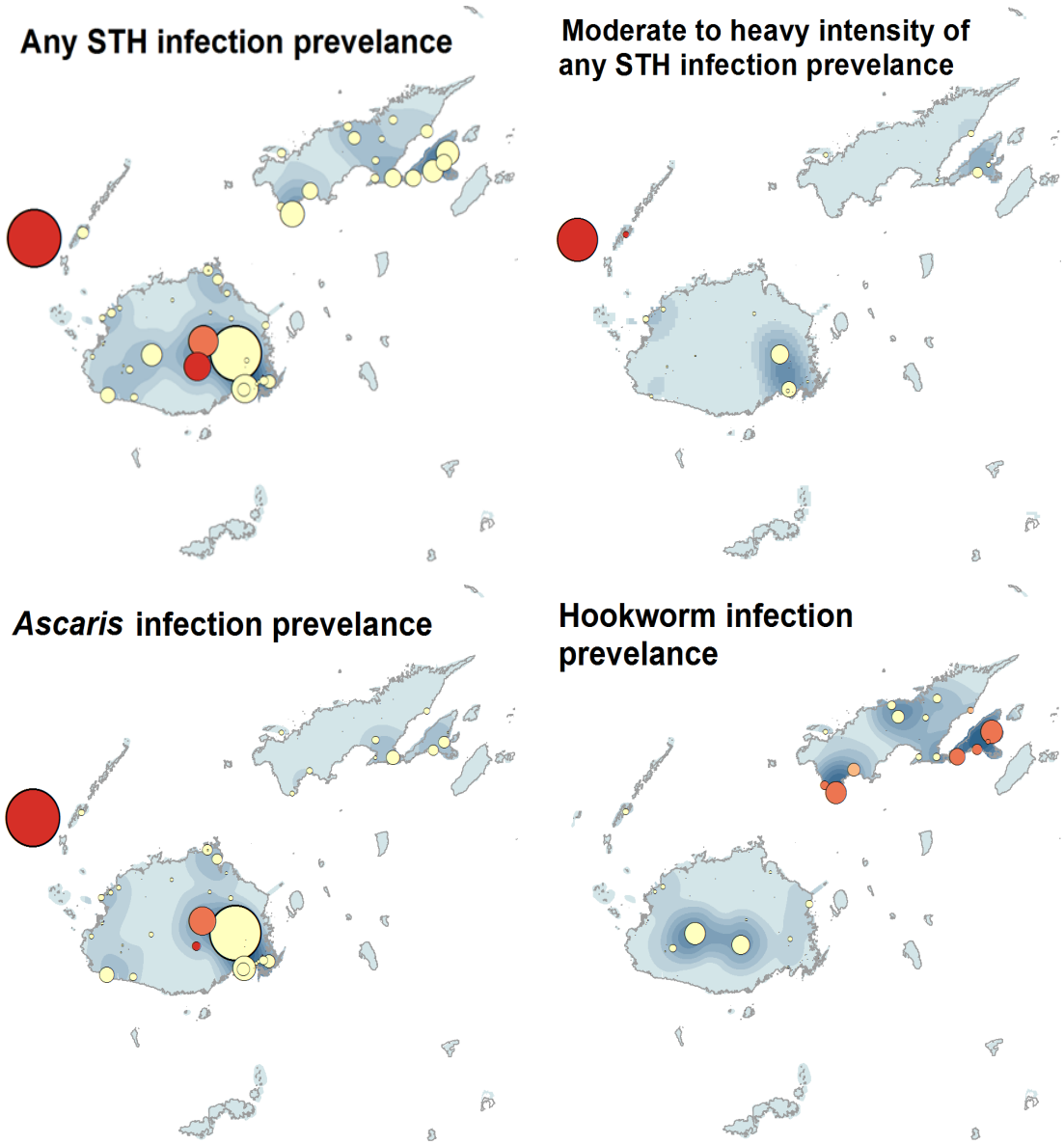


Figure 6.11 Spatial clustering trends and density distribution of STH infection prevalence among school children of 2 main islands of Fiji, 2014-2015



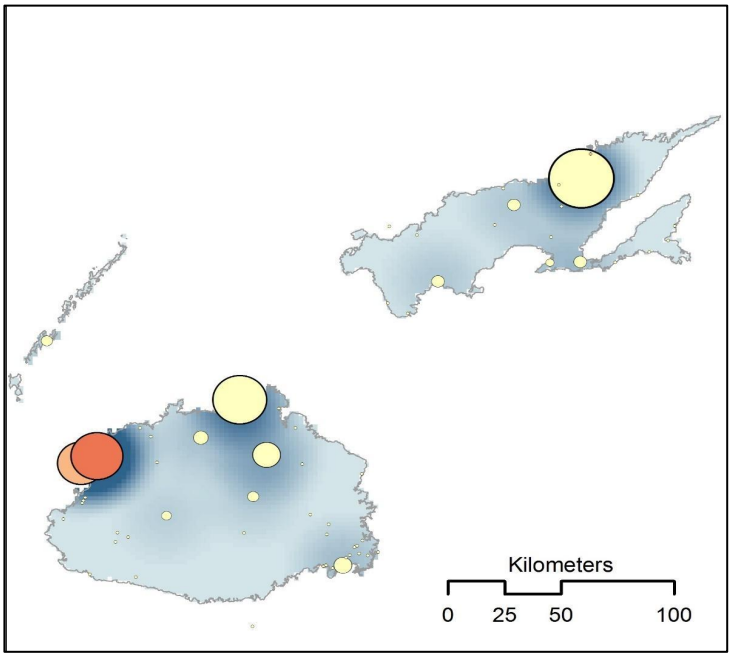
N.B.: The colour codes in the circles are from Z Renderer which is used for symbolizing standard deviations including the output from the hotspot analysis. The red-orange colored circles for high prevalence areas with positive z scores. A high positive z-score indicates a spatial clustering of high prevalence levels. The higher the z-score is, the more intense the clustering is. A z-score near zero which is in yellow, indicates no apparent spatial clustering.

For kernel density estimation, a smooth continuous surface defines the level of risk for that area. As such, deep blue denotes high risk of the infection, while light blue low risk.

Using the Getis-Ord Gi* method (optimized hotspot analysis using inverse distance), hotspots for any STH infection were identified, which were clustered rather than being randomly distributed (*P*-value < 0.05) (Figure 6.11). This is mainly derived from *Ascaris* infection, as it is shown in the case of species-specific infection distributions. As for hookworm infection, hotspots were clustered around the southern shore of the Northern Division with statistical significance, rather than being randomly distributed (Figure 6.11). Figure 6.11 presents the Gi* analysis identifying a global trend with a surface density map (kernel density).

Using the same methods, we concluded that *Giardia* spp. infections at schools were clustered rather than being randomly distributed, around Lautoka city of the Western Division (Z-score = 3.36, *p*-value < 0.05) (Figure 6.12). Figure 6.12 shows results of the Gi* analysis identifying a global trend with a surface density map (kernel density).

Figure 6.12 Spatial clustering trends and density distribution of *Giardia* spp. infection prevalence among school children of 2 main islands of Fiji, 2014-2015



N.B.: The colour classification for Getis-Ord Gi* significance levels and prevalence levels follows that of Figure 6.11

6.3.3.2. Sentinel site surveillance in the Eastern Division

School level *Ascaris* and hookworm infection prevalence in the Eastern Division showed also varied more widely for *Ascaris* infections (Figure 6.13a) than hookworm infection (Figure 6.13b). Nearly all schools in Lomaiviti sub-Division had Ascaris infected cases, while there were no cases in Rotuma sub-Division. Hookworm infected cases were only found in schools in Kadavu and Lomativiti sub-Divisions (Figure 6.13b).

Figure 6.13a Sketch map of 24 schools and their school level *Ascaris* infection prevalence in the Eastern Division of Fiji, 2015

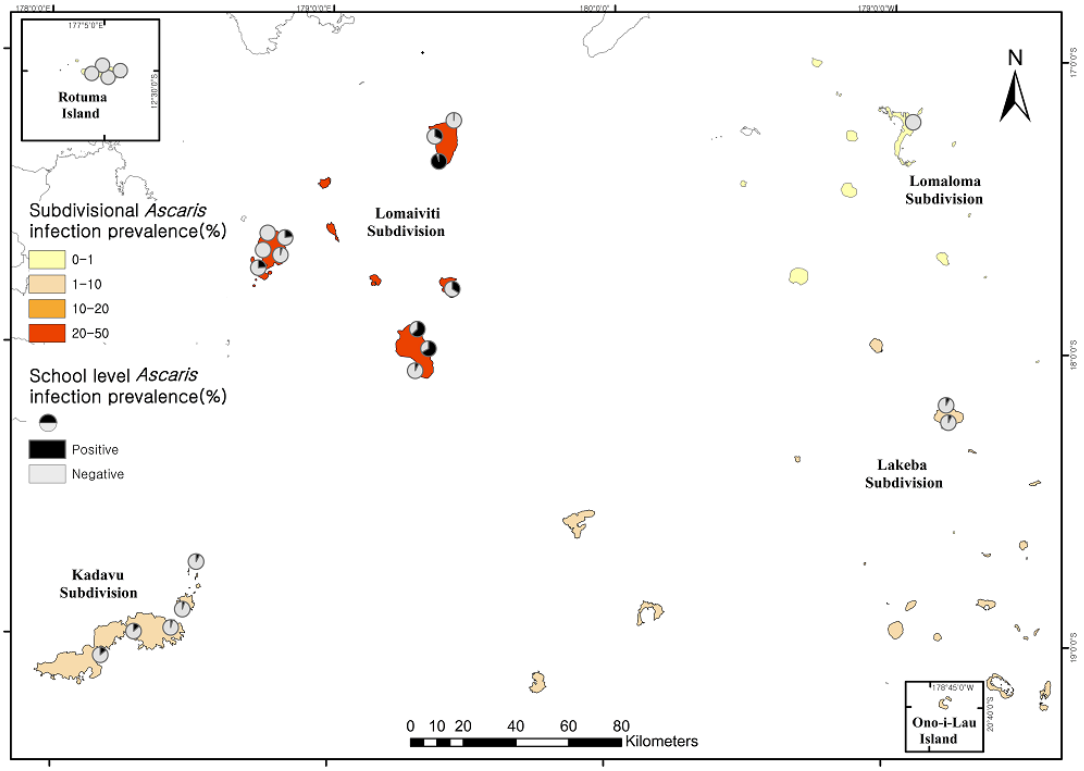
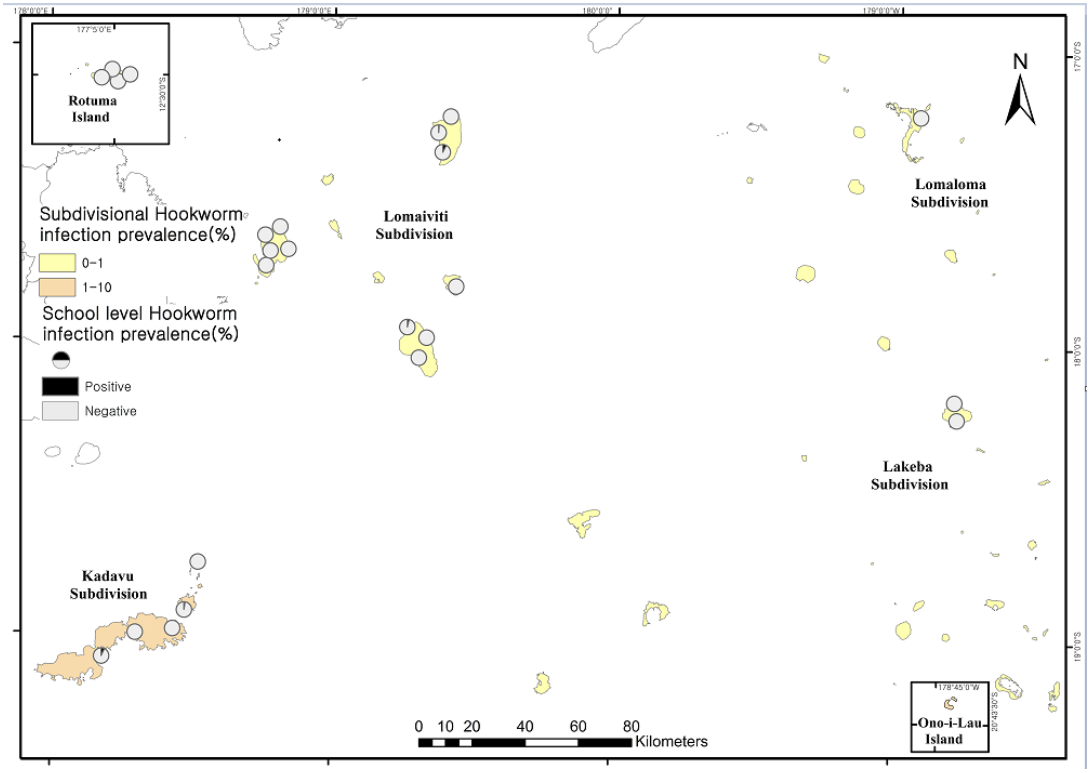


Figure 6.13b Sketch map of 24 schools and their school level hookworm infection prevalence in the Eastern Division of Fiji, 2015



6.4. Discussion

6.4.1. Low levels of endemicity but clustered intestinal parasite infection in Fiji

The survey of 69 primary schools across three Divisions through LF TAS as well as sentinel site surveillance of 24 schools in the remaining Division showed that overall STH infections in school-age children of Fiji were at a low level in the majority of the country, which was long considered to be endemic (WHO 2017f). The residual infections were *Ascaris* spp. infections followed by hookworm infections, while *Trichuris* infections were rarely seen. The absence of *Trichuris* infections in two main islands was also confirmed by real PCR, which will be discussed later in detail in Chapter 8. For hookworm and *Trichuris* infections, no moderate or heavy intensity infection cases were found during the survey. This is in contrast to the most recent survey conducted in Fiji: A survey conducted in 2005 examined school children from five villages in Taveuni Island (Thomas et al. 2005). A total of 258 children aged 5–15 years were surveyed, and the overall prevalence of *Ascaris*, hookworm, and *Trichuris* infections were 33%, 14%, and 17%, respectively. Though children in the same localities were not included in our assessment, it can be inferred that that the large-scale nationwide preventive chemotherapy through various programme platforms over a decade, especially including LF MDA, may have impacts on the control of STH infection in the country as shown in other endemic countries (Drabo et al. 2016). Collecting up-to-date epidemiological information is important not only to assess the impact of LF MDA on STH but also to plan ahead for more tailored interventions for STH infections in children.

The most notable finding from our study is large variations in STH infection prevalence at school levels. There were schools with no remaining STH endemicity at all, while several had any STH infection prevalence>50%. From hotpot analysis using the Getis-Ord Gi* method, we found that the spatial distribution of STH infections at school levels across two islands was not random but was clustered. Cases of *Ascaris* infections were grouped at schools in inland areas of the Central Division and in an island of Yasawa Island groups; and cases of hookworm infections around the southern shore of the Northern Division. The focality of the infections across Fiji is notable as it was not observed in previous studies conducted, but this is consistent with recent findings from the impact assessment of the national school-based deworming programme, where the impact varied markedly by county and school (Nikolay et al. 2015). Though it is uncertain to make decisions as pre-chemotherapy baseline as well as school-level treatment coverages are not available, it might be possible that: i) Varying performance of mass deworming interventions through LF and NIMS programmes by localities would have

influenced the epidemiological situations of STH; and ii) There were innate geographical diversities of the underlying intensity of transmission of the STH infections, which has influences on the rate of reinfection between the treatments (Nikolay et al. 2015).

As for intestinal protozoan infections via cross-sectional approach among class 1 and 2 schoolchildren, it appears that gastrointestinal protozoan infections are not rare in two main islands of Fiji. Given the insensitivity of single stool sampling, the actual prevalence could be higher, and it would have been better to attempt further faecal sampling such as three consecutive day stool samples (Osman et al. 2016), which was not practiced because of logistical challenges in the field. Nevertheless, the finding was further confirmed by real-time PCR, showing that *Giardia* infection prevalence of every 10th sample collected in the Western Division reached up to 19.7%, as similarly observed in other recent studies in the different part of the world (Osman et al. 2016). This is mainly owing to higher sensitivity and specificity of the applied molecular technique (Verweij and Stensvold 2014), but it could also reflect the actual high level of the infection endemicity at the time of the survey.

We found that the spatial distribution of *Giardia* infections at school level across two islands was not random, but was clustered, and cases of the *Giardia* infections were grouped at schools, mostly around the urban centres such as Lautoka, the second biggest city in Fiji, and Ra town. From the spatial analysis in the Western Division using the Getis-Ord Gi* method, we were able to confirm that there was a real hotspot of *Giardia* infections in these areas at the timing of the survey. This is unexpected given that *Giardi*a infections are mainly from the use of unprotected water source (Heymann 2014) of which distribution is more prevalent in rural settings (Hunter et al. 2010). Nevertheless, as a study in the post-earthquake camps of Colombia indicated, giardiasis could emerge during the major events which alter the existing water and sanitary conditions (Lora-Suarez et al. 2002), and episodes of flooding and heavy water run-off could subsequently contaminate water and foods with *Giardia* cysts from infected human or animal wastes (Lora-Suarez et al. 2002; Baldursson and Karanis 2011). Thus it may be possible that *Giardia* infections in these major urban areas originated from the contaminated water and foods by the floods, caused by Cyclone Kofi in Feb 2014 (Fiji Meteorological Service 2014), which was just before our stool samples were collected. In this regard, we propose enhanced surveillance efforts including water quality testing in the disaster-affected areas as part of the preparedness plan to explore whether there is contamination of water sources or any increased level of endemicity of *Giardia* infections. The uptake of the rapid test kits for the diagnosis of intestinal protozoa infections can be also considered. These should be implemented urgently whenever there are major events which can alter the water and sanitation conditions.

Apart from the findings from real-time PCR in the Western Division, gastrointestinal protozoan infection prevalence levels in our study are lower than those of recent surveys targeting schoolchildren with similar methods in urban (Bahmani et al. 2017) and rural areas in Iran (Sarkari et al. 2016) with better standards of living at national level (UNDP 2016). These findings suggest that the epidemiological profiles of gastrointestinal protozoan infections could differ by the local factors such as being urban or rural, and justifies epidemiological investigation on protozoan infections utilizing more sensitive diagnostic techniques such as real-time PCR in different settings.

6.4.2. Study implications on Fiji's national intestinal parasite infection control, school health, and WASH programmes

Fiji was one of the first countries in the Pacific which initiated nation-wide preventive chemotherapy against LF using the combination of DEC and albendazole (WHO WPRO 2006), with the support from the PacELF. The NIMS programme established in 2010 was the first national STH infection control programme in the country. The results from the National Nutritional Survey in 2004 showed that anaemia was endemic in Fiji, and among the STH infections, particularly hookworm would be one of the attributable causes, though the baseline hookworm endemicity at that time was unknown. Regardless of the scarcity of data, the national programme decided to include albendazole in the scheme and to conduct mass deworming together with the supplementation of other micronutrients. The decision was based on the followings: 1) firstly, Fiji has been long considered as STH endemic and the prevalence of anaemia was high among school-aged children (Schultz et al. 2007), and it is also well known that hookworm infection is a risk factor for anaemia in children (Smith and Brooker 2010; Chami et al. 2015; Drabo et al. 2016); 2) secondly, deworming is proved to be the most cost-effective public health interventions and its benefits on children have been well described and strongly recommended (Smith and Brooker 2010; Hicks et al. 2015; Drabo et al. 2016; Croke et al. 2017) especially LF MDA rounds had been stopped; and 3) finally adding albendazole or mebendazole to the iron and vitamin A supplementation programme to treat school-age children and preschool age children would not pose extra costs for the programme, given that albendazole tablets are mostly secured from the donation by the partner.

The current study results suggest that Fiji is not far from achieving the goal of elimination of STH infections as a public health problem, as the overall moderate and heavy intensity infection is just around 2% in two main islands (WHO 2012b). However, data before commencement of the LF MDA and NIMS are not sufficient to assess whether this low level of prevalence is a direct impact of deworming activities, or it was low even before commencing the interventions. With the help of community-wide MDA against LF, national deworming coverage for school-aged children reached 60% for a decade from 2002 when it was started. Unfortunately, this level of coverage was not maintained, because the geographical coverage of LF MDA started to decrease since 2010 (Table 3.2). Even if the deworming coverage of schoolchildren achieved through the NIMS scheme was above 75% in its first year, the programme was not able to achieve the global target equally among school-aged and preschool-aged children until 2014 (Table 6.1). The NIMS scheme was under review since its completion of 5-year piloting since 2016 (personal communication with MHMS), which leaves populations under the risk of the infections except those in the Eastern Division, Taveuni sub-Division, and Malolo Medical Area who are covered from the LF MDA. There is a strong need to intensify the efforts for STH infection control and revive the momentum.

Although nationwide LF MDA with DEC and albendazole had contributed hugely in achieving coverage of deworming activities in Fiji not only by including schools but communities, as of 2015, the population at risk for LF who requires preventive chemotherapy is only around 5% of the total population (Table 3.2). To consolidate the impact achieved, the national STH programme should explore the best platform to access to at-risk populations such as preschool and school-aged children as well as women of childbearing age: Firstly, like Burkina Faso, the country which is a good candidate to pursue interruption of STH transmission at national level via LF MDA (Anderson et al. 2015; Drabo et al. 2016), LF MDA in areas with LF endemicity should be counted; Secondly, resuming of school based mass deworming as a stand-alone approach or in combination of students health check-ups should be of priority; and implementation research may be needed to explore what would be the best delivery channels especially for preschool children and women with child-bearing age, for instance, either as a facility or as community-based in a post-MDA surveillance setting (WHO 2015a; Drabo et al. 2016), which had substantially lower coverage compared to school-aged children under NIMS. The results of the review of the NIMS scheme will potentially help the national programme identifying possible options to pilot drug distribution strategies for preschool children and women of childbearing age.

Following the global recommendation on treatment options for STH, Fiji will now need preventive chemotherapy once a year (WHO 2015a). If strategies can differ at Divisional level or sub-Divisional level, the Western Division can implement mass deworming once every two years and there are sub-Division which can even stop mass deworming. However, there are still areas with high endemicity at sub-Divisional as well as school levels, and this is primarily due to having a few hotspot schools in the area, a caution should be taken to address this geographical heterogeneity. The fact that such hot spots still exist after many years of nation-wide deworming through LF MDA and NIMS suggests that there is a need to urgently resume deworming activities, as well as to combine additional WASH interventions. Attempts to explore possible local factors in these areas which contribute to persistently elevated levels of endemicity should be also made (Drabo et al. 2016), theoretically, repeated annual preventive chemotherapy for children and adults could reduce *Ascaris* and hookworm infection to ground level (Anderson et al. 2015; Truscott et al. 2015; Drabo et al. 2016). Further research is needed especially on the impact of limited or poor water supply, hygiene-related behaviours such as washing hands or open defecation, and sanitation conditions in these hot-spots in Fiji (Strunz et al. 2014). The STH control programme is encouraged to work with the existing structure of environmental health within the MHMS for the delivery of WASH interventions to the population, as preventive chemotherapy alone in these areas will not be sufficient in interrupting transmission (Truscott et al. 2014; Drabo et al. 2016).

With the total number of school-aged children in the study areas up to 200, 000 (Fiji Bureau of Statistics 2009), several hundreds of children could have been infected with gastrointestinal protozoa including *Giardia* spp.. Whilst not all the infected children would develop morbidity, it is likely that in schools with these infections there have been occasions of water or food being contaminated which is known to be a source of the disease (Hunter et al. 2010). Thus, it may be necessary to establish the overall public health importance of the infection in the country and what would be a core set of interventions for WASH improvement at the school level, in order to break the transmission cycle of these protozoan infections. Considering that the *Giardia* infection prevalence levels were even higher in urban areas in our study and even several hotspots existed, attention should be equally paid to urban areas as well as rural areas. Enhanced surveillance efforts are commended in the disaster-affected areas.

6.4.3. Application of LF TAS in assessing intestinal parasite infections

The usefulness of LF TAS in assessing the epidemiological profile of intestinal parasite infection especially for STH as a platform to determine deworming strategies upon stopping LF MDA has been well demonstrated in other countries (Chu et al. 2014; Drabo et al. 2016). Drabo et al. reported the study from Burkina Faso, in which 6-7 year-olds and 10-14 year-olds in community settings and also 10-14 year-old children in school-settings (Drabo et al. 2016) were tested. The overall STH infection prevalence was higher among 10-14-year-olds who were tested in communities, with most of the infections from hookworm. In Tonga, ten schools out of 127 were set aside for the STH infection prevalence assessment, targeting class 3 students separately from the LF target of class 1 or 2 children (Chu et al. 2014). These findings would have led to the recommendations in the new guideline for assessing the epidemiology of STH during a TAS (WHO 2015a): the target population for STH survey is 8-10-year olds for school-based surveys and 6-7-year-olds for household surveys. This recommendation also reflects the findings that children aged 5 to 12 years had a higher percentage of *As. lumbricoides* and *T. trichiura* infections than individuals in the other age groups (Smith et al. 2001).

Nevertheless, our study is unique in the sense that the assessment took place in complete synchronization at the school level with the LF TAS, by having the same target group. This relieves a burden of mobilizing and running several different teams at the same time, so adding the STH component is proved to be feasible and efficient in the limited resource settings. One concern would be underestimating overall STH infection prevalence by having 6-7-year-old children as the target population. However, as it is shown in the study from Burkina Faso, the overall STH infection prevalence among 10-14-year-olds could have been higher, though it was not significantly different (Drabo et al. 2016). Moreover, the dominant STH species in Fiji is *Ascaris,* and in fact, it was shown that in the sentinel site surveillance of the Eastern Division, the *Ascaris* infection prevalence level was higher among younger children. Therefore, we believe that it is less likely to underestimate the overall burden of STH infections in our study and targeting 6-7-year-olds equally for the TAS and STH survey would be an alternative option in resource-constraint settings, to utilize the best benefit of resources.

Another difference in our study design compared to the new guideline for assessing the epidemiology of STH during a TAS published in 2015 is that we had sub-sampled the schools but did the census at the school level, and the sample sizes were greater in numbers than what is recommended (WHO 2015a). Currently, the recommended sample size for assessing the epidemiology of STH using cluster survey in the TAS-STH survey guideline is 332 (WHO

2015a). This is about nine times smaller compared to that of the original TAS design (WHO 2011c) for the population > 18,000, as the hypothesis to be tested differ. However, the new guideline for the STH assessment did not specify how to recruit this smaller number of participants for STH than the LF TAS. One option would be to select one single class in selected schools for the LF TAS and then randomly choose 11 students in that class, considering the minimum number of clusters was 30 (WHO 2011c). Alternatively, schools can be further sub-sampled and number of students per school can be increased. We have followed the latter, by sub-sampling 20 or 30 schools but enrolling all available students as in the TAS. The decision was based on the practical consideration, as selecting 11 or more children would be more time-consuming at schools as it requires preparation of the student's list and individual level randomization. Also, it was encouraged following the discussion among the health and education authorities that opportunities for testing should be available for everyone in the same class, with the sense of ensuring equity, as the study was part of the public health initiative rather than research. In addition, the recommended sample size of 332 was based on a design effect of 2.0 for cluster sampling (WHO 2015a). In our study, the actual design effect was 2.5, and the required sample size was 415 (2.5*166), which was in fact achieved in our survey with sufficient power up to 80%, in estimating the STH infection prevalence. Also, we applied diagnostic techniques more sensitive to intestinal protozoan infections, while the new guidelines recommend Kato-Katz and mini-FLOTAC for the examination of stool samples (WHO 2015a). We preferred FEC and added real-time PCR, as mini-FLOTAC would not detect protozoan cysts without changes of the solutions. Molecular techniques may not be available in every setting, but FEC is simpler and less costly, so it may be beneficial to consider in areas with the higher possibility of intestinal protozoan infections on top of Kato-Katz smear technique or mini-FLOTAC, when it comes to consider TAS-STH combined surveys.

We also conducted the sentinel site surveillance in all age groups attending the primary schools in Eastern Division, and the design of the survey is more close to that of conventional school-based surveys (WHO 2011a). The result does not show significantly different STH infection prevalence, even if we relied on solely KK technique and may have underestimated the prevalence, especially that of hookworm (Tarafder et al. 2010). Likely, the fact that there were no single positive STH infected case in all four schools of the Rotuma sub-Division should be noted with caution, given that the schools in the area were visited lastly and the samples were processed after weeks from the timing of the collection due to logistic difficulties, which would significantly impact the hookworm egg detection. Thus, it may have been possible that these were false negatives, and actual burden of the infection in the island could be higher,

which also lowered the overall infection prevalence in the Eastern Division in turn. Nevertheless, this separate sentinel site surveillance was conducted right before the annual LF MDA round in 2015, that had allowed sufficient time from the last cycle of drug distribution for re-infection to occur (Drabo et al. 2016) and likely explain the high level of the discrepancy of the overall STH prevalence across the sub-Divisions. These warrants follow up sentinel site surveillance in the Division especially in Rotuma and Lomaiviti sub-Divisions, to explore whether this very low or high level of endemicity is sustained.

There are number of limitations in this study. Firstly, the age group selected for STH infection prevalence assessment through LF TAS is limited to class 1 and 2 students, and it is possible that epidemiological profile of the STH infection in older children or who do not attend the schools may be different. For, instance, given that the risk of hookworm infections may be higher in the adult population (Anderson et al. 2015), our results may have underestimated the true burden of the diseases. We tried to overcome this limitation by expanding the age groups in the sentinel site surveillance in the Eastern Division and demonstrated the association between the age and *Ascaris* infection prevalence. However; it was not feasible for hookworm infections to do it as the number of positive cases was too few. We also believe the discrepancy between children at schools and at communities would be minimal for primary school students, given that the enrolment rate is very high. All in all, the results may represent the better distribution of *Ascaris* and *Trichuris* infection, but may not represent that of hookworm of all population at risk, similar to the example of Burkina Faso (Drabo et al. 2016).

As for intestinal protozoan infections we may have also underestimated the true prevalence, as we had relied on the detection of protozoa cyst only from a single specimen per person. Given that microscopic examination of protozoan infections is time-consuming and dependent on the operator's skills and expertise, newly available antigen-based detection methods using rapid detection tests (RDT) could be attractive alternatives (Goudal et al. 2017). However, we rely on locally available techniques as RDT are not readily used in Fiji for public health surveillance and tried to overcome it in the Western Division's survey by adding real-time PCR as a quality control tool as discussed in Chapter 8. Similar to STH infections, having only class 1 and 2 students in the sample may not reflect the actual epidemiological profile of protozoan infections among schoolchildren on Fiji, considering age groups may have impacts on the risk of being infected (Osman et al. 2016). Another similar design of the survey with a wide range of age groups may be warranted to have a more representative picture.

6.5. Conclusion

In conclusion, through large-scale preventive chemotherapy, Fiji has successfully controlled STH infection transmission in school-age children in the most part of the country and not far from the programmatic goal of eliminating STH infections as a public health problem (WHO 2012b). Also, the level of endemicity of intestinal protozoan infections was overall low, but there was clustering of *Giardi*a spp. infection in the Western Division. Future research is needed on potential risk factors which allow especially *Ascaris* infections to persist after a decade of preventive chemotherapy. By adding stool sample collection and FEC to LF TAS, we were able to shed new light on the distribution of intestinal parasite infections across the island. LF TAS provides a access platform to assess the up-to-date epidemiological profile of intestinal parasite infections for a country in the stage of post MDA surveillance and should be actively utilized as a monitoring and evaluation tool for other NTD programmes. Strategies need to be developed to consolidate the lessons learned and capacity built from the experience of implementing the LF MDA and NIMS, and to carry them over to target elimination of STH infections as a public health problem.

Chapter 7

Factors associated with soil-transmitted helminth infections on Fiji

Chapter 7. Factors associated with soil-transmitted helminth infections on Fiji

7.1. Introduction

Soil-transmitted helminth (STH) infections are one of the most common infections worldwide, affecting more than a quarter of the world's population (Boonjaraspinyo et al. 2013), mostly affecting those who reside in the underprivileged communities (WHO 2017h). In Oceania, STH infections remain as a public health problem especially where access to safe water and improved sanitation facilities is limited, but hot and humid tropical climate plays additional roles, even if preventive chemotherapy against STH infections had been initiated and national programmes to improve water, sanitation, and hygiene conditions exist.

Transmission dynamics of STH infection is complex, and there are multiple factors involved (Muller 2002), such as socioeconomic status, human behaviours, and environmental parameters (Gazzinelli et al. 2012). The infections are mediated via ingestion of eggs or contacts with hookworm larvae in the soil, and the primary control strategies are preventive chemotherapy through mass drug administration (MDA) either with albendazole (a single oral dose of 400 mg) or mebendazole (500 mg), targeting at-risk populations once or twice per year (WHO 2012a). While preventive chemotherapy can reduce morbidity from helminth infections (Strunz et al. 2014), reinfection occurs rapidly after treatment with anthelmintic (Jia et al. 2012). At six months after treatment, the infection prevalence of all three worms returned to half of the initial level. At 12 months, the prevalence of *As. lumbricoides* and *T. trichiura* infections reach to the initial pre-treatment prevalence, while levels of hookworm reinfection continued to fluctuate at about half pre-treatment levels (Jia et al. 2012). Thus, chemotherapy alone will not sustain the interruption of transmission, especially in high-endemic areas (Gabrie et al. 2014).

7.1.1. Individual demographical characteristics and STH infections

In most helminth infections, prevalence peaks in childhood (Loukas et al. 2016) and school-aged children comprise the largest group that is most vulnerable to the infection (Crompton and Savioli 2006). Ascariasis is the most common infection in children 2 to 10 years old, and prevalence levels decreases when the age is over 15 years old (Muller 2002). However, as for hookworm infections, the age-specific epidemiology is different, and infection prevalence

increases until it plateaus in adulthood (Anderson et al. 2013). In high-endemicity settings, *Trichuris* infection prevalence also may rise rapidly and attains a plateau of between 80% and 100% after the age of two to four years (Bundy 1986). The prevalence then remains high and relatively constant throughout adulthood (Bundy 1986).

In regard to gender and helminth infections, studies have consistently identified that hookworm infection prevalence is especially higher in boys than in girls (Gabrie et al. 2014), but it is unclear whether this is due to differences in exposure or physiology (Gabrie et al. 2014). In a study conducted at Thai-Myanmar border, the females were significantly more likely to be infected with *As. lumbricoides* and/or *T. trichuria,* and significantly less likely to be harbouring hookworm (Nacher et al. 2003). However, there are also studies reporting that there was no significant difference in STH infections between genders (Phongluxa et al. 2013; Anuar et al. 2014).

Lack of sanitation and inadequate hygiene at individual level, such as the absence of hand washing after defecation and before eating, or walking barefoot, play a great role in transmitting the STH infections (Bartram and Cairncross 2010; Strunz et al. 2014; WHO 2015e). Equally, provision of clean water for hand-washing and improved sanitation at household and community levels is believed to reduce contacts with contaminated soil and helminth eggs, and transmission of the infection (WHO 2011a; WHO 2015e). Therefore, better targeted and jointly implemented water, sanitation, and hygiene practices (WASH) and NTD control strategies such as preventive chemotherapy have been strongly advocated for the longer-term control and elimination of STH infections (Bartram and Cairncross 2010; WHO 2015e). Specifically, a recent meta-analysis on the relationship between WASH access and practices and STH infections showed that water-related access and practices, especially with treated water, were associated with lower odds of STH infections (Nasr et al. 2013; Strunz et al. 2014; Campbell et al. 2017). Availability or use of latrines was associated with lower risk of any STH infection (Strunz et al. 2014), and soap use or its availability for hand-washing was also significantly associated with lower odds of any STH infection (Balen et al. 2011; Strunz et al. 2014; Campbell et al. 2017). Handwashing, both before eating and after defecation, was associated with lower odds of *As. lumbricoides* infection (Nasr et al. 2013; Strunz et al. 2014), and handwashing after defecation alone was associated with reduced odds of any STH infection (Balen et al. 2011; Strunz et al. 2014). The meta-analysis also found evidence of a strong association between wearing shoes and lower odds of hookworm infections (Jiraanankul et al. 2011), which was also associated with lower odds of any STH infection (Strunz et al. 2014).

7.1.2. Geospatial and socioeconomic characteristics and soil-transmitted helminth infections

As for geospatial determinants, soil types and temperature play a key role in the transmission of STH infections: For *Ascaris* and *Trichuris* spp., clay soils are favourable for the survival of eggs (Nolf 1932), as they dry out less in drought and have better adhesive properties (Muller 2002). The type of soil is also critical for the development of larvae for hookworm eggs, which migrate up against the water flow and down again when the top layer of soil is drying (Muller 2002), and the hookworm larvae will not be able to survive in clay soils so well as in sandy soils (Mabaso et al. 2003). *Ascaris* eggs will not embryonate below 18ºC but can survive for many weeks at low temperatures, and continue to develop when the temperature is raised (Nolf 1932; Muller 2002). The optimum temperature for hatching of hookworm eggs is around 20-27ºC, and they fail to develop at thermal maxima that exceed 40ºC (Mabaso et al. 2003). For the ova of *Ancylostoma duodenalis*, they can develop at a minimum of 14ºC, which is lower than those of *Necator americanus* (Muller 2002). *Trichuris* infection is more common in countries with a constant temperature between 22 and 28ºC, and also with heavy rain-falls and dense shade (Muller 2002).

There is also an increasing interest in the impact of climate change on the endemicity of STH infections (Gazzinelli et al. 2012). Increasing temperatures and fluctuation of rainfalls is believed to alter eco-system, though the actual effects on helminth transmissions are not well understood (Morgan and Wall 2009). These findings suggest that there is a need to identify factors more attributable to the transmission of the STH infections in a particular setting, in order to design effective control strategies in the locality (Gazzinelli et al. 2012; Gabrie et al. 2014).

STH infections are closely linked to poverty, and they also result from poverty (Gazzinelli et al. 2012). Displaced populations, including refugees, and urban slum dwellers are particularly vulnerable to the disease (Hotez et al. 2009). Poor and marginalized communities specifically suffer from: 1) undernutrition, which leads victims to micronutrient deficits and lower resistance to infections with helminth; 2) degraded and high-risk environments lacking adequate housings, resulting in close contact with soils contaminated with helminth eggs; and 3) limited access to social services including education and healthcare, which can help the population to be exposed to preventive measures against the infections (Gazzinelli et al. 2012).

7.1.3. Justification of the study

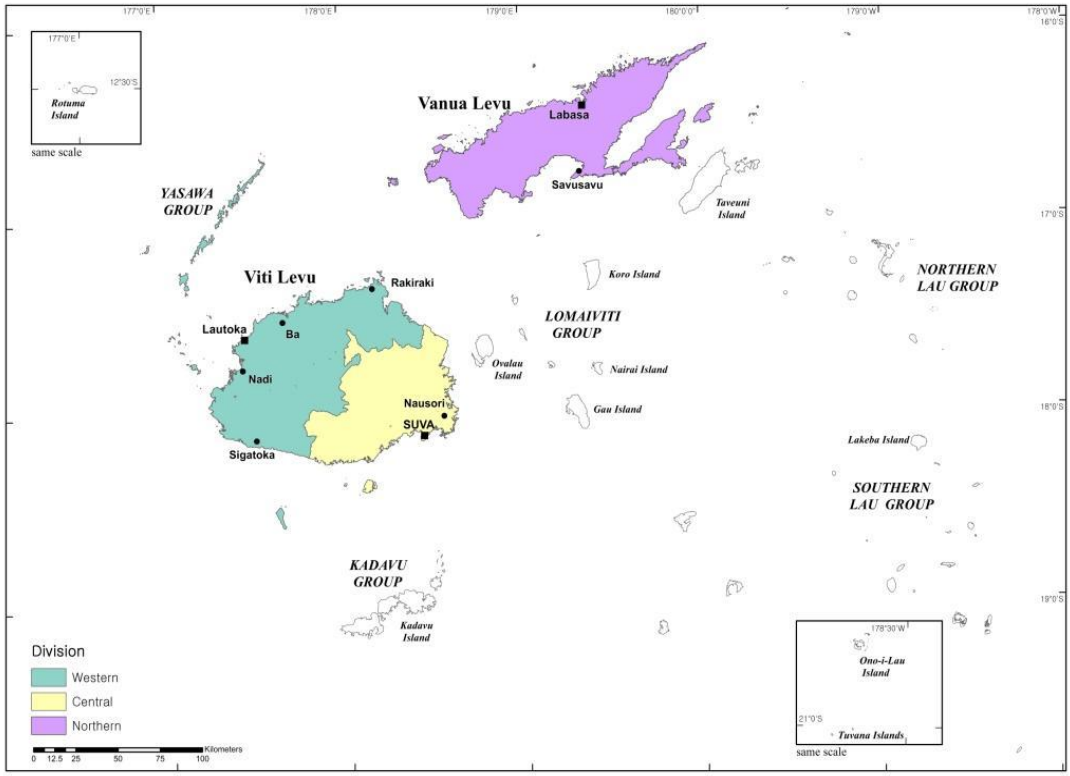
It is increasingly accepted that the health problems should be understood in the context of human-environmental relations (Stokols 1996), and dynamics of STH infections are believed to be the results of continuous interactions between human and other physical, socioeconomic, and environmental determinants (Gazzinelli et al. 2012). As a precondition, there is a need for a comprehensive study to explore the relationship between STH infections and individual behaviours, as well as environmental parameters in particular settings (Gazzinelli et al. 2012). However, in Oceania, the data on the association are scarce to the extent that assists contextualization of effective interventions other than mass drug administration of anthelminthic (Kline et al. 2013). Fiji is not the exception and there has been limited information on the demographical, WASH-related and environmental parameters associated with the infections. Therefore, the study was planned to explore the important relationship to be able to support the national programme in the development of the effective targeted interventions for the STH infections in the country (Gazzinelli et al. 2012), especially within the local context considering the island setting where no information on the factors associated with the infection is known.

7.2. Methods

7.2.1. Study site

The present study was nested within the epidemiological assessment to explore the geographical distribution of intestinal parasite infections in Fiji, conducted in conjunction with the three different LF TAS for three Divisions (Western, Central, and Northern) of two main islands of Fiji, Viti Levu and Vanua Levu (Figure 7.1). A detailed description of the study area was provided in the previous chapters (Chapter 4 and 6). The Taveuni sub-Division was excluded, as it formed an independent evaluation unit (EU) out of the Northern Division following the 2013 LF mid-term assessment and was not included in the LF TAS for the Northern Division.

Figure 7.1 Sketch map of Fiji showing 3 Divisions, except Taveuni sub-Division, and their boundaries targeted for analysis of factors associated with intestinal parasite infections



7.2.2. Study design and study population

The study was school-based and cross-sectional, and details on sample size calculations and field implementation had been described in Chapter 6. The list of schools as well as the Ministry of Education's classification of schools based on their locations, such as urban and rural, was obtained from the respective District Education Offices. Briefly, the fieldwork took place during February-March 2014 for the Western Division and July-August 2014 for the Central Division, as well as February-March 2015 for the Northern Division except the Taveuni sub-Division. Altogether 70 schools, 30 urban (10 each from the Division) and 40 rural (20 from the Western and 10 each from the Central and Northern), were randomly sampled out of 209 schools which were systematically selected for the three LF TAS. Schoolchildren specifically attending class 1 and 2 (usually aged 6-7 years) were invited to participate, as these were the recommended age group for the LF TAS. The questionnaires on parameters were sent to students' parents or guardians, as these children were not old enough to respond to the questionnaires and to provide information. An estimated eligible study population was 3,895 class 1 and 2 students.

7.2.3. Stool sample collection and parasite determination

One stool sample was requested from each participant to determine presence and intensity of intestinal parasite infections using Kato-Katz method, and/or the formol-ether-acetate concentrate technique (FEC). With the help of their parents/guardians, children were requested to bring their fresh morning stool samples on the date of the survey to their schools. Samples were placed in cool boxes with ice packs to maintain the cold chain and transported to the Fiji Centre for Communicable Diseases Control (FCCDC) in Suva, where they were refrigerated until sample preparation and examination for parasites on the same or next day. Helminth eggs were identified by their characteristic features and systematically counted. Details of laboratory procedures are described in Chapter 6.

7.2.4. Definition of variables of interest

Definitions of outcome variables are as follows: (1) Any STH infection indicated being positive with ova of at least one species of STH, marked as a binary variable (zero or one); (2) Any moderate or heavy intensity STH infection were defined as being positive either with egg counts as ≥5,000 egg per gram (epg) for *Ascaris*, ≥1,000 epg for *T. trichiura*, or ≥2,000 for hookworm, marked as a binary variable (zero or one), if egg counts were available in the KK examination considering the public health importance of this type of STH infections (WHO 2011a); and (3) Mono *Ascaris, Trihuris,* or hookworm infection was denoted as being positive with ova of *Ascaris*, *Trihuris*, or hookworm, marked as a binary variable (zero or one).

7.2.5. Definition of participants' demographic and water, sanitation, and hygiene (WASH) characteristics and data collection procedures

Students' demographic and WASH-related information were collected through structuralized questionnaires, distributed together with the consent forms and answered by children's parents/guardians. Data collected included: basic demographic information such as age and gender, individual behaviours associated with STH infections such as handwashing, shoes wearing, and the use of utensils for meals, and household level water sources and the type of sanitary facilities.

In order to describe the child's tendency of performing his/her behaviours, parents/guardians were requested to evaluate frequencies of each activity based on their own judgment by selecting one of three choices: not at all, not always but sometimes, or usually. For students' primary water source and the type of sanitation facilities at their home, choices were

provided following the Fijian Ministry of Health and Medical Services' environmental standards to classify the relevant terms: (1) source of water either to be spring, well, rainwater tanks, piped water into household from private or local source, or piped water directly from the Fiji Water Authority; and (2) the type of latrine either to be river, bush, pit latrine, water-seal or pour-flush. Lastly, parents/guardians also were asked to answer the question that they could recall their children took a deworming or hookworm tablet ever. The positive response was recorded when there was a circle on top of 'yes' in the questionnaire form. If there was a circle on top of 'no' or no circle at all in the form, these were all considered as a negative response, given that cultural similarity in both instances.

For the school level water and sanitation characteristics, the school headmaster was interviewed to choose an option to report their primary water source and the type of sanitation facilities at the school, following the MHMS' environmental standards to classify the relevant terms as described above.

7.2.6. School-level geospatial and socioeconomic data collection procedures

The coordinates of surveyed schools were collected by the survey team member using a handheld GPS device, and the location was estimated on the Google Earth where there was an error. Remotely sensed data relating to elevation (USGS 2017), forest and soil types (FAO/UNESCO 2016), Enhanced Vegetation Index (EVI) and Normalised Difference Vegetation Index (NDVI) (IRI 2017), and mean and maximum temperature and total annual and seasonal rainfall (Barker and Price 2012; WorldClim 2017) were extracted at the location of each of the surveyed schools. Population density and estimated percentage of population below the poverty line using Cost of Basic Needs approach were also derived using the location of each of the surveyed schools, which were available at the smallest administrative unit (Tikina) level (World Bank 2011).

7.2.7. Data management

The results of parasitological examinations and demographic and WASH characteristics obtained from the questionnaires were entered into a Microsoft Office Excel spreadsheet 2007 (Microsoft). For the frequency of individual behaviours such as handwashing, shoes wearing, and use of utensils for meals, the ordinal number was assigned to each variable as zero for not at all, one for not always but sometimes, and two for usually and treated as categorical variables. For the variables related to water sources and sanitation facilities, each of them was categorized and the ordinal number was assigned as follows: (1) Major source of water as zero, denoting

baseline for any surface water, spring, well, or rainwater tank, one for piped water into household from private or local sources, and two for piped water directly from the Fiji Water Authority; and (2) Type of latrine as zero, denoting baseline for river or bush, one for pit latrine, and two for water-seal or pour-flush.

7.2.8. Statistical analysis of the association between children's demographic and WASH characteristics and STH infections

Statistical analyses were conducted with the STATA Release 12 (College Station, TX: StataCorp LP), and STATA svyset command was used to specify primary sampling unit variables and sampling weight. For the associations between the demographical and WASH characteristics and odds of being STH infected, a multi-level logistic regression model was applied, accounting for intra-cluster correlation among the students attending the same school or sub-Division (Kawachi and Berkman 2003). A random intercept logistic model was fitted using STATA gllamm command for a generalized linear mixed model with random effects based on a logit link function for the estimates of the log odds of being STH infected. Bivariate and multivariate analysis of associations between covariates and outcome variables such as any STH infection was undertaken, and factors associated with inter-school variation in the infection prevalence were also explored. A manual stepwise forward logistic regression of significant variables was used to find best predicting models with P-value<0.2. School level effects on STH infections were examined by adjusting for the effects of differences in the distribution of individual-level risks between schools, and school level variance was then evaluated for the different characteristic of the study participants.

The linearity of the association between the type of the household water sources and sanitation facilities, and school level any STH infection prevalence was explored using locally weighted scatterplot smoothing (LOWESS) curves, which carries out a locally weighted regression of y variable on x variables and displays the graph. In general, the curve minimizes the variance of the residuals or prediction error, which allows consequently no assumptions about the form of the relationship, and the form to be discovered using the data itself.

7.2.9. Statistical analysis of the association between geospatial and socioeconomic characteristics and school-level STH infections

The individual-level data for outcome variables were aggregated for each school, and STH infection prevalence at school level was calculated. For measuring the association between geospatial and socioeconomic variables and STH infections at school level via modelling over-

dispersed count outcome variables, which were equal to the number of STH infected cases at each school, Poisson, Negative Binomial, Zero-inflated Poisson, and Zero-inflated Negative Binominal models were compared according to the Akaike information criterion (AIC) and the Bayesian information criterion (BIC), and Likelihood ratio test, in order to select the most appropriate regression model (Chipeta et al. 2014). Table 7.1 shows the results of the model selection which became the ground for the choice of Zero-inflated Poisson model.

Table 7.1 Comparisons among distinct count regressions according to the Bayesian information criterion, Akaike information Criterion, and Likelihood Ratio Test

Models being compared	Bayesian information criterion (BIC) and akaike information criterion (AIC)		Likelihood ratio test *P*-value
Poisson	251.5892	294.0372	
			0.000
Negative Binomial	237.757	282.4391	
Zero-inflated Poisson	221.4126	268.3288	
			0.1681
Zero-inflated Negative Binomial	224.3375	273.4878	

Fourteen geospatial and three socioeconomic variables were available for testing, and incidence rate ratio (IRR) was calculated for independent variables, where regression coefficients were interpreted as the difference between the log of expected counts. Continuous independent variables (i.e. all geospatial variables except the forest type and dominant soil type, as well as socioeconomic variables) were used by weighted Zero-inflated Poisson regression analysis for an association with the STH infection positivity. If the analysis showed at least moderate evidence of an association with the STH infection positivity (*P*-value 0.05) then the continuous variable was divided into two or four categories using the halves or quartiles and treated as ordered categorical variables. Multivariate analysis was conducted in a backward stepwise elimination method, starting with all variables with at least weak evidence of an association (*P*-value 0.10). Variables were eliminated from the model one-by-one, using the likelihood ratio test until a final model that explains the dependent variable was reached. Test for linearity was used to decide whether the independent variable was to be used together with other independent variables with co-linearity.

7.3. Results

7.3.1. Study population and its demographic characteristics

Demographic details of the study population were described in Chapter 6. Briefly, 1,890 children from class 1 and 2 across 69 schools (30 Western, 19 Central, and 20 Northern) out of 70 targeted schools participated in the study, where 1,839 stool samples were available for microscopic examination using KK and/or FEC. The age distribution of the participants was between 4 to 10 years old, and most of them (93.4%) were either 6 or 7 years old. There were 932 girls and 958 boys among whose gender status was available.

7.3.2. Individual and household level WASH characteristics of the study population

Individual and household WASH characteristics varied across Divisions: More than half of students were reported to wash their hands before eating or after toilet use (68.6%), to use utensils during the meals (60.9%), and to wear shoes (59.7%), by being denoted as 'usually'. However, in the Central Division, less than half of students (46.2%) were marked to wear shoes as 'usually'. The proportion of students who did not practice these behaviours, being marked as 'not at all', was the highest in the Northern Division consistently for washing hands (6.2%), using utensils (13.1%), and wearing shoes (15.1%). In one-third of the study participants, their parents/guardians answered that they could recall previous ingestions of deworming tablets of their children (Table 7.2).

In the Western Division, more than half of the students reported that piped water from the Fiji Water Authority was their main water source at home, while it was the piped water from private or local sources in the Central (52.6%) and Northern Division (71.3%). The majority (86.3%) of the households had either water-seal or pour-flush as their type of latrines, and pit latrine or open space use such as river or bush was overall infrequent, mostly being reported in the Western Division (15.1%) and the Northern Division (4.2%).

Table 7.2 Distribution of individual and household WASH characteristics of the study participants in the Western, Central and Northern Divisions* of Fiji, 2014-2015

Characteristics	Western (n=932)	Central (n=553)	Northern (n=405)	All (n=1,890)
% Distribution by hand washing behaviour before eating or after toilet use				
Yes, usually	71.8	53.6	79.3	68.6
Not always but sometimes	27.8	45.5	14.5	30.0
Not at all	0.4	0.9	6.2	1.4
% Distribution by utensil use during the meals				
Yes, usually	57.6	60.3	76.8	60.9
Not always but sometimes	39.0	35.6	10.1	34.1
Not at all	3.4	4.1	13.1	5.0
% Distribution by shoes wearing behaviour				
Yes, usually	62.3	46.2	70.3	59.7
Not always but sometimes	36.5	51.7	14.6	36.9
Not at all	1.2	2.1	15.1	3.4
% Who recall deworming tablet ingestion	33.8	46.2	25.3	35.4
% Distribution by main water source at home				
Piped water, Fiji Water Authority	52.7	30.4	13.6	41.8
Piped water, private or local	35.3	52.6	71.3	44.5
Others (Rainwater tank, borehole, river or stream)	12.0	17.0	15.1	13.7
% Distribution by home latrine type				
Water seal/pour-flush	84.5	92.9	87.3	86.9
Pit latrine	15.1	6.4	8.5	12.1
River or bush	0.4	0.7	4.2	1.0

N.B.: ***** Taveuni sub-Division is excluded.

7.3.3. School level WASH characteristics of the study area

Table 7.3 Distribution of school-level WASH characteristics distribution by the Western, Central and Northern* Divisions of Fiji, 2014-2015

School WASH characteristics	Western (N=30)	Central (N=19)	Northern (N=20)	All (N=69)
% Distribution by number of students enrolled in class 1 and 2				
<35	35.9	31.9	35.8	34.6
>35 and <75	33.1	34.5	32.7	33.5
>75 and <150	16.3	5.6	31.5	15.6
>150	14.7	28.0	0.0	16.3
% Students in urban schools	29.2	27.1	1.8	23.8
% Distribution by main water source at school				
Piped water, Fiji Water Authority	49.7	43.9	27.7	44.1
Piped water, private or local	20.5	27.4	44.6	26.7
Others (Rainwater tank, borehole, river or stream)	29.8	28.7	27.7	29.2
% Distribution by school latrine type				
Water seal/pour-flush	100.0	100.0	93.4	98.9
River or bush	0.0	0.0	6.6	1.1

N.B.: *****Taveuni sub-Division is excluded.

As for school level WASH characteristics, the Northern Division was distinguished from two other Divisions, by having no school with more than 150 class 1 and 2 students registered and with very low percentage (1.8%) of urban schools. Overall, the most frequently reported main source of water at schools in the Divisions was the piped water from the Fiji Water Authority (44.1%), while in the Northern Divisions, it was the piped water from private or local sources (44.6%). It was also the only Division which reported a school without on-site toilets and open space toilet use by students (Table 7.3).

7.3.4. Association between children's demographic and WASH characteristics and STH infections

Upon exploring the best model to describe the dataset given, a two-level (students at level 1 nested within schools at level 2) was fitted. In mono-variate analysis, the children aged 4 to 6 were more likely to be infected with any STH compared to the children aged 7 to 10 (OR=1.70, 95% CI 1.18-2.46%), and by class, it was class 2 students who were less likely to be infected (OR= 0.68, 95% CI 0.47-0.96%) (Table 7.4). Current age and class were closely associated (ϱ= 0.81) and only current age was retained during the later stage of analysis. There was no statistically significant difference in the risk of infection per sex. Table 7.4 presents crude and adjusted odds ratios of any STH infection, by selected demographic and WASH characteristics of the surveyed children.

Children who were reported to wash their hands before eating or after toilet use, and to use utensils during the meals, either 'usually' or even 'not always but sometimes' were at reduced risk of any STH infection, but there was no statistical significance. Wearing shoes lowered the risk of any STH infection, while the difference was only statistically significant when students were reported to do it 'usually', compared to 'not at all' (OR 0.43, 95% CI 0.18-0.99%) (Table 7.4). Children whose parents/guardian could recall deworming tablet ingestions had a lower risk of any STH infection, but it was not statistically significant (OR 0.90, 95% CI 0.57-1.43%). As for household WASH characteristics, having piped water either from private or local sources or from the Fiji Water Authority had a protective effect against any STH infection, but it was only statistically significant in the latter (OR= 0.43, 95% CI 0.19-0.96%). Children resided in the households with water-seal or pour-flush type latrines were also less likely infected with any STH (OR 0.31, 95% CI 0.11-0.93%).

Table 7.4 Mono-variate and multivariate analysis of students' demographic and WASH characteristics and any STH infection from multilevel mixed regression

Characteristics		Mono-variate		Multivariate	
		Crude odds ratio (95% CI)	*P*-value	Adjusted[b] odds ratio (95% CI)	*P*-value
By current age (years)					
	4~6	1.70 (1.18-2.46)	**0.005**	1.72 (1.11-2.68)	**0.016**
	7~10	1.00[a]		1.00[a]	
By class					
	1	1.00[a]		-	
	2	0.68 (0.47-0.96)	**0.031**	-	
By gender					
	Female	0.88 (0.63-1.23)	0.453	0.89 (0.57-1.37)	0.585
	Male	1.00[a]		1.00[a]	
By hand washing behaviour before eating or after toilet use					
	Yes, usually	0.36 (0.11-1.12)	0.077	0.69 (0.41-1.17)	0.171
	Not always but sometimes	0.58 (0.17-1.93)	0.373	1.00[a]	
	Not at all	1.00[a]			
By utensil use during the meals					
	Yes, usually	0.58 (0.25-1.34)	0.205	1.05 (0.66-1.71)	0.901
	Not always but sometimes	0.57 (0.23-1.42)	0.229	1.00[a]	
	Not at all	1.00[a]			
By shoes wearing behaviour					
	Yes, usually	0.43 (0.18-0.99)	**0.047**	0.56 (0.33-0.97)	**0.040**
	Not always but sometimes	0.94 (0.38-2.30)	0.890	1.00[a]	
	Not at all	1.00[a]			

Continued.

Characteristics	Mono-variate		Multivariate	
	Crude odds ratio (95% CI)	*P*-value	Adjusted[b] odds ratio (95% CI)	*P*-value
By recalling deworming tablet ingestion of the child				
Yes	0.90 (0.57-1.43)	0.650	0.93 (0.57-1.52)	0.782
No	1.00[a]		1.00[a]	
By main water source at home				
Piped water, Fiji Water Authority	0.43 (0.19-0.95)	**0.037**	0.51 (0.27-0.98)	**0.045**
Piped water, private or local	0.87 (0.43-1.75)	0.695	1.00[a]	
Others (Rainwater tank, borehole, river or stream)	1.00[a]			
By home latrine type				
Water-seal/pour-flush	0.31 (0.11-0.93)	**0.037**	0.33 (0.10-1.03)	0.058
Pit latrine	0.44 (0.13-1.52)	0.195	0.38 (0.10-1.38)	0.139
River or bush	1.00[a]		1.00[a]	

N.B.: a: Reference value; b: adjusted for all other selected variables

When school level characteristics were considered in mono-variate analysis, the children attending schools in urban areas were considerably less likely to be infected with any STH than the children attending the rural schools (OR 0.30, 95% CI 0.12-0.73%), and this was consistent with the lower risk of any STH infection found among schools with the registered number of class 1 and 2 students greater than 75 (Table 7.5). This was predictable given the high level of association between schools in urban locations and the number of registered class 1 and 2 students > 75 (ϱ= 0.76). Piped water from the Fiji Water Authority as the main source of water at schools was found to be associated with lower risks of any STH infection, when compared to the ther sources such as rainwater tanks, boreholes, river or streams (OR 0.34, 95% CI 0.15-0.76%), and this was a consistent finding when the main water source at households was piped water from the Fiji Water Authority. Having water-seal or pour-flush type of latrines at schools did not lower the risk of any STH infection, in contrast to the results of the household latrine types.

Table 7.5 Mono-variate analysis of school-level characteristics and any STH infection from multilevel mixed regression models

School-level characteristics	Any STH infection	
	Odds ratio (95% CI)	*P*-value
By number of students enrolled in class 1 and 2		
<75	1.00[a]	
>75	0.57 (0.25-1.28)	0.172
Urban location		
Yes	0.30 (0.12-0.73)	**0.008**
No	1.00[a]	
By main water source at school		
Piped water, Fiji Water Authority	0.34 (0.15-0.76)	**0.009**
Piped water, private or local	0.75 (0.31-1.86)	0.540
Others (Rainwater tank, borehole, river or stream)	1.00[a]	
By school latrine type		
Water/pour-flush	1.80 (0.16-20.23)	0.631
River or bush	1.00[a]	

N.B.: a: Reference value

The school-level effects on any STH infection was examined by adjusting for the effects of differences in the distribution of individual and household-level risks between schools. Using multilevel logistic regression models, school-level variances were evaluated for the different characteristics of children. Briefly, a comparison between the null model and the random intercept model indicated that two-thirds of the school-level variation was accounted for by the individual characteristics considered in the model. When school-level characteristics were added to the model as explanatory variables, none remained significant and it does not explain the much between-school variability. Individual and household-level risks were only retained in the model as there was no statistically significant interaction between school-level covariates and any individual characteristics with previously statistically significant effects.

Allowing for school level variations in the multivariate model, children who were 4-6 year old were more likely to be infected with any STH, compared to the 7-10-year-olds (Table 7.4). Children who were reported to wear shoes 'usually' had a lower chance of being any STH eggs positive, in contrast to the students who were reported to wear shoes less frequently such as 'not always but sometimes' or 'not at all'. As for the main water source at home, those having piped water from the Fiji Water Authority had the lower risk of any STH infection, compared to the children whose home water sources were either piped water from private or local sources or others (rainwater tank, borehole, river or stream). These explanatory variables with statistically significant ß coefficients were then allowed, one by one, to vary across schools, but no variable had significantly varying effects on any STH infection prevalence, confirming the final model as a random-intercept one rather than random coefficient.

The linearity of the association between the coverage of having the piped water from the Fiji Water Authority as the main water source, and sanitation facilities either with water-seal or pour-flush type latrines at students' households and school-level any STH infection prevalence was explored by using locally weighted scatterplot smoothing (LOWESS) (Figure 7.2a and 7.2b). Overall, there is a declining trend of any STH infection prevalence at school level when the coverage levels of having the piped water from the Fiji Water Authority as the main water source and sanitation facilities either with water-seal or pour-flush type latrines at students' households increase.

Figure 7.2a LOWESS curve for students' household piped water from the Fiji Water Authority (FWA) coverage at each school and school level any STH infection prevalence

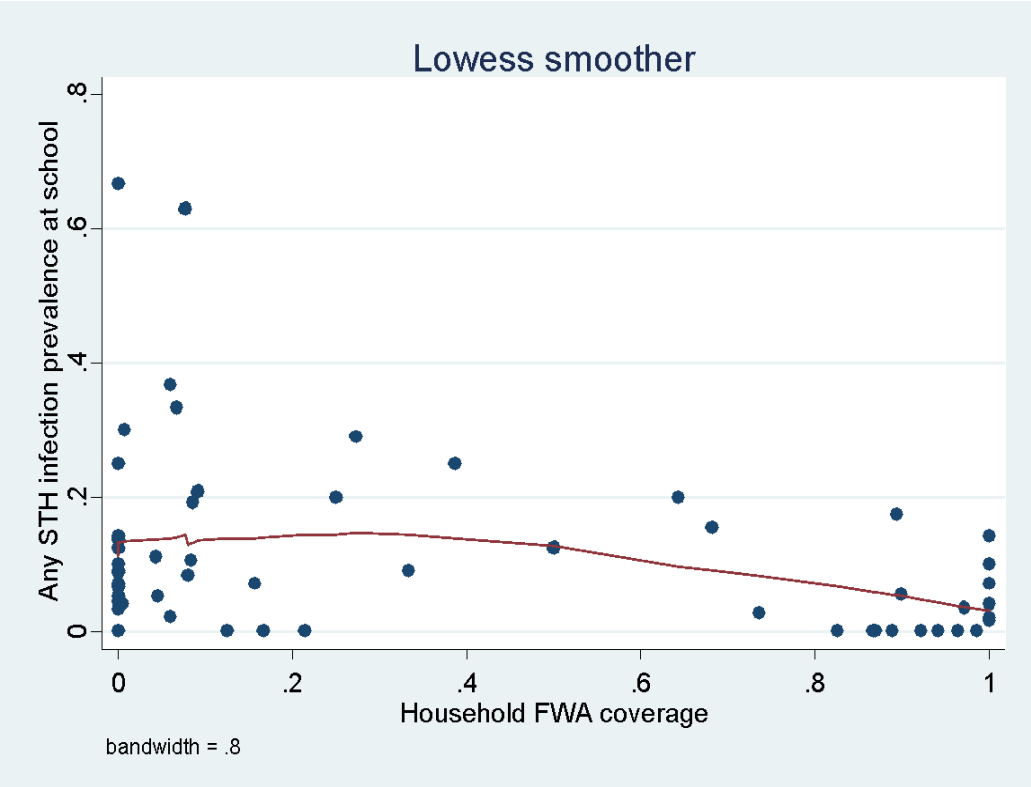
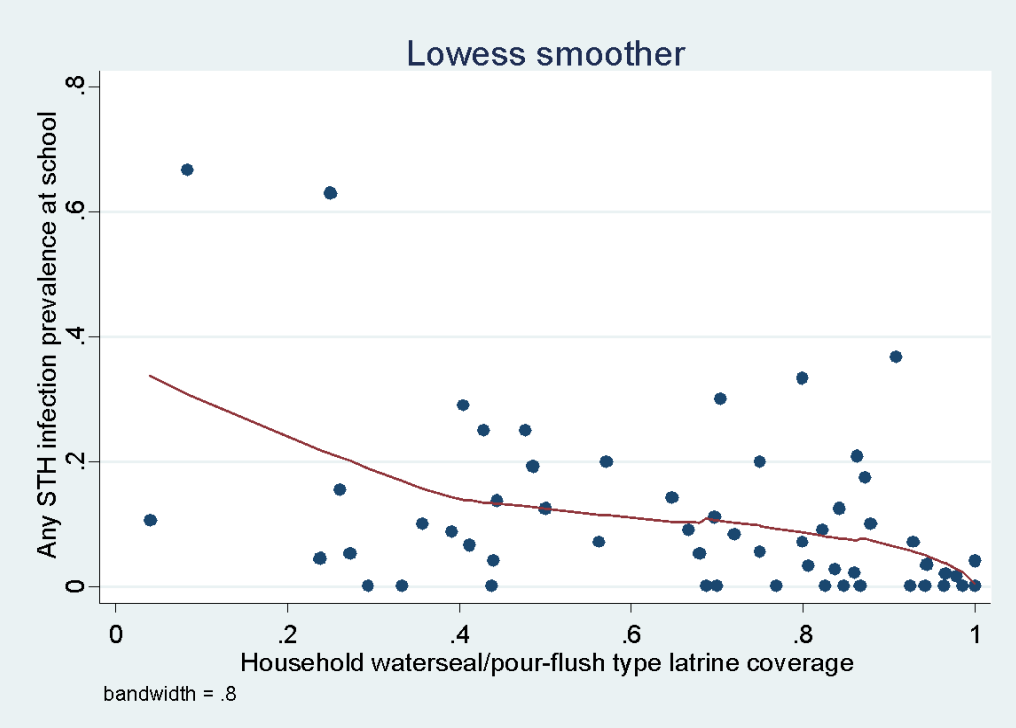


Figure 7.2b LOWESS curve for students' household water-seal or pour-flush type latrine coverage at each school and school level any STH infection prevalence



7.3.5. Association between geospatial and socioeconomic characteristics and school-level STH infections

In the multivariate analysis for the factors associated with STH infections, covariates such as forest type, dominant soil type, mean maximum temperature of the warmest month, population density, poverty gap index showed their statistical significance even after adjusting all other covariates included in the model for *Ascaris* infections (Table 7.6). As for the hookworm infections, after adjusting all other covariates in the model, factors such as elevation, dominant soil type, maximum EVI and mean maximum temperature of the warmest month, population density showed IRR with statistical significance. It was only mean maximum temperature of the warmest month which was associated with the increased incidence rate for the upper-half values compared to their lower half values for both species (Table 7.6).

Table 7.6 Results of multi-variate analysis of the associated incidence rate of *Ascaris* and hookworm infections at schools of the Western, Central and Northern Divisions of Fiji, 2014-2015

Characteristics	*Ascaris* infection			Hookworm infection		
	IRR*	95% CI	*P*	IRR*	95% CI	*P*
Elevation						
≤1 m	1.00[a]			1.00[a]		
1-20 m	0.45	0.19, 1.02	0.057	0.51	0.06, 4.23	0.531
20-80 m	1.67	0.87, 3.21	0.125	6.58	1.94, 22.34	**0.003**
>80 m	0.83	0.35, 1.96	0.658	13.70	3.20, 58.58	**0.000**
Forest						
Dry	1.00[a]			-		
Wet	8.15	2.22, 29.93	**0.002**	-		
Dominant soil type						
Chromic Cambisols	1.00[a]			1.00[a]		
Eutric Cambisols	0.76	0.09, 6.53	0.803	2.10	0.22, 19.49	0.516
Ferralic Cambisols	0.44	0.07, 2.65	0.373	8.12	1.25, 52.73	**0.028**
Dystric Gleysols	0.01	0.00, 0.13	**0.000**	2.20	0.12, 41.02	0.598

Characteristics	*Ascaris* infection			Hookworm infection		
	IRR*	95% CI		IRR*	95% CI	
EVI (Enhanced vegetation index)						
Maximum						
≤0.65	-			1.00[a]		
>0.65	-			2.01	1.01, 4.00	**0.047**
Minimum						
≤0.25	1.00[a]			-		
>0.25	0.83	0.50, 1.40	0.496	-		
Mean maximum temperature of the warmest month						
≤31.1 °C	1.00[a]			1.00[a]		
>31.1 °C	4.79	1.72, 13.31	**0.003**	6.47	1.58, 26.40	**0.009**
Total annual rainfall						
≤2 m	-			1.00[a]		
2-2.5 m	-			0.81	0.22, 3.01	0.753
2.5-3.0 m	-			12.81	0.87, 189.62	0.064
>3.0 m	-			2.19	0.30, 16.37	0.446
Total rainfall during the trade season (May-October)						
≤0.45 m	1.00[a]			-		
0.45-0.60 m	1.51	0.35, 6.50	0.580	-		
0.60-0.75 m	2.76	0.45, 17.12	0.273	-		
>0.75 m	0.84	0.11, 6.62	0.865	-		
Population density (per 100 m2)						
≤0.4	1.00[a]			1.00[a]		
0.4-0.8	2.05	0.33, 12.79	0.442	0.23	0.08, 0.70	**0.010**
0.8-1	0.38	0.11, 1.27	0.116	3.95	0.57, 27.40	0.164
>1	6.96	2.06, 23.60	**0.002**	0.09	0.01, 0.88	**0.038**
Poverty rate						
≤30 %	-			1.00[a]		
30-40 %	-			0.23	0.03, 1.73	0.152
40-50 %	-			3.84	0.36, 40.93	0.264
>50 %	-			1.60	0.18, 14.65	0.674
Poverty gap index						
≤7 %	1.00[a]			-		
7-11 %	0.18	0.04, 0.88	**0.034**	-		
11-20 %	0.06	0.01, 0.43	**0.005**	-		
>20 %	0.04	0.01, 0.33	**0.003**	-		

N.B.: a: Reference value and IRR denotes incidence rate ratio, which is the estimated rate ratio for a one unit increase of the variable, given the other variables are held constant in the model.

7.4. Discussion

7.4.1. Factors associated with STH infections on Fiji

Though STH infections are still widely prevalent, studies on this topic in Oceania as well as in Fiji, are limited, and here we present an in-depth investigation of the factors associated with STH infections in Fijian school children living in both urban and rural communities. In the mono-variate analysis, several factors were found significantly statistically associated with STH infections, but once controlling for covariates, the strength of association for some factors disappeared. Thus, the following discussions focus on the risk factors included in the multivariate models.

7.4.1.1. Demographic and WASH-related characteristics associated with STH infections

It is well known that the risk of infections decreases with age for *As. lumbricoides* and *T. trichiura*, while it increases for hookworm infection (Muller 2002; Gabrie et al. 2014). In our study population, we observed a pattern that increased age has a putatively protective effect on any STH infection even if our study population included only class 1 and 2 students. This finding may be due to the fact that the majority of any STH infection came from *Ascaris* species rather than hookworm, as described in Chapter 6. This is also in line with the findings of our previous study in the sentinel sites surveillance of the Eastern Division (Figure 6.6), where the wider age range of children from class 1 to class 8 students were included and the oldest age group had the lowest level of *Ascaris* infection prevalence. One plausible explanation would be that older children would adopt hygienic behaviours better than their younger counterparts, but we did not measure this association among the wider population age groups in our study. Another explanation would be that students who were older had been exposed more frequently to albendazole distribution, which was similarly observed among class 2 students, given that class 1 students were the new entrants to the school and had been excluded from the school-based deworming.

Our data show a marginal statistically significant difference between children practicing shoes wearing behaviours regularly and children who do not or do it occasionally: The former had only a half of odds of being infected by any STH infection, implying that shoes wearing has a protective effect on any STH infection. A recent meta-analysis (Ziegelbauer et al. 2012; Strunz et al. 2014) supports evidence of a strong association between wearing shoes and lower odds of hookworm infection (k = 5, OR 0.29, 95% CI 0.18–0.47%). Wearing shoes was also associated with lower odds of infection with any STH (k = 3, OR 0.30, 95% CI 0.11–0.83%)

(Strunz et al. 2014) as shown in our study population, even if hookworm infections were not the major species of infection but *Ascaris* species was. The proportion of children who did not practice regular shoes wearing reached more than one-third of the study participants, and it may be higher, in reality, considering that this is self-reported shoes wearing by caregivers rather than the actual observation of the practice. The findings underscore the needs for studies to explore whether local populations are unaware of the health benefits of wearing shoes or what are the barriers for children to wear shoes regularly in Fijian settings (Paige et al. 2017).

A major finding from this study was that having a main water supply from the Fiji Water Authority had the statistically significant contributing effect in lowering any STH infection risk compared to any other water source. Using piped water from private or local source did have lowering impacts on the STH infection prevalence, but there was no statistical significance in univariate analysis. This is similar to what had been reported in the recent meta-analysis, where using treated water (filtered or boiled) was associated with lower likelihood of having any STH infection, but piped water was not (Strunz et al. 2014). Water is a critical component of WASH resources (Mogaji et al. 2017). According to our observations from the field, having a main water supply from the Fiji Water Authority provides not only quality-controlled water but also less interrupted supply, compared to other sources. Thus it also may act as a surrogate of other WASH characteristics, such as availability of functioning washing stations, rather than a factor associated with the infection of a casual nature (Nikolay et al. 2015).

Only 12.1% of the study participants lived in the households with pit latrines and another 1.0% without an adequate option for faeces disposal. Our findings are consistent with data from the WHO/UNICEF Joint Monitoring Programme stating that 92.8% of homes had improved sanitation facilities (WHO and UNICEF 2015). It has been documented well that the lack of improved sanitary facilities increases children's risk of acquiring STH infections (Conlan et al. 2012; Gabrie et al. 2014) especially of *Ascaris* species (Scolari et al. 2000). In our study, having the water-seal or pour-flush type of latrines at home showed the protective effect of any STH infection compared to no latrine at home in monovarietal analysis, but it lost its statistical significance in multivariate analysis. Nevertheless, it should be noted that we did not measure the actual accessibility or functionality of the sanitation facilities at home. Conversely, it is possible that not all children were using or willing to use the latrines at home, thus actual usage of the improve sanitation facilities would be lower. These may explain why our multivariate analysis did not identify having the water-seal or pour-flush type of latrines at home as one of the protective factors for STH infections.

7.4.1.2. Geospatial and socioeconomic characteristics associated with STH infections

Studies have shown that the intensity of STH infection transmission is influenced by climatic factors such as the land surface temperature and normalized difference vegetation index (Pullan et al. 2008; Brooker et al. 2015). In our study, *Ascaris* and hookworm infections favoured different geospatial and socioeconomic conditions in the study area. Contrasting relationships with vegetation indices were observed for *Ascaris* and hookworm infections. *Ascaris* infections clearly favoured 'wet' forest, but this association was not observed in the cases of hookworm infections. In addition, a higher half of maximum vegetation indices were associated with increased incidence rate ratio for hookworm infections, which was not observed for *Ascaris* infections, suggesting that there are differences in transmission dynamics according to STH species. The positive association between increased vegetation indices and higher incidence rate ratio for hookworm infections is consistent with findings with other studies (Saathoff et al. 2005; Wardell et al. 2017). Given that EVI and NDVI are a measure of vegetation and thus a proxy for environmental moisture and shade studies (Wardell et al. 2017), this finding further supports that hookworm requires soil moisture for survival and transmission (Udonsi and Atata 1987; Brooker and Michael 2000). In comparison, similar relationships with mean maximum temperature of the warmest month was observed for *Ascaris* spp. and hookworm infections, implying that in tropical areas there were conditions that both species favour. The latter is expected given that maximum survival rates of the larvae occur at 20-30°C for hookworm and between 28 and 32°C with the development of *As. lumbricoides* (Brooker et al. 2006).

STH infections flourish in impoverished areas with inadequate sanitation and overcrowding (Brooker et al. 2006), and it is assumed that *Ascaris* infections are more prevalent in urban areas whereas hookworm is more often found in rural areas (Crompton and Savioli 2006). Findings from our study are in line with the assumption: When the population density is in the highest quartile, the incidence rate ratio for *Ascaris* infections was increased compared to the lowest quartile, while it was the other way around for hookworm infections. Though it was only statistically significant for both species in the highest quartile, the reversed trend was clearly observed (Table 7.6). The increases of the poverty gap index was associated with the decreased incidence rate ratio for *Ascaris* infections. However, given that the poverty gap index is higher in the rural area (World Bank 2011), it is difficult to clearly delineate which would be the predisposing factors for another.

7.4.2. Next steps for the control of STH infection transmission in Fiji

The result presented here is relevant to the control of STH infections in Fiji in developing more tailored sets of interventions as per risk factors, as preventive chemotherapy alone will not be sufficient to eliminate the infection. School-aged children in certain localities are still at increased risk of STH infections, especially those in hot-spots, thus other measures to prevent children from STH infections such as health education and improved water and sanitation following situation analysis of the locality should be actively pursued. For instance, given that main water supply was a significant factor for the infection in our study and uninterrupted and sufficient water supply is a pre-condition for functioning sanitation facilities in the case of water-seal and pour-flush types and maintaining hygienic behaviours such as hand washing, the delivery of integrated approaches as a package should be a considered wherever possible (Strunz et al. 2014; Erismann et al. 2016). Thus, it is recommended to apply an integrated and inter-sectoral control approach to promote the expansion of water treatment and safe storage provided by the Fiji Water Authority, as well as improvement of the toilet conditions combined with proper health education on hygienic behaviours would be the critical to reduce the parasitic intensity and to reduce morbidity from STH infections among school children in Fiji. In response to the findings, the National Framework on STH infections Prevention and Control (attached in Annex), developed by the writer, was adapted by the Ministry of Health and Medical Services (MHMS) in March 2017.

As for the realization of the framework, fortunately, the MHMS of Fiji had already launched an inter-sectoral approach as a key initiative of delivering primary health care to improve and achieve the ultimate health outcomes of the population of Fiji where MHMS is a leading agency in service delivery, community collaboration, and creating partnership but the roles of other sectors are also highlighted (MHMS 2017). The inter-sectoral approach to prevent and control parasitic infections is proved to have benefits on schoolchildren's physical development and educational achievement (Gazzinelli et al. 2012), but also improvements in WASH infrastructures and health-related behaviours are key to achieve sustained control and elimination of NTDs (WHO 2015b; Erismann et al. 2016). This, in turn, will contribute to ways of moving forward with implementing the Sustainable Development Goals (SDGs) agenda (UN 2017), not only for the goal number 3, "ensure healthy lives and promote well-being for all at all ages", but also for the goal number 6 "ensure availability and sustainable management of water and sanitation for all" (Bangert et al. 2017; UN 2017). This inter-sectoral approach at country level has been adapted in the national STH infection control strategy (MHMS 2017)

where the SDG core indicators for the goal numbers 3 and 6 were also included as part of its monitoring and evaluation frameworks (UNSG 2017).

As this study showed, schools are an ideal platform to reach children and their caregivers to provide health services and information. For a longer-term success, it would be important not only to resume mass deworming for at-risk populations but also to complement integrated WASH interventions by utilizing schools as entry points of service delivery. A new project called 'WHO Health Promoting Schools in Fiji' in partnerships with WHO, was launched by the government of Fiji in 2016 from the funding provided by the Government of the Republic of Korea, where the writer played a critical role in mobilization of the fund based on this study's finding to justify the establishment of the project. An in-depth assessment has been conducted in order to identify the best set of interventions including school deworming and WASH interventions and to understand the possible impacts of these interventions on schoolchildren' health (MHMS, unpublished). It will provide opportunities to link the school health programmes to STH infection control primarily at selected schools but eventually also at children's households. Given that inter-sectoral intervention hold promise to make a lasting impact on STH infections by combining school-based and community-based interventions that go beyond WASH (Erismann et al. 2016), the project could set the local example to prove it by including health education, behavioural modification, as well as WASH components on top of the preventive chemotherapy for STH infection control via close collaboration with the Ministry of Education.

7.4.3. Limitation of the study

Our study has several limitations. Firstly, the analysis of factors was restricted to the variables that we had measured, thus it may not capture the whole picture of the local STH transmission dynamics (Greenland et al. 2015). Since it was the first attempt to use LF TAS as a survey platform to assess the STH epidemiology in Fiji, we used the simple questionnaire for the collection of WASH-related data, to ensure that surveys were highly feasible and efficient to be conducted in the field. In this regard, measurement of the individual and school level WASH characteristics was based on self-reporting, rather than direct observations by the survey team; therefore, it is possible that the frequency of desirable hygiene practices was over or under-estimated, and the functionality or accessibility of water and sanitation infrastructures was not appropriately reflected in the answers. It may be useful to directly assess more detailed WASH variables at both levels, as long as other opportunities and additional resources are available.

Moreover, STH infection prevalence levels were assessed only among class 1 and 2 school-children, not in the community, and school-based surveys may not be sufficient to represent the STH transmission dynamics of the local population. Given that the National iron and micro-nutrient supplementation (NIMS) programme targeted pre-school age children (pre-SAC) and school-age children (SAC), and hookworm is found to the main species of STH infection in the Northern division, which can be with higher intensity infections for an older group of people compared to our study participants (Nikolay et al. 2014), it may be necessary to appraise STH infection status among pre-SAC and older children at the community to explore whether other factors are involved for these age groups.

Lastly, measurement of the WASH covariates was undertaken following the infection, whereas causality is time-bound, and causes should precede their dependent effects. Thus, the association reported here may not be a nature of causation (Benjamin-Chung et al. 2015). We also assumed that the value of the covariate measured at a time point had been consistent over the time, which could not be always realistic especially for individual hygiene practices. Similarly, covariates derived from remotely sensed data are a spatial aggregation of a spatially continuous phenomenon (Stanton 2017), with the own boundaries of each cell being defined by the organizations from which the data were sourced. These cells are aggregated or disaggregated, to ensure a consistent spatial resolution, and the method by which the data are processed can have an impact on the association between the data and the point-level infection prevalence (Stanton 2017).

7.5. Conclusion

We examined the associations between demographical, WASH as well as other environmental/socioeconomic variables and STH infections in a comprehensive manner, which captured different levels of exposures at home and school among Fijian school children especially for WASH. We utilized the data obtained from the STH infection prevalence surveys and household and school-based WASH infrastructure and behaviours assessment, adapting the LF TAS as a survey platform. Results suggest that there are mixed impacts of household and school WASH characteristics on the prevalence of infection as reported in other studies (Freeman et al. 2013). Impact of geospatial and socioeconomic risk factors differed across individual worm species, which is expected as they reflect the divergent mechanisms of the infection. This study provides important data for the national STH infection control programme regarding what would be the essential component of the programme and how to link to other programmes in delivering effective control strategies in order to achieve the new

goal of eliminating STH infections in Fiji. Furthermore, the results provided here were acknowledged by the health authorities in setting up the baseline of the core programme indicators to track progress in terms of integrated NTDs control in line with SDG goals (WHO 2015e).

Chapter 8

Pilot assessment of the epidemiology of *Strongyloides stercoralis* infections on Fiji and application of molecular techniques as a diagnostic quality control tool for intestinal parasite infections

Chapter 8. Pilot assessment of the epidemiology of *Strongyloides stercoralis* infections on Fiji and application of molecular techniques as a diagnostic quality control tool for intestinal parasite infections

This Chapter has been published in a modified form in the Parasite Epidemiology and Control, Volume 1 (2016) 263–267. The published article is enclosed in the Appendix.

8.1. Introduction

Strongyloidiasis is a chronic parasite infection caused by a nematode, *Strongyloides stercoralis* (SS) and it presents in tropical and subtropical regions as well as in temperate climates. Currently, WHO estimates that around 30–100 million people are infected worldwide (WHO 2015d), with prevalence rates in some cohorts being as high as 50% (Muller 2002). Behaviours of *S. stercoralis* differ from other intestinal nematodes that larvae are excreted in the stool but can develop into infective form in the human intestine and then penetrate the mucosa or perianal skin, resulting in a prolonged cycle of autoinfection (Olsen et al. 2009). Like other STH infections, the risk of infection is associated with poor hygiene, putting children at higher risk (WHO, 2015d). Without appropriate treatment, the infection would not resolve by itself and may persist for life. However, as of now, no public health strategies for controlling the infection has been in place.

8.1.1. Epidemiology of strongyloidiasis in Oceania

Oceania is a region of tropical and sub-tropical islands in the Pacific where one-quarter of the population is impoverished placing them at increased risk of several neglected tropical diseases (NTDs) (Kline et al. 2013). Amongst others, lymphatic filariasis (LF) and soil-transmitted helminthiasis (STH) are particularly widespread in the region . The epidemiology and importance of *Strongyloides stercoralis* infection, however, is not well-known, (Kline et al. 2013; Olsen et al. 2009) outside of Australia (Miller et al. 2014), Papua New Guinea (Igra-Siegman et al. 1981) and the Solomon Islands (Pattison and Speare 2008).

8.1.2. Epidemiology and control of strongyloidiasis in Fiji

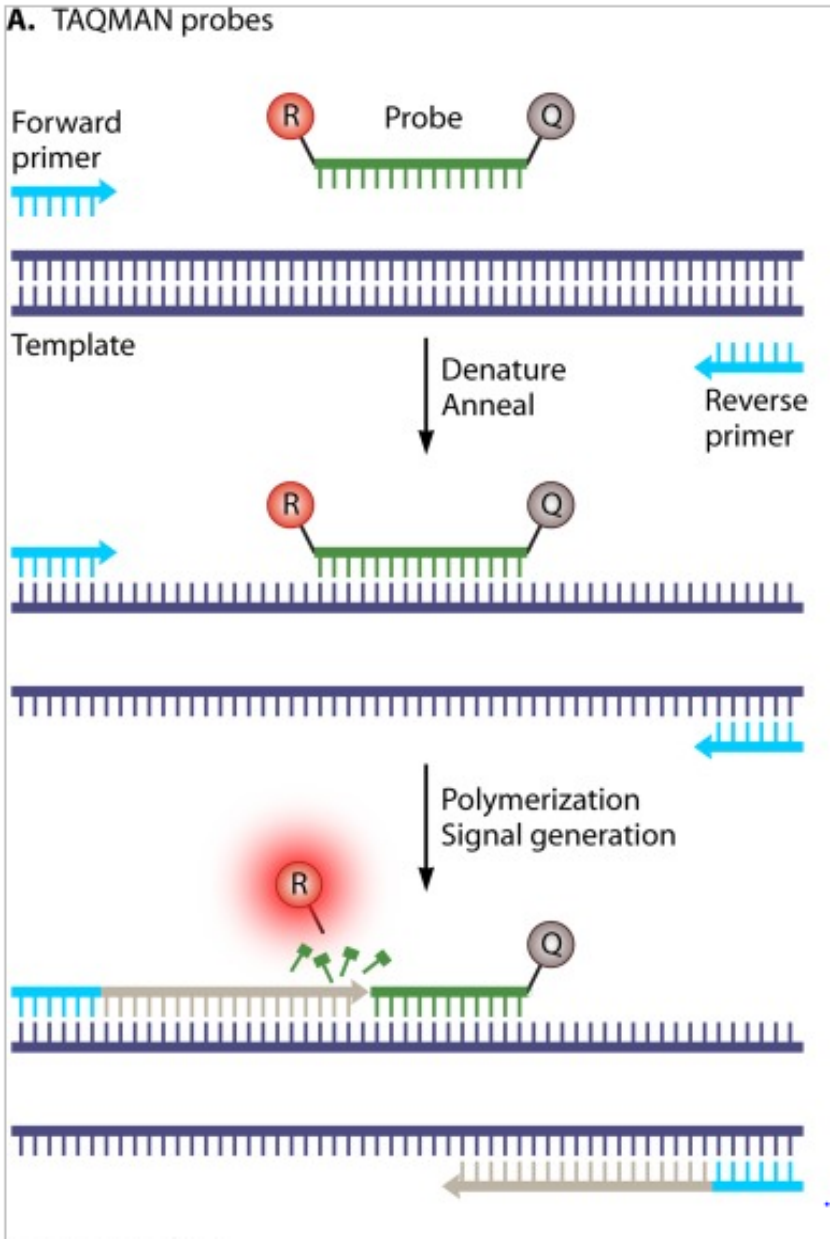
With the fourth largest population in Oceania (Fiji Bureau of Statistics 2009), some 835,000 Fijians reside mostly on two major islands, Viti Levu and Vanua Levu, out of the 100 or so currently inhabited. Throughout Fiji, there has been a long history of interventions against LF and STH (WHO 2017f) and un-updated epidemiologic profiles are described in the previous Chapters (Chapter 3 and 6). However, typical of other islands in the vicinity, given the warm and humid climate and often-inadequate sanitation, the occurrence of *Strongyloides stercoralis* infection is likely (Muller 2002), but not well known. Previous parasitological surveys have attempted to determine the general prevalence of STH, but with insensitive diagnostic methods used, such as direct faecal smears (Lott 1980; Jansen et al. 1991), all geohelminth infections have likely been under-reported including *Strongyloides* spp. infections. Nevertheless, several clinical cases of strongyloidiasis in travellers or migrants from Fiji have been reported, which point towards existing on-going transmission (Coulter et al. 1992), which needs to be affirmed locally.

8.1.3. Diagnosis of intestinal parasite infections with real-time PCR

To assess STH infections within a population Kato-Katz thick-smear technique by counting the number of eggs per gram of faeces (Verweij et al. 2007) would be the most common method currently used. However, microscopic examination of Kato-Katz smears has limitations as its sensitivity and specificity could be hampered especially for hookworm species: 1) species identification between hookworm eggs and *Oesophagostomum bifurcum* may not be possible due to the fact these eggs are morphologically similar; and 2) hookworm eggs may not be visible unless the slides are read within 30 minutes (Verweiji et al, 2007). Also, Kato-Katz smear technique does not allow the diagnosis of intestinal protozoan infection, nor that of *Strongyloides stercoralis*, given that it relies on the detection of eggs.

Over the last few decades, a variety of nucleic acid-based methods have been developed for the diagnosis of intestinal parasite infections (Verweij and Stensvold 2014). Based on the advantages such as increased sensitivity and specificity and simpler standardization of diagnostic procedures, they emerge as a valuable tool for surveys and surveillance studies (Verweij and Stensvold 2014). Real-time PCR (polymerase chain reaction) is one of the DNA based technique where the production of amplicons is measured in "real time" during the amplification process (Klein 2002), and it has also overcome the drawbacks of PCR from the early years, such as contamination risk from amplified products (Verweij and Stensvold 2014).

Figure 8.1 The example of probe-based real-time PCR chemistry: Hydrolysis or TaqMan probes (Verweij and Stensvold 2014)

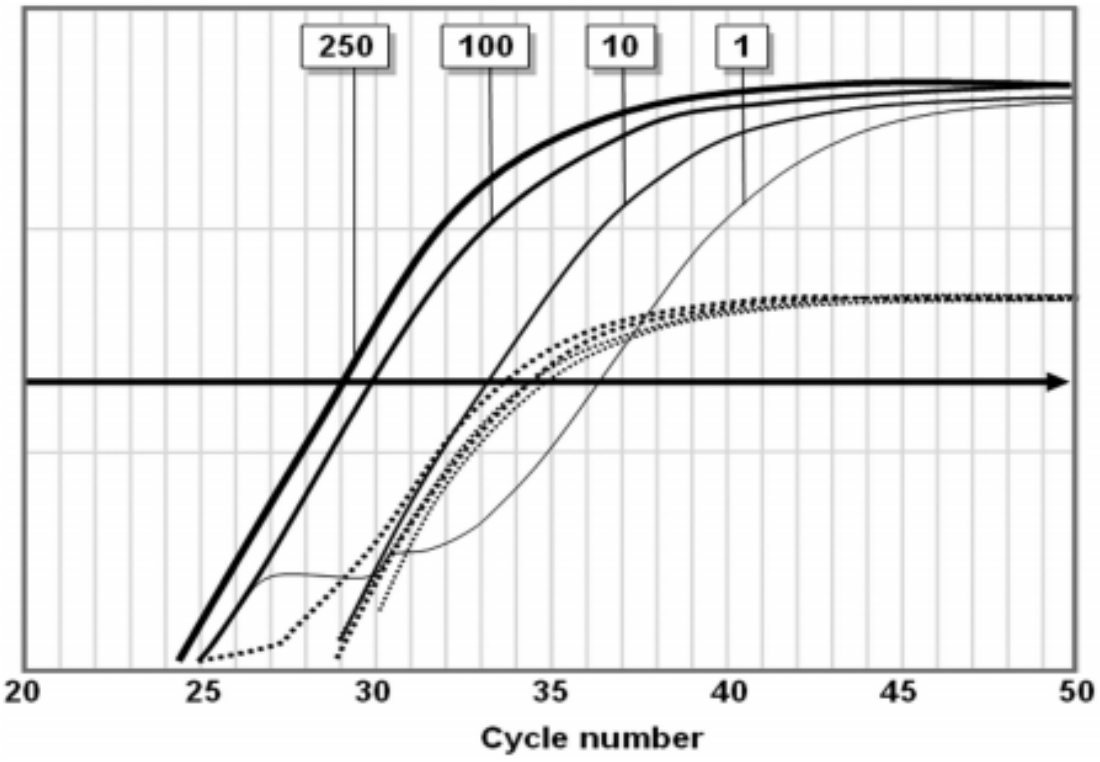


N.B.: The fluorescent molecule at the 5' end of the probe is separated from the quencher molecule at the 3' end of the probe, resulting in a fluorescent signal that can be measured after each amplification cycle

The most commonly used probe-based methods is the use of hydrolysis or TaqMan probes (Figure 8.1) (Verweij and Stensvold 2014): To establish the PCR assay, genomic DNA was isolated using the QIAamp Tissue Kit (The 5'-to-3' exonuclease activity of *Taq* polymerase cleaves the hybridized probe during the elongation phase of the amplification reaction. The amplification cycle at which the level of the fluorescent signal exceeds the background fluorescence (threshold cycle, Ct-value) is directly correlated with the initial amount of target

DNA in the sample (Figure 8.2). Separate measurement of probes with different fluorophores emitting fluorescence at different wavelengths enables the implementation of multiplex PCR of similar-sized DNA fragments with the same efficiency (Verweij and Stensvold 2014). DNA samples can be stored and used for genetic characterization and molecular typing. With the advance of technology for real-time PCR, it is also possible to combine more than one target organism in a multiplex assay simply. However, the technique requires highly skilled human resources and a substantial amount of labour time compared to the conventional microscopy, as well as the costs for the machines and consumables, though it provides species specific diagnosis with higher sensitivity compared to the conventional microscopic examination techniques (Verweij and Stensvold 2014).

Figure 8.2 Detection of parasite DNA from faecal suspension containing 1, 10, 100 or 250 oocysts of a parasite (ten Hove 2009)



N.B.: The continuous curved lines show an increased fluorescent signal of the parasite-specific probes and the dotted curved lines are from corresponding internal controls. Samples with a higher concentration of initial DNA templates will cross the fluorescent threshold (horizontal arrow) at a lower amplification cycle number.

8.1.4. Justification of the study

To shed light on the occurrence of SS infections on Fiji, which had not been conducted in the area so far with diagnostic tests specific and sensitive enough to detect strongyloidiasis in the stool samples, our study aimed to pilot epidemiological assessments by taking advantage of the existing national LF and STH programme evaluation activities, rather than conducting a stand-alone initiative. We detailed how a transmission assessment survey (TAS) for LF and STH survey provided a convenient access opportunity to determine the extent of SS infections in school-aged children. In addition, we demonstrated the feasibility of using multiplex PCR as a quality control tool for the diagnosis of intestinal parasite infections in a resource limited setting, by archiving part of stool samples and transferring them to the reference laboratory with the availability of molecular techniques.

8.2. Methods

8.2.1. Study area

The survey was conducted in the Western Division, which is a drier western half of Fiji's biggest islands, Viti Levu (Figure 3.5). The area is further classified ecologically into Lowland dry zone in the western half, also called as Strong Dry zone, and Lowland intermediate zone in the east, called as Moderate Dry zone (FAO 2009). Annual mass drug administration (MDA) using albendazole and diethylcarbamazine citrate (DEC) for LF was conducted from 2001 to 2009, resulting in a very low LF antigen prevalence (< 1%) in the survey conducted in 2007 (Ministry of Health and Medical Service, data unpublished). This warranted the first TAS (TAS 1) in 2011 revealing one only positive child for LF antigen in the blood, and subsequently, the second TAS (TAS 2) was planned for 2014 which forms the access opportunity for this investigation. Since 2010, annual albendazole distribution targeting pre-school and school-aged children was also started in the surveyed area as part of the National Micronutrient Supplementation Programme together with ferrous sulphate tablets administration as detailed in Table 6.1 (Vasu 2015).

8.2.2. Study design and sampling strategy

There are 249 primary schools registered at the Ministry of Education in the Western Division, and 77 schools were systematically selected for TAS 2, using Survey Sample Builder (The Task Force for Global Health, Georgia, USA) as previously described. Furthermore, 30 schools were sub-sampled for STH infection prevalence assessment, 10 schools per each of

three ecological zones (10 urban and rural in Strong dry zone and 10 in Moderate dry zone), following the WHO guidelines (WHO 2011a). The spatial coordinates of each school were recorded by a hand-held GPS unit (e-trex, Garmin Ltd, Kansas, USA) in decimal degrees.

8.2.3. Data collection procedure and microscopic examination of specimens

Before initiating the LF antigen test via finger-prick, class 1 and 2 students who were born between 2007 and 2008 were asked to submit stool containers with their fresh morning stool. Then stool samples were transported to the national parasitology reference laboratory, using cool boxes. The Baermann concentration technique (BC), as described in Chapter 2 (Stothard et al. 2008) was applied for the detection of *Strongyloides* spp. larvae for all stool samples arrived at the lab for three days specifically designated for the strongyloidiasis surveillance. To save BC processing time, between 4 and 8 stools were pooled and examined simultaneously.

8.2.4. Molecular diagnosis of strongyloidiasis and other intestinal parasite infections

8.2.4.1. Sample collection

Seeking a wider appraisal across the Western Division of Fiji on the intestinal parasite infections, a selection of samples identified positive for all geohelminths as well as gastrointestinal protozoa by coproscopy together with a systematic sub-sample (every 10th negative samples) were filtered through a 212-micron metal sieve and preserved in 95% ethanol, then transported to the reference laboratory located in the Netherlands for molecular diagnosis of intestinal parasite infections, since this was not locally available in Fiji. In the reference laboratory, the samples were stored at -20°C until detection of parasite DNA loads by multiplex PCR. DNA isolation, amplification and detection were performed blinded to previous microscopic results (Meurs et al. 2017).

8.2.4.1. DNA extraction

For extraction of DNA in faecal samples, the Powersoil DNA Isolation Kit (Mo Bio, Carlsbad, CA USA) was used with minor modifications. Briefly, Zirconia/Silica 0.5 mm beads (Daintree Scientific, St. Helens, Tasmania, Australia) were used in place of supplied beads. Each extraction used 370 μL of the Powerbead solution and 0.2 g faeces. The Precellys®24 Lyser/Homogeniser (Bertin Technologies, Montigny-le-Bretonneux, France) (setting "6500 1x60 015") was used in a one-minute homogenization step in place of vortexing. Further alterations to kit protocol included: processing the entire supernatant through each step to

avoid loss of material, reducing the volume of Solution C4 to 1 ml, and an additional standard dry filter centrifugation step following Solution C5 removal. DNA was eluted from the column in a 100 μL of buffer and stored at -20˚C prior to PCR. A phocine herpesvirus (PhHV) only control was included in each batch processed as a comparison.

8.2.4.2. Multiplex PCR optimisation

Extracted DNA was run in two pentaplex real-time PCR reactions (Llewellyn et al. 2016). The first was an assay for *N. americanus*, *Ancylostoma* spp. (*An. duodenale*, *A. ceylanicum*), *Ascaris* spp., *T. Trichiura* and PhHV; the second was an assay for *E. histolytica*, *Cryptosporidium* spp., *G. duodenalis*, *S. stercoralis* and PhHV (Verweij et al. 2007; Verweij et al. 2004; Llewellyn et al. 2016). The Rotor-Gene 6000 (Qiagen, Melbourne, VIC AUS) was used for all PCR assays (Basuni et al. 2011). For amplification reaction mixture consisted of Hotstar Taq mastermix (Qiagen), 5mM MgCl2 (Bioline(Aust)), optimized primer and probes as well as 2 μL of template DNA in a total volume of 20 μL was prepared, where details of the primers and probes are listed in Table 8.1. For the STH multiplex PCR, the DNA amplification was performed using the following conditions: 5 minutes at 95˚C, followed by 40 cycles of 95˚C for 9 seconds and 60˚C for 60 seconds. The protozoa, other nematodes and *S. stercoralis* multiplex PCR were performed using the following conditions: 15 minutes at 95˚C followed by 40 cycles of 95˚C for 30 seconds, 55˚C for 30 seconds and 72˚C for 30 seconds. Assay optimisation was initially undertaken for each target in conventional single-plex PCR with positive control genomic DNA as a template (*An. duodenale, N. americanus* and *As. lumbricoides*, *T. trichiura*, *E. histolytica, G. duodenalis*, and *C. parvum;* and *S. stercoralis*) (Llewellyn et al. 2016).

The concentrations for each primer pair within each multiplex reaction were optimised in single plex PCR by primer limiting experiments using plasmid controls of each PCR. Ct-value comparison between multiplex and single plex PCR assays were also performed, with concentrations selected for greatest sensitivity without detrimentally affecting consecutive PCR reactions. Further optimisations were also performed to compare the ability of the multiplex PCR to detect mixed infections with dilutions of target organisms tested across a range of background DNA. The fluorescence threshold was set at 10% for *N. americanus*, *Ancylostoma* spp., and *Cryptosporidium* spp.; 20% for *G. duodenalis*; and 30% for *Ascaris* spp., *T. trichiura*, *E. histolytica*, *Strongyloides* spp., and the PhHV control. The PhHV Ct-value was monitored for each sample, with values more than two cycles higher than the expected Ct-value considered unsuccessful, with repeat extraction required. The expected PhHV Ct-value was determined by comparison to the PhHV-only control and surrounding samples.

Table 8.1 An overview of multiplex PCR for stool samples from class 1 and 2 students in the Western Division of Fiji, March 2014 (Llewellyn et al. 2016; Cunningham, Stothard, et al. 2018)

Target	Oligonucletide Sequence 5'—3'		Product Size	Gene Target	Final Conc. nM
Necator americanus	Forward	CTGTTTGTCGAACGGTACTTG C	101bp	ITS2	200
	Reverse	ATAACAGCGTGCACATGTTGC			200
	Probe	FAM-CTGTACTACGCATTGTATAC—MGBNFQ			100
Ancylostoma spp.	Forward	GAATGACAGCAAACTCGTTGT TG	71bp	ITS1	100
	Reverse	ATACTAGCCACTGCCGAAACG T			100
	Probe	VIC-ATCGTTTACCGACTTTAG—MGBNFQ			100
Ascaris spp.	Forward	GTAATAGCAGTCGGCGGTTT CTT	88bp	ITS1	60
	Reverse	GCCCAACATGCCACCTATTC			60
	Probe	ROX - TTGGCGGACAATTGCATGCG AT- IBRQ			100
Trichuris trichuria	Forward	TCCGAACGGCGGATCA	56bp	ITS1	60
	Reverse	CTCGAGTGTCACGTCGTCCTT			60
	Probe	CY5.5 - TTGGCTCGTAGGTCGTT-BHQ-2			100
Phocine herpes virus	Forward	GGG CGA ATC ACA GAT TGA ATC	81bp	gB	40
	Reverse	GCG GTT CCA AAC GTA CCA A			40
	Probe	VIC-TTT TTA TGT GTC CGC CAC CAT CTG GAT C-BHQ2			100

Continued

Target		Oligonucletide Sequence 5'—3'	Product Size	Gene Target	Final Conc. nM
Entamoeba histolytica	Forward	AACAGTAATAGTTTCTTTGGTTAGTAAAA	135bp	SSU rRNA	200
	Reverse	CTTAGAATGTCATTTCTCAATTCAT			200
	Probe	ROX—ATTAGTACAAAATGGCCAATTCATTCA—IBRQ			80
Giardia duodenalis	Forward	GACGGCTCAGGACAACGGTT	63bp	SSU rRNA	200
	Reverse	TTGCCAGCGGTGTCCG			200
	Probe	CY5—CCCGCGGCGGTCCCTGCTAG—IBRQ			100
Cryptosporidium spp.	Forward	CAAATTGATACCGTTTGTCCTTCTG	150bp	COWP	300
	Reverse	GGCATGTCGATTCTAATTCAGCT			300
	Probe	HEX—TGCCATACATTGTTGTCCTGACAAATTGAAT—IBFQ			75
Strongyloides spp.	Forward	GGGCCGGACACTATAAGGAT*	471bp	SSU rRNA	100
	Reverse	TGCCTCTGGATATTGCTCAGTTC			100
	Probe	CY5.5—ACACACCGGCCGTCGCTGC—BHQ-2			100

N.B: bp denotes base pair; ITS denotes internal transcribed spacer; SSU rRNA denotes Small subunit ribosomal ribonucleic acid; COWP denotes Cryptosporidium oocyst wall protein; and gB denotes glycoprotein

8.3. Results

8.3.1. Geographical distribution of strongyloidiasis in the Western Division of Fiji

A total of 111 faecal samples were examined using BC as a set of 19 pooled samples. Multiple *Strongyloides* spp. looking larvae were found in one sample, out of a pooled batch comprised of 7 stool samples from school 6 (Figure 8.3). When BC was performed with each of 7 samples within that batch, we identified one stool sample positive with the *Strongyloides* spp. larvae (Table 8.2). The infected child, a 6-year old boy, was subsequently followed-up in an effort to ascertain the clinical manifestations. The child did not present with abdominal pain, diarrhoea, cough, urticaria or pruritus nor sign of malnourishment, wheezing or skin abnormality. Also, laboratory tests indicated no eosinophilia or anaemia. This child was judged to be asymptomatic for SS infections, and treatment with albendazole was administered at 400 mg, once per day, for 7 days. Parents of the child were interviewed and stool samples from his family members, including 4 siblings, were tested using BC. None was found positive for *Strongyloides* spp. upon these examinations.

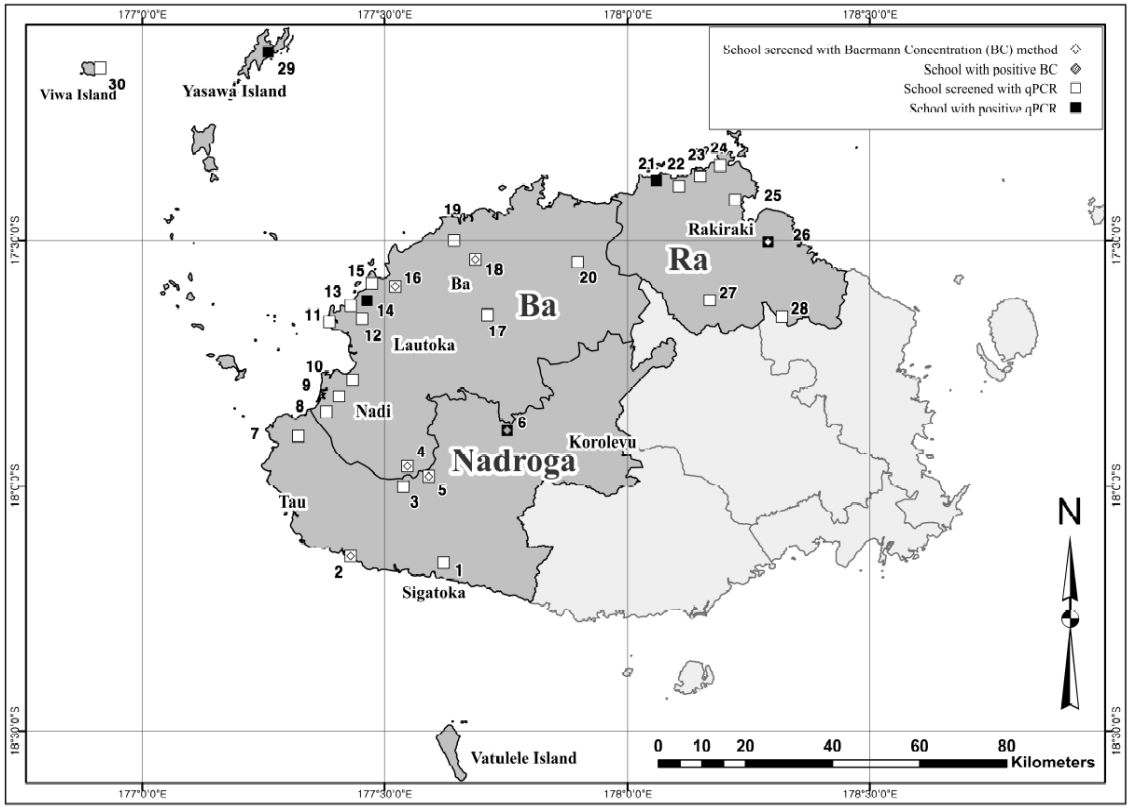
Table 8.2 Screening of strongyloidiasis among class 1 and 2 students in the Western Division of Fiji, March 2014

	Baermann concentration technique (BC)	PCR	Total
Number of students screened	111	173	262*
Number of students infected	1	6	6**
Prevalence (%)	0.9	3.5	

NB: * Stool samples of 22 students were screened by both BC and PCR; **Number includes 1 positive and 1 negative by BC; BC denotes Baermann concentration technique

To shed light on the geographical distribution of SS infections, altogether 173 faecal samples from children of 30 schools in Western Division were tested by multiplex PCR. In total, 6 samples (2 from class 1, and 4 from class 2 students) were positive for SS, including one identified by BC, at schools across the Western Division (Figure 8.3, school 6, 14, 21, 26, and 29) and overall prevalence of SS infections was 3.5 % (Table 8.2).

Figure 8.3 Sketch map of selected schools and the results of BC and (or) multiplex PCR at each school in the Western Division of Fiji, March 2014



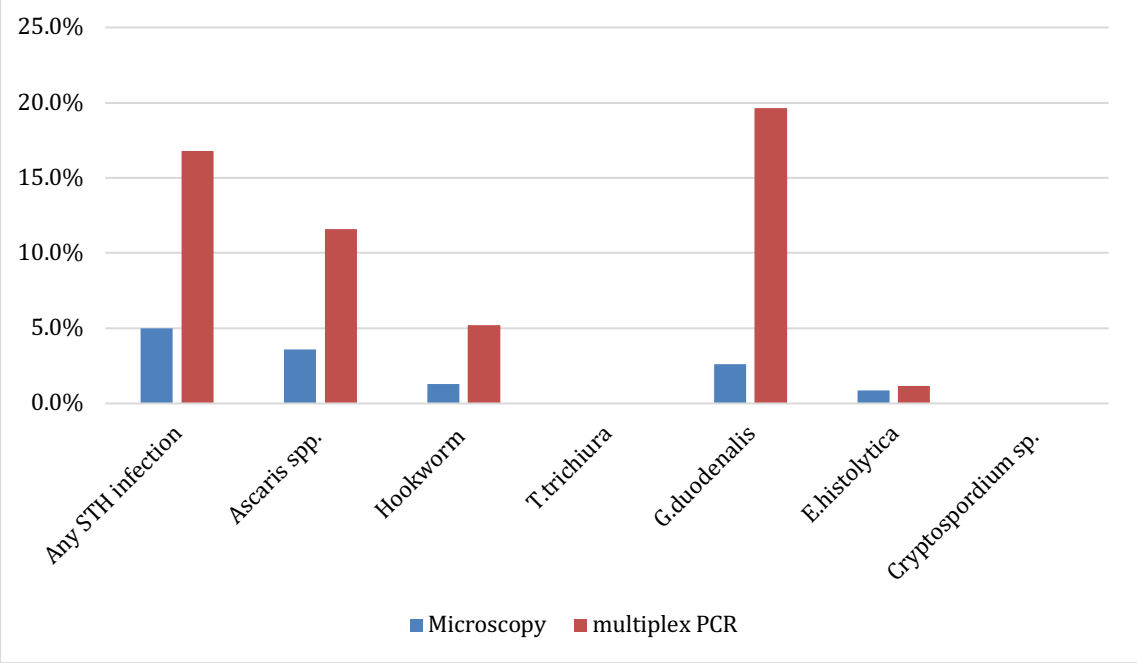
N.B.: *Strongyloides* spp. was found by BC at school No. 6 only, while by multiplex PCR, SS infections were found at 5 schools (6, 14, 21, 26, and 29)

8.3.2. Molecular diagnosis of intestinal parasite infections in the Western Division of Fiji

Apart from SS infections, the multiplex PCR technique also confirmed the presence of *As. lumbricoides*, *N. americanus*, *An. duodenale* and the absence of *T. trichiura* in the Western Division for STH infections. As for intestinal protozoan infections, it also confirmed the presence of G. *duodenalis* and *E. histolytica*, and the absence of *C. parvum*. The most common single intestinal parasitic infection was *G. duodenalis* infections, followed by *As. lumbricoides* infections (Figure 8.4).

For the detection of single intestinal parasitic infection, comparison of the prevalence of intestinal parasite infection apart from strongyloidiasis between microscopy and multiplex PCR showed that the observed multiplex PCR prevalence was consistently higher across nearly all target organisms (Figure 8.4).

Figure 8.4 Overall intestinal parasite infection prevalence comparison between multiplex PCR and microscopy, among school children in the Western Division of Fiji, March 2014



N.B.: Microscopic diagnosis for STH infection is based on Kato-Katz thick smear or FEC, wherever it was positive for eggs, while for intestinal protozoa infection it is solely on FEC.

Along with a higher detection ratio of all individual target organisms, the multiplex PCR was superior in terms of sensitivity in detecting poly-parasitism among the samples examined (Figure 8.5a). Increased levels of being multiple species infections was discovered: The proportion of being single species infection was lower, while being double and triple species infection was higher in multiplex PCR in comparison to microscopy. An additional sample was found to have four intestinal parasites (*As. lumbricoides*, *N. americanus*, *An. duodenale* and *S. stercoralis*), which was only detected by multiplex PCR.

Co-infection with the two genera of hookworms (*N. americanus* and *An.duodenale*) either with *As. lumbricoides* or not , was also detected in the multiplex PCR (Figure 8.5b), which resulted in an increased level of being polyparasitism.

Figure 8.5a Pie graphs depicting total number of parasites per sample for microscopy (I) and multiplex PCR (II) among stool samples collected in the Western Division of Fiji, March 2014

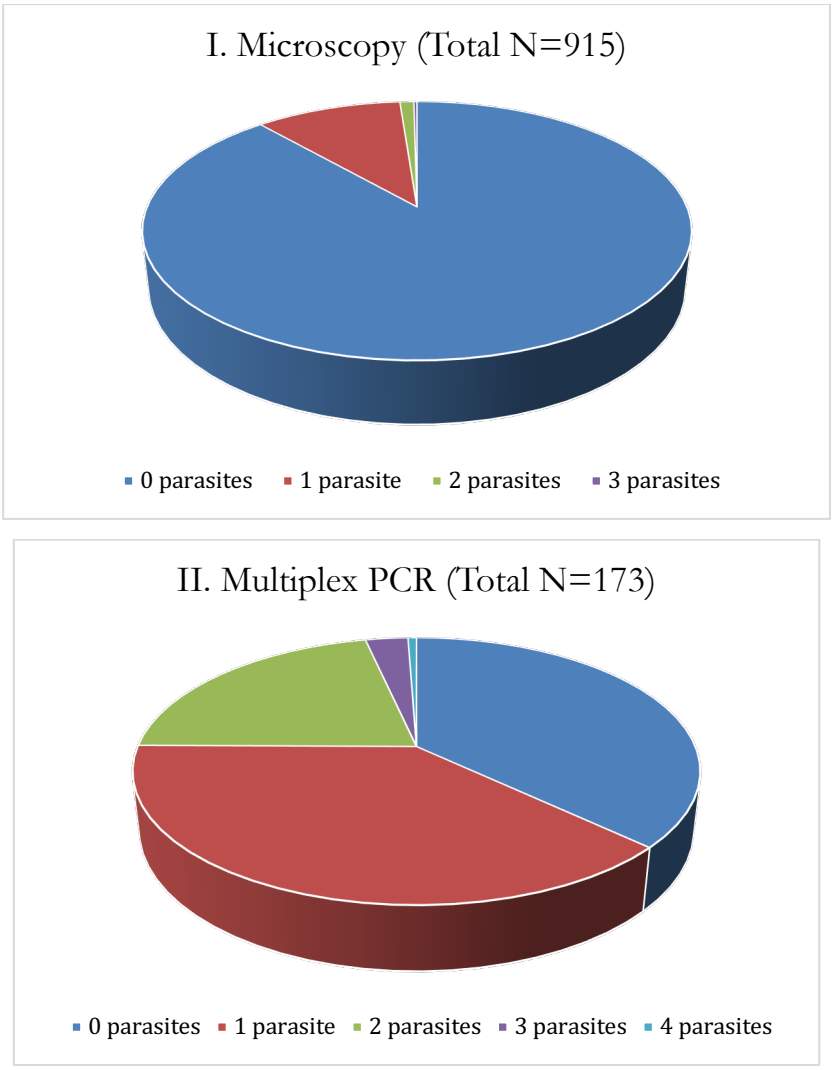
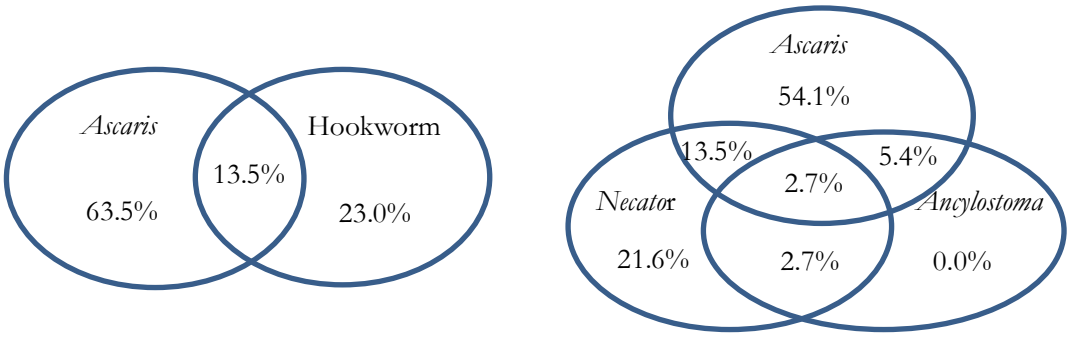


Figure 8.5b Venn diagrams depicting the specific division of *Ascaris* spp. and hookworm co-infection for microscopy and multiplex PCR among stool samples collected in the Western Division of Fiji, March 2014



8.4. Discussion

8.4.1. Low levels of strongyloidiasis in the Western Division of Fiji

Having a full appraisal of SS infections in the Oceania region continues to be problematic due to the present diagnostic difficulties in both operational and reference diagnostic settings (Kline et al. 2013). Previously, there were only three studies in Fiji with limited focus on strongyloidiasis from 1960 to 1980 (Lott 1980; Jansen et al. 1991). In 1966, a survey in a rural area of Naitasiri, Central Division reported SS infection prevalence levels of 3% among villagers of 7 villages (Jansen et al. 1991), with differences in ethnic groups. A much higher prevalence level was reported in a survey undertaken in a village near Rewa river, with an SS infection prevalence level-up to 50% among Fijian ethnicity of all ages groups (Jansen et al. 1991). Lastly, a study that covered 60 school children (age 5-15) on the island of Ovalau, Eastern Division, using unstained faecal smears, reported the SS infection prevalence of 13%, equally in girls and boys (Lott 1980).

In our study, we have found that SS infections exist among class 1 and 2 school students in three different eco-zones of the Western Division using BC and multiplex PCR (Figure 8.3). The general prevalence of SS infections is estimated to be up to 3.5% via molecular technique and we believe the infection is endemic (>1 %) in the assessed area (Muller 2002). Considering that there are approximately 45,000 primary school students in the Western Division, some 1,500 children are likely to be infected with SS, but it may be possible that the actual burden of the infection is greater, considering a poor diagnostic sensitivity from a single stool sample (Mirdha 2009).

Whilst the infected child encountered during this survey cannot be considered symptomatic, management of asymptomatic SS infections is recommended since the infection may persist for many years without appropriate drug treatment (Mirdha 2009). Considering that treatment with albendazole is lengthy and shows variable results, the preferred option would be single dose administration of ivermectin (Marti et al. 1996). However, most of these potential cases will not have opportunities of being treated, since neither the country is endemic to onchocerciasis nor ivermectin is included in the Essential Medicines List. We encourage that the health authorities in Fiji to consider treatment needs with ivermectin to be available in near future.

8.4.2. Utilization of the molecular technique as a quality control tool for the diagnosis of intestinal parasite infection

We have demonstrated an example of applying the multiplex PCR technique as a quality control tool for the conventional microscopic examination for the diagnosis of intestinal parasite infections and assessed its feasibility. It has shown that the results were helpful to back up the microscopic findings especially in the low endemicity setting, as it was found in the case of *T. trichiura* and *C. parvum* infections: For instance, it would have been difficult to conclude that there is no trichuriasis in two main islands of Fiji, even if there was no egg found in KK and FEC unless it was confirmed by multiplex PCR, considering that the sensitivity of the latter is much higher. With the expected success of the several national STH programmes in lowering the endemicity through preventive chemotherapy as seen in the Fiji' case, it will be useful as a confirmatory tool for the non-existence of the specific species.

It was also beneficial to confirm the presence of both species of hookworm infections, namely *N. americanus*, and *An. duodenale*, in the county, which was not been documented previously (Kline et al. 2013), but was only possible with the level of specificity that PCR provides. In addition, the PCR technique demonstrated that the level of intestinal parasitic infection with multiple species were higher than observed with microscopy. This is of importance to the assessment of disease burden, given that polyparasitism could have more severe impacts on health by elevating the risk of morbidity, than single parasite infections (Supali et al. 2010).

A large disparity in the prevalence rates between techniques was noted for the detection of hookworm, *Ascaris* and *Giardia* which had been previously observed (Llewellyn et al, 2016). Multiplex PCR detected a large number of positive samples not detected in either of microscopic techniques. As a consequence, microscopy was inferior to multiplex PCR in detecting polyparasitism, which was present in over half of all positive samples by multiplex PCR. A small number of samples in fact were also positive by microscopy but negative in multiplex PCR. This may be caused by variation in the dispersion of larvae, eggs, and oocytes within the subsamples used, which is innate for stools to be inhomogeneous (Llewellyn et al, 2016), the non-uniform excretion into stool (Basuni et al, 2011), and heavy dependency on the technical expertise of the users when it comes to microscopy. Alternatively, there might have been issues with PCR sensitivity which requires further optimization as reported previously, especially for the detection of *S. stercoralis* (Verweij et al. 2009; Knopp et al. 2014). Selected characteristics were compared for microscopy and molecular technique on the diagnosis of intestinal parasite infections with stool samples.

Table 8.3 Characteristics of microscopy and molecular technique for the diagnosis of intestinal parasite infections (WHO 2015a)

Characteristic	Microscopy*	Molecular technique
Standard	Used regularly in endemic countries	Relatively new and requires very high standard
Specimen	Fresh or fixed faeces	Fixed faeces
Sensitivity	Good sensitivity for moderate- or heavy-intensity infection Poor sensitivity for very light-intensity infection Will not detect *S. stercoralis* larvae	Good sensitivity for moderate- or heavy-intensity infection Poor sensitivity for very light-intensity infection as well as *S. stercoralis* infection
Detection of polyparasitism	Less sensitive	More likely
Specificity	Less species-specific	Highly species-specific
Equipment	Requires equipment that is simple to use, low cost and recyclable; readily available in endemic countries	Requires equipment that is complex to use, high cost and non-recyclable; not readily available in endemic countries
Ease of implementation	Very easy or mostly easy	Not easy, needs guidance and experience
Requirement for fixative	No fixative or 2 ml of 5% solution of formalin required to fix each faecal sample required	95% ethanol or
Personnel	Should be trained	Should be highly trained
Timing	Examination of faecal specimens within 4–6 h of collection (Kato-Katz) Faecal specimens can be fixed after collection and examined within 1–2 weeks (mini-FLOTAC or FEC))	Examination of faecal specimens within weeks or months
Dependency on person	More likely	Less likely
Logistic implications	If the site at which specimens are collected is more than 3–4 h from a laboratory, processing, and examination should be done on site. (Kato-Katz) Specimens can be collected on site, fixed and transported to a central laboratory for examination (mini-FLOTAC or FEC)	Specimens can be collected on site, fixed and transported to a central laboratory for examination.

N.B.: *: Microscopy includes the Kato-Katz thick smear, mini-FLOTAC, and FEC techniques

8.4.3. Limitation of the study

Our study has several limitations. The actual SS infection prevalence could have been higher as we had examined only a single stool sample per student rather than consecutive 3-day samples (Mirdha 2009), and also through pooling of samples rather than individually examining them. As it was the first time to assess the epidemiological feature of the SS infections in Fiji, we simply adapted BC instead of the Harada-Mori technique or the Koga-agar plate culture method, which could be more time and labour consuming, and opted to secure its maximal feasibility in a local laboratory setting by applying it to several batches of the samples. Furthermore, only samples received during the specifically designated 3 days were examined with BC, owing to the logistic constraints in the field based on the human resources availability, which limited the coverage of the schools surveyed. The SS infection prevalence could have been more precise if we had covered the greater number of schools.

Regarding the sampling strategy for multiplex PCR, we had half of them systematically selected, while the inclusion of another half was based on the need of confirmative diagnosis as a quality-control purpose, which may have impacts on the level of representativeness. This may be less robust, depending on the level of clustering and heterogeneity of geo-helminths and intestinal protozoan infections in the area (Smith et al. 2015), in assessing the overall burden of the infections. At the same time, it might have overestimated the actual burden in the area. However, we have included them based on the fact that the prevalence estimates in two groups do not differ statistically in the case of SS infection prevalence estimation. Another limitation in our study in terms of the application of multiplex PCR was that we did not consider quantitative aspects of the technique, which could provide ideas on the infection intensities. It has been demonstrated that multiplex PCR could enable more accurate determination of infection intensity for the infection such as *Ascaris,* hookworms and *Giardia* compared to microscopy (Llewellyn et al. 2016).

One might question that why multiplex PCR was not used as a main diagnostic technique for intestinal parasite infections if it was eventually available. Given that the total number of samples collected in the Western Division was up to 1,000 and we were only able to include 20% of the available stool samples, it would have required 5 more times of human and financial resources than it actually needed, if a PCR technique was applied. Instead, we used it as a quality control tool considering that the shorter turn-around time of the conventional microscopic techniques with the in-country availability as well as the fact the national programme's principle to utilize in-country capacity as much as possible in establishing the baseline data and monitoring the progress of the national programme. Thus, the molecular

technique was primarily adapted as a quality control tool and it was arranged by establishing new collaborative partnerships between the Fiji MHMS and a partner organization in the Netherland, the Tilberg Hospital, which provided valuable diagnostic services for the processing and analyzing the archived stool samples in support of the Fiji national programme.

8.5. Conclusion and next steps

In summary, we were able to shed new light on the distribution of SS infections in the Western Division of Fiji by using the LF TAS/STH as a pilot manner, a practical platform to integrate SS infection surveillance. *S. stercoralis* infection is endemic in the part of the country and access to adequate diagnosis and treatment should be available. Using species-specific diagnostic tools such as BC or multiplex PCR from achieved stool samples could be helpful to find further future synergy within the TAS/STH survey sampling framework (Chu et al. 2014). Application of molecular techniques as a quality control tool by achieving stool samples for the conventional microscopy-based diagnosis of intestinal parasitic infection is beneficial and should be more actively pursued.

Chapter 9

General discussion

Chapter 9. General discussion

The present thesis has contributed to obtaining a better knowledge about the key communicable diseases' epidemiology in Oceania and provides evidence-based information for the health authorities in Wallis and Futuna and Fiji, to guide the respective national programmes to move forward in achieving their programmatic goals. To do so, we have utilized lymphatic filariasis (LF) transmission assessment surveys (TAS) as a major backbone of the survey and adapted several additional diagnostic methods from a classical microscopic examination of the stool samples to rapid diagnostic tests, as well as DNA based molecular technique, for hepatitis B virus (HBV), intestinal protozoa, and soil-transmitted helminth infections including *S. stercoralis*. On top of the epidemiological profiles of intestinal parasite infections assessed, we also identified factors associated with the intestinal parasite infections as well as spatial mapping. Moreover, the thesis provided the actual examples how the national programmes would make evidence-based programmatic decisions based on the information obtained from the surveys, in order to meet the control or elimination goals set by the international community.

The thesis consists of studies which primarily utilized LF TAS as access opportunities for the monitoring and evaluation of the national control or elimination programme. In particular, the thesis

a) Assesses the impact of the mass drug administration in Wallis and Futuna (WAF) and Fiji, in order to provide the evidence to make stopping mass drug administration (MDA) decisions or as critical part of post-MDA surveillance activities;

b) Assesses the revalence levels of HBV surface antigen (HBsAg), as well as HBV vaccination coverage and its timeliness among school-aged children in WAF, where national HBV vaccination programme was commenced 20 years ago for the elimination of HBV infection;

c) Assesses the prevalence of intestinal parasite infections at national and sub-national levels of Fiji and the distribution patterns the school-level infections using spatial analysis, to evaluate the impact of the control programme in integration with LF elimination and as part of micronutrient supplementation programme for STH infections;

d) Explores factors associated with STH infections on Fiji, not only limited to water, sanitation, and hygiene (WASH) but also including environmental and socioeconomic

characteristics, to obtain baseline information for health authorities to consider effective strategies to apply; and

e) Assesses the prevalence of *Strongyloides stercoralis* infections in the Western Division of Fiji as a pragmatic trial using Baermann concentration technique and provides an example of using a DNA-based molecular technique as a quality-control tool of surveillance activities for intestinal parasite infections.

9.1. Impact assessment for the national lymphatic filariasis elimination programme

9.1.1. Wallis and Futuna

In light of the results presented, there is evidence of interrupted LF transmission in the territory, with the circulating filariasis antigen (CFA) prevalence less than 1% (WHO 2011c), which satisfies the global target of below 1% antigen prevalence in *Aedes*-endemic areas for LF elimination. As it is discussed previously, it is likely that continued rounds of MDA had contributed to the interruption of LF transmission in Wallis and Futuna given that vector management against LF had not been an ongoing activity in the last decade. Therefore, it is recommended stopping MDA and moving to the post-MDA surveillance phase to detect any LF recrudescence in the territory. The final TAS is recommended in five years of time considering that the last round of MDA was conducted in 2011, in order to confirm that the disease has been eliminated as a public health problem in Wallis and Futuna.

9.1.2. Fiji

Nation-wide post-MDA surveillance activities recently conducted in Fiji have shown that a significant reduction in LF antigen prevalence has been achieved since the commencement of MDA in 2002 with DEC and albendazole. The results confirm that more than 95 % of people across Fiji is now considered to be at very low or no risk of LF infection transmission, which is a major step towards the national programmatic goal, as well as the projected goal of eliminating LF by 2020 set by the Global Programme to Eliminate Lymphatic Filariasis (GPELF) (WHO 2010b). Furthermore, the study presents a detailed narrative of the WHO TAS implementation (WHO 2011c) process in an island setting with its potential use in assessing the impact of key communicable diseases as a survey platform.

Now the country programme will further continue the surveillance efforts following the predetermined monitoring and evaluation timeline (Table 4.3): The final TAS (TAS 3) for the Western Division except for the Malolo Island Medical Area and the second TAS (TAS 2) in the Central division, as well as TAS 3 for the Northern Division will be followed. As examples presented here, the country LF programme may continue to explore the application of TAS as a survey platform to assess the epidemiology of other key communicable diseases of public health importance. Recent discussions included possibilities of integrated implementation with the national immunization programme on the assessment of HBV vaccination's impact on HBV infection prevalence (MHMS, personal communication). Efforts will be also to be maintained in implementing further MDA rounds for the Malolo Island Medical Area which will join the Eastern Division and the Taveuni sub-Division. It should be noted that the Fiji programme's success can largely be attributed to the combination of the followings: 1) Strong political will of the government to tackle the infection by continuing the MDA implementation without interruption for longer than a decade; and 2) the excellent collaborative network established by the national programme with partner organizations which have been able to provide key financial and technical support in overcoming challenges and continuing the momentum towards achieving the elimination goal.

9.2. Impact assessment for the hepatitis B virus vaccination programme in Wallis and Futuna

Our results show that Wallis and Futuna has likely reduced early childhood hepatitis B virus transmission, being close to the WHO's Western Pacific Regional HBV infection control goal of reducing chronic HBV infection prevalence to <1% among children (WHO WPRO 2013). As these surveyed children were born even before Wallis and Futuna adopted the HBV birth dose vaccination policy, it may be possible that prevalence in younger cohorts is even lower. This low level of HBV infection could be the result of a successful HBV vaccination programme which was introduced in 1992, considering not only given that pre-vaccine prevalence was high but also reported 3-dose HBV vaccination coverage has been at least 90% collected by WHO/UNICEF. This is further verified by our findings that coverage with three doses of HBV vaccines is about 96% among the survey participants. Nevertheless, the impacts of the HBV vaccination program could have been greater, given that less than half of children received all three doses of the vaccine following the currently recommended schedule. Timely vaccination should be more actively encouraged according to the immunization schedule introduced in 2006.

9.3. Epidemiology of intestinal parasite infections and factors associated with the STH infections in Fiji

Overall STH infections in school-age children of Fiji were at a low level in the most part of Fiji, even if the country was long considered to be endemic. The residual infections were mostly from *Ascaris* spp. followed by hookworm infections, while *Trichuris* infections were rarely seen. Through large-scale preventive chemotherapy including LF MDA, it is likely that the country has successfully controlled STH infection transmission among school-age children and is not far from the programmatic goal of eliminating STH infections as a public health problem (WHO 2012b). However, there are still areas with high endemicity at sub-Divisional and at school levels, suggesting that there is a need to urgently resume preventive chemotherapy for preschool and school-aged children as well as women of childbearing age against STH infections, with the special attention to these hot-spot areas especially for complementary WASH interventions.

In a cross-sectional approach among class 1 and 2 schoolchildren on two main islands of Fiji, it appears that gastrointestinal protozoan infections are not rare. Given the insensitivity of single stool sampling, the actual prevalence could be higher, especially for *Giardia* infections, which was further confirmed by real-time PCR showing that *Giardia* infection prevalence of every 10th sample collected in the Western reached up to 22.4%. The spatial distribution of *Giardia* infections at school level was clustered, and cases of the *Giardia* infection were grouped at schools mostly around urban centres of the Western Division, such as Lautoka, the second biggest city, and Ra town. As giardiasis could emerge during the major events which alter the existing water and sanitary conditions (Lora-Suarez et al. 2002), it may be possible that this level of *Giardia* infection endemicity in these major urban areas originated from the contaminated water and foods by the floods, caused by a cyclone which hit the area just before the stool samples were collected. Having a better appraisal of intestinal protozoan infections continues to be problematic owing to present difficulties in both operational and diagnostic settings, but we were able to shed new light on the distribution of the infection including *Giardia* across the island by utilizing LF TAS as a survey platform and adding the FEC technique.

The study also provides an in-depth investigation of the factors associated with STH infections in Fijian school children living in both urban and rural communities: having a main water supply from the Fiji Water Authority at home and shoe-wearing had protective effects on the STH infections. Therefore, the programme is encouraged to work with the existing structure for the integrated delivery of water, sanitation, and hygiene (WASH) interventions to

the population in the hot-spot areas, as preventive chemotherapy alone in these areas will not be sufficient in interrupting transmission. For a longer-term success, it would be important to continue the current deworming programme as well as to complement integrated WASH interventions to gain and sustain benefits by reducing reinfection and transmission of STH infections (Erismann et al. 2016) by utilizing schools as entry points of service delivery. A practical discussion is currently ongoing with partners with the launch of the new project named 'Health Promoting Schools in Fiji' where nutrition, STH infection control, and WASH are three main thematic areas for school children in selected schools. Assuming that the county is going to resume mass deworming and implement other complementary interventions, it may be useful to integrate the approaches with other initiatives wherever feasible which could be more sustainable in a long run, as it took several decades to eliminate STH infections at national level even for the countries such as the Republic of Korea (Muller 2002). Strategies to consolidate the lessons learned and capacity built from the experience of implementing the LF MDA and TAS and to carry them over to tackle STH infections in a systematic way would be warranted.

By using the LF TAS/STH survey as a platform in a pilot manner, the study also shed new light on the distribution of *S. stercoralis* infections in the Western part of the island in Fiji, Via applying species-specific diagnostic tools such as the Baermann concentration technique and real-time PCR from achieved stool samples, the study was able to confirm that the infection exists in the country, which in turn will bring the attention on the access to adequate diagnosis and treatment of *S. stercoralis* infections in the country. A pilot of the application of molecular techniques such as real-time PCR as a quality control tool for the conventional microscopy-based diagnosis of intestinal parasitic infection was beneficial especially in a low endemic setting, which confirmed the absence of *T. trichiura* and *C. parvum*, as well as the existence of both species of hookworm and *E. histolytica*.

9.4. Application of LF TAS as a public health programmes' monitoring and evaluation platform

At country level, this thesis has demonstrated that LF TAS provides a convenient access platform to assess the up-to-date epidemiological profiles of intestinal parasite infections and other communicable diseases when the country programme is in the stage of post MDA surveillance, and they should be actively utilized as a monitoring and evaluation tool for other NTD programme as well as other public health interventions (WHO. 2011b), given that the median cost per TAS was up to $21,170 (including the costs for rapid diagnostic tests,

personnel, and transport (Brady et al. 2017). Several factors are to be considered for the decisions to be made by programme managers. Firstly, the net school enrolment ratio for the school-aged children is critical, as this is the first step to define the survey strategy for TAS. In the examples of WAF and Fiji, the thesis demonstrated that TAS primarily guarantees the access opportunities to the students at primary schools, with the high levels of net school enrolment ratio. However, at the same time, this would define the accessible age group from 6 to 11 or so, depending on the schooling systems in the country, which would limit the chances of using TAS as a platform. For instance, thought it targets mainly students, the global school-based student health survey (GSHS), a collaborative surveillance project designed to help countries measure and assess the behavioural risk factors and protective factors in 10 key areas among young people aged 13 to 17 years (WHO 2018a), would not be an optimal example to be considered, considering that the differences in target age groups. Nevertheless, TAS will be still useful to evaluate any other interventions in school-settings, such as school-based deworming, supplementation of any nutrients at schools, health education or school immunization programmes. Even if school-based TAS are not feasible, TAS still provide windows to access to at least 30 communities in a designated EU, which would be suitable to include preschool-age children below 6 years old as well as women in child-bearing age (15-49 years old), in countries where the net school enrolment ratios are lower than 75% (WHO, 2011b). There are several public health programmes which enclose these non-school aged children as their major target of the interventions, in line with improving maternal and child health. For example, blood concentrations of antibodies to tetanus antitoxin among women in child-bearing age is one of the most critical indicators for the validation of the elimination of mother to child transmission of tetanus (WHO 2018b), while the prevalence levels of anaemia among adolescent girls, pregnant mothers or women in child-bearing age are important markers now to decide whether mass deworming for these at-risk population would be required or not (WHO 2017h).

Furthermore, the required sample size per EU and the sampling frame should be carefully reviewed before TAS are considered to be utilized as a platform. By its nature and its robust sampling framework based on the lot quality assurance, TAS would guarantee at least 1,692 for the areas with its principal vector for LF as non-*Aede*s spp. and 3,080 children for the areas with its principal vector for LF as *Aede*s spp., when the total number of children exceeds 50,000 (WHO, 2011b) and clusters are to be used instead of systematic sampling. This scale of the sample size and an access to the schools and communities would allow several programmes to conduct the assessment as required. For instance, the sample sized of the traditional EPI

cluster survey methodology, which choose a fixed sample of 7 children in 30 clusters (7 x 30) to guarantee a maximum absolute confidence interval width of ± 10% at an assumed coverage level of 50%, and design effect of 2, would have been easily met with the TAS design (W H O 2015). Likewise, as seen the example of the HBsAg seroprevalence assessment, any survey relying on the cluster designs would be a good candidate for TAS to be considered as a platform. However, with the recent changes in the recommended survey methodology for the immunization coverage survey, where a probability-based sample is introduced, and the probability of an individual being selected, which will vary from cluster to cluster, is considered to conduct a weighted analysis (WHO, 2015). The sample size follows these principles will have more clusters than in the traditional 7 x 30 design with the higher number of total survey participants. Nevertheless, this robust new methodology also highlights the needs of taking account of multiple potential survey goals and to determine the most feasible combination of goals to address in the survey, which in turn proposes the potential for TAS to be integrated into it, rather than to be a platform. In any case, there should be a thorough review on how to calculate sample sizes for multiple objectives, how to review the priorities of each objective, and how to compromise where necessary (WHO, 2015).

Likewise, there are also other platforms which have potentials to be used as a tool for monitoring of evaluation frameworks of the public health programmes. Cunningham, Odoom, et al. demonstrated that a well-developed network of laboratories, the global polio laboratory network (GPLN), can be used as collaborative opportunities for the surveillance of NTDs, namely on STH infections and schistosomiasis, via examining stool samples collected for the polio surveillance. This approach can be used for a wider range of diseases which would both benefit the efforts to control, and also increase the scope of the network as a diagnostic platform (Cunningham, Odoom, et al. 2018). Also, with the widening of the scope of NTDs, especially for scabies which was newly added to the list, TAS could be considered as a surveillance platform. Scabies is recognised as a disease of public health importance, and is responsible for significant morbidity due to secondary bacterial infection (Romani et al. 2015). Physical examinations of children during TAS would provide chances to assess the prevalence of skin conditions. Moreover, considering *Sarcoptes scabiei* is sensitive to ivermectin, and with the newly available recommendations of introducing ivermectin as a part of triple-drug therapy against LF, the ivermectin-DEC-albendazole (IDA) regimen (Fischer et al. 2017), assessing the impact of IDA on scabies during TAS could be a critical piece of information for the health authorities in making programmatic decisions on scabies.

9.5. Key recommendations

With the remarkable success achieved by the LF elimination programme in Oceania (Ichimori and Graves 2017) and the current recommendations of repeating TAS every 2-3 year at least for 5 years of the post-MDA surveillance period, there will be multiple opportunities for the country programme in Oceania as well as in other regions that TAS could be used as access platforms for school-aged children. The lot quality assurance sampling (LQAS) method for CFA prevalence assessment is acceptable for other large public health programmes both in the rural and semi-urban implementation units, and therefore should be actively utilized in different settings to provide a broad picture of the impact of communicable diseases control or elimination programmes. However, when integration is considered, it is important to plan ahead and to consider each disease-specific goal carefully. This needs to be done properly at the initial stage of planning, and thus having good collaborative networks among different vertical programmes would matter significantly. As shown in Wallis and Futuna's as well as in Fiji's case, if integration efforts are undertaken in a systematic way, this can lead to strengthening the existing health system mostly by local capacity building and the best utilization of resources.

For LF elimination programmes in countries which had MDA based implementation for more than several decades, there may be challenges in stopping the interventions all at once. Thus, advocacy about the programme's progress and its strategic directions should be well communicated to the stakeholders and as well as with the public. As the survey coverage is a key to make programmatic decisions based on the survey findings, the main suggestion for the Fiji LF programme would be to pay special attention to conduct more effective social mobilization during the TAS preparation, in order to ensure that the survey coverage especially in urban settings to be improved. More intensive awareness campaigns would be necessary not only to share necessary information about the survey to convince the parents in providing their consents to the survey participation but also to ensure high levels of school attendance of the students during the survey period. A similar recommendation to the WAF immunization programme to implement timely vaccination by intensifying social mobilization on the importance of making timely visits to health centres following the recommended vaccination schedules.

With the total number of school-aged children in the study areas in Fiji up to 200, 000 (Fiji Bureau of Statistics 2009), several hundreds of children could have been infected with gastrointestinal protozoa including *Giardia* spp. considering findings of our study. Whilst not all the infected children would develop morbidity, it is likely that in schools with these infections

there have been occasions of water or food being contaminated which is known to be a source of the disease (Hunter et al. 2010). Thus, we recommend establishing the overall public health importance of the infection in Fiji, and identifying what would be a core set of interventions for WASH improvement at school level as a part of the integrated approach, reflecting this finding. Considering that the *Giardia* infection prevalence levels were even higher in urban areas and even several hotspots existed, attention should be equally paid to urban areas as well as to rural areas. Enhanced surveillance efforts should be considered in the disaster-affected areas on *Giardia* infections.

Discussions on the improvement of the operational aspects of the STH infections control programme for STH infections are ongoing in Fiji, and the findings of this thesis will help inform important policy decisions on how best to improve the programme outputs, with the focus on the inter-sectoral approaches for the effective delivery of WASH, on top of preventive chemotherapy as a backbone strategy. The following suggestions should be considered as the programme progresses to the critical stage. Mass deworming coverage should be improved without disruption of the service delivery. Special mechanisms for the effective provision of tablets for preschool age children and women of childbearing age should be considered, given the low coverage as facility-based approaches in the past. A close collaboration with the health promotion programme under the Wellness Unit and the environmental health programme for health education and access to improved water and sanitation would be required as part of 'inter-sectoral approaches', which will shorten the duration of deworming required to interrupt STH infection transmission to decrease the level of heterogeneity of the infection in the country.

In linking with the existing WASH programmes, preventive measures should be advocated more actively not only against STH infections including *S. stercoralis* but also against intestinal protozoan infections by expanding the current scope of the programme in a long run and the primary preventive measures such as personal hygiene (wearing shoes, washing hands after defecation and defecating in the toilet) and sanitation should be reinforced against the broader scope. *S. stercoralis* infection screening should be considered in the STH infection surveillance activities in Fiji, employing Baermann method or Koga agar plate culture or both whenever possible. Strongyloidiasis should be considered among patients presenting symptoms especially among those who are immune-compromised.

9.6. Suggestions for future work

Based on the results and observations made in these studies, the following research gaps need to be addressed in the future:

1. What would be the best setting to have access to school-aged children in the post-validation era not only for LF but also for other public health programmes?

Our findings in this thesis provide clear evidence that TAS is a convenient platform to access school children in assessing the epidemiological profile of key communicable diseases and in the monitoring of the programme impacts. However, with the advances of the LF programme, the country programmes eventually will not require any more TAS and the country will move forward to preparing its dossier for the validation of LF elimination as a public health problem. This implies that there will be no more population-based survey platform as a form of TAS in the country. Currently, LF elimination validation dossier requires the national programme to describe how to sustain post validation surveillance activities and also about potential platforms (WHO 2017a), but actual examples are yet to be shared among international communities given that certification of validation has been only recently awarded for a few countries. Therefore, demonstration of utilization of the access platforms by other initiatives such as routine students' health check-ups for the disease-specific surveillance purpose will be beneficial to continue the efforts in the post-validation era.

2. Whether targeting at-risk population for deworming would be the ideal mechanism to achieve the elimination of STH infection as a public health problem or community directed mass drug administration would be beneficial, where community-based LF MDA is no longer required?

Transitions from LF MDA (community directed targeting all age groups to deworming of the at-risk population via risk-group specific approaches took place in several countries such as Fiji, where post-LF MDA surveillance status had been achieved in respective evaluation areas. Ideally, the STH control programme can be built on the success of LF programmes, as it delivered at least five years of community-wide MDA with albendazole, which could have provided the epidemiological settings for interrupting the transmission of STH infections. However, levels of drug pressure were not always well-maintained following transitions from community direct approaches to risk-group specific approaches, as it was seen in Fiji's case. In this regard, DeWorm3 study (Natural History Museum 2015) will provide interesting findings once it is completed: Clusters are randomized to the intervention arm of the trial receiving twice yearly community-wide MDA, targeting eligible individuals of all ages or to the

control arm of the country's standard of care treatment targeting pre-school and school-age children. It is assumed that community-wide treatment will result in a lower prevalence of the predominant STH infections in areas where LF programmes have reduced the prevalence and intensity of STH infections and also is sufficient to interrupt transmission of each individual STH species, whereas programmes targeted at pre-school and school-age children will not achieve such interruption (Natural History Museum 2015).

3. What would be the gold standard for the diagnosis of STH and intestinal protozoan infections in public health surveillance in a low endemicity setting and how can it be validated?

There is a need to develop and/or validate "Gold-Standards" test using a small amount of stool specimens either using coproscopic or molecular diagnostic techniques for a reliable screening purpose of intestinal parasite infections. Some studies had proposed duplicate Kato-Katz or all samples to be examined for parasites' genetic material via quantitative PCR, but each approach has a number of strengths and weaknesses, which need to be explored further especially in the low prevalence settings.

4. How best to link the NTD control and elimination programmes with the existing WASH programmes?

Given that the WASH and NTD sectors have a common target population, who lacks access to safe and reliable water sources and sufficient sanitation or the tools to practice good hygiene behaviours, it is surprising that historically they have worked in parallel (Ogden et al. 2014). It is partly because they have different focuses. While the most of NTD control and elimination programme have disease-oriented approaches, WASH sectors have been built upon the types of interventions to be provided. Recognizing the importance but the lack of coordination, there have been suggestions regarding how to improve it: Monitoring and evaluation tools to help WASH sectors to collaborate with the disease control programme for joint measurements of the program impact on NTDs are to be developed; and advocacy tools to help WASH sectors drive funding to interventions with proven health impacts are to be provided. Nevertheless, the successful country examples are yet to be shared which could stimulate similar initiatives in other endemic resource-limited countries.

Appendix

Annex and bibliography

Appendix

Annex. Fiji National Framework on STH Infection Control and Elimination, 2017

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