Comparative Accuracy and 'Field Friendly' Effectiveness of Diagnostic Tools for Lymphatic Filariasis and Neurocyticercosis in Papua New Guinea and Timor-Leste with Consideration on the Impact of Parasitic Reduction Programs

Thesis submitted by

David Mark REEVE

B. Sc (Hons) (University of Otago, 1996)

PGDipDevStud (Massey University, 2002)

For the degree of Doctor of Public Health in the

School of Public Health, Tropical Medicine and Rehabilitation Sciences

James Cook University

20 July 2010

Statement of Access

I, the undersigned, the author of this work, understand that James Cook University will make it available for use within the University Library and, via the Australian Digital Thesis network, for use elsewhere.

I understand that, as an unpublished work, a thesis has significant protection under the Copyright Act and I do not wish to place any further restriction on access to this work.

.....

David Reeve

Statement of Sources

Declaration

I declare that this thesis is my own work and has not been submitted in any form for another degree or diploma at any university or other institution of tertiary education. Information derived from the published or unpublished work of others has been acknowledged in the text and a list of references is given.

.....

David Reeve

Declaration on Ethics

The research presented and reported in this thesis was conducted within the guidelines for research ethics outlined in the National Statement on Ethics Conduct in Research Involving Humans (1999), the Joint NHMRC/AVCC Statement and Guidelines on Research Practice (1997), the James Cook University Policy on Experimentation Ethics; Standard Practices and Guidelines (2001), and the James Cook University Statement and Guidelines on Research Practice (2001). The proposed research methodology received clearance from the Medical Research Advisory Committee of Papua New Guinea (MRAC No: 06/02, 06/05), James Cook University Experimentation Ethics Review Committee (H1423) and The Townsville Hospital District Ethics Committee (32/03).

.....

David Reeve

Statement of Contribution of Others

In Timor-Leste, the staff of the World Health Organization, with special acknowledgement to Dr Alex Andjaparidze, Dr Megan Counahan and Salavador Amara and from the Ministry of Health, Antonio Da Costa and the District Health Service in Suai and Lautem. These individuals and organisations arranged the logistics of the sentinel sites and assisted in collecting samples and demographic data. Associate Professor Wayne Melrose examined the faecal concentrates for protozoa, ova and cysts,

In Papua New Guinea, from the Ministry of Health, Leo Sora Makita and Norma Sargon, and the numerous Health Staff at New Ireland, West New Britain, East New Britain, Oro and Bougainville assisted in organisation and participated in collecting samples and performing the ICT test.

In Japan at Asahikawa University, Dr Toni Wandra assisted in testing specimens for neurocysticercosis and tested the specimens using recombinant glycoproteins.

Assistance was provided by Sharon Cooke who arranged and collected specimens from patients with Crohn's disease.

The thesis was edited by Dr Lisa Lines of Elite Editing & Tutoring, and editorial intervention was restricted to Standards D and E of the Australian Standards for Editing Practice.

Acknowledgements

I acknowledge many people who assisted and provided support.

From James Cook University, Dr Wayne Melrose who provided direction and organised the logistics in the various work that was undertaken, Dr Natkunam Keethesam who provided guidance with the lymphocyte proliferation assays and Dr Jeffrey Warner who was always there for a sounding board. Dr John Croese from Townsville Hospital and Professor Rick Speare provided direction in experimental design for the immunological response of Crohn's patients to hookworm infection.

In Japan at Asahikawa University, Professor Akira Ito and Associate Professor Hiroshi Yamasaki who ran the 2nd International Seminar on Technology Transfer for Immunodiagnosis of Cysticercosis and Echinococcosis. In England at the University of Nottingham, Professor David Pritchard and Dr Alan Brown who provided guidance and assistance in the immunology of hookworm.

Abstract

This research has contributed to the field of parasitology by evaluating several diagnostic tests in the area of lymphatic filariasis and determining their suitability for field surveys. A lymphatic filariasis survey in Papua New Guinea (PNG) using the TropBio Assay showed a wide range of prevalence with locations of high prevalence among areas of low prevalence that could be reservoirs of infection if not covered by the filariasis elimination program. Additionally, the results of parasitological surveys conducted in Timor-Leste and PNG are presented, which show the presence of human parasites in these countries previously unreported in the literature. Finally, research is presented that suggests that the presence of intestinal parasites may confer some benefit to the human host.

Diagnostic tests are just as important as the medications, vaccinations and therapies used to prevent and control population health issues. Without these tests, measurement of many health problems could not occur. They are also required to determine if activities designed to address a particular health problem are succeeding. Standardised and validated diagnostic tests are therefore a mandatory requirement for monitoring and evaluation of many health programs.

An essential element for determining whether a disease is eradicable is an effective diagnostic tool. Prioritised research needs for eliminating lymphatic filariasis include defining the comparative accuracy of diagnostics and taking advantage of improving user friendliness. In 2005, a new test kit became available for detecting infection by *Brugia* spp. in infected individuals. The *BRUGIArapid* cassette uses a recombinant antigen, BmR1, to detect antibody present in serum and whole blood samples. The

literature was evaluated to determine the relationship between prevalence of seropositivity using tests incorporating BmR1 and prevalence of microfilaraemia. Additionally, the test was evaluated to determine its suitability for use in field conditions. The *BRUGIArapid* cassette was used in several sites throughout Timor-Leste. A literature review showed acceptable sensitivity and specificity and minimal cross-reactions with other parasitic infections. There was a linear relationship between the prevalence of microfilaraemia and prevalence of seropositivity to BmR1. The equation predicts that a location with 1% microfilaraemia prevalence will have a 9.3% (95% PI, 5.2 –13.3) seropositivity to BmR1 prevalence. The test was quick and easy to use at the field sites. Problems noted with the test were poor fitting reagent bottle lids that leaked buffer during transport and testing, inaccurate instructions and a failure of the test pad to clear blood from the reading area at the recommended reading time. Changes were made to the design of the buffer bottles and test instructions were updated. The *BRUGIArapid* cassette was accepted for use by the World Health Organization (WHO) in Brugian filariasis elimination programs.

There have been mixed reports of the sensitivity of the filter paper version of the TropBio *W. bancrofti* ELISA. This technique was evaluated as part of the baseline surveys undertaken in PNG for the elimination of filariasis program. Nocturnal blood was collected and tested for microfilaraemia and by the ICT, TropBio ELISA and the filter paper version of the TropBio ELISA kit for antigenaemia. The absorbent pad from the ICT was removed and tested by the TropBio ELISA kit. To reduce the complexity of the TropBio ELISA the necessity of the boiling step to inactivate rheumatoid factor was investigated. A modified field version of the TropBio ELISA, the fast friendly field test, that has no boiling step and is read visually, was evaluated and compared to the standard test. The filter paper technique showed poor sensitivity (67.2%, 95% CI: 62.1–

72.1) although it was similar to the ICT (63.6%, 95% CI: 58.6–68.4) when compared to the serum TropBio ELISA. Using the filter paper from the ICT had better sensitivity (83.2%, 95% CI: 74.7–89.7) but was poor when used at another site (41.7%, 95% CI: 22.4–63.4) when compared to the serum version. Paired measurements using boiled and unboiled specimens were significantly correlated ($r=0.97$, $p<0.001$). The fast friendly field test had 96.0% (CI, 79.7–99.9) sensitivity and 98.4% (CI, 94.2–99.8) specificity compared to the serum version. The filter paper technique is unsuitable for due to its poor sensitivity. The boiling step appears unnecessary in the standard TropBio ELISA. The fast friendly field version shows acceptable sensitivity and specificity but may be cumbersome in field settings.

Baseline surveys of *Wuchereria bancrofti* lymphatic filariasis prevalence were conducted at two localities in each of the PNG provinces of New Ireland, West New Britain, East New Britain, Bougainville and Oro in 2006 prior to the beginning of mass drug administration for the Filariasis Elimination Program. These data were collected as part of the monitoring and evaluation requirements for the program. Venous blood was collected between the hours of 1900 and 0100, a thick blood smear prepared and examined for microfilariae and the serum tested by the TropBio ELISA. There were no antigenaemic individuals found in Rorovana, Bougainville and the prevalence ranged from 1.0% (95% CI: 0.2–1.8) at Oro Bay, Oro to 64.7% (95% CI: 59.5–69.9) in Sipai, Bougainville. Microfilaraemia was not found at the two sites in the Oro province with the highest prevalence found at Kokopo, East New Britain (22.6%, 95% CI: 15.1–30.1). Overall, antigenaemia rose with age with a peak prevalence in the 40–44 year old age group. Excluding Rorovana, there was no difference in antigenaemia prevalence ($p=0.29$) between the genders but males had a higher prevalence of microfilaraemia compared to females ($p<0.01$). West New Britain had undergone a mass drug

administration (MDA) one month before the baseline prevalence testing had started. In Kokopo, West New Britain there was a 36.4% (95% CI: 30.0–42.8) antigenaemia prevalence but no cases of microfilaraemia. PNG shows a wide range of lymphatic filariasis prevalence. Concentration of lymphatic filariasis in small communities could act as a reservoir source for surrounding districts if these are missed during the MDA. Successful baseline surveys were conducted using the TropBio ELISA. As this test quantifies the amount of antigen, rather than simply giving an ordinal positive or negative result, comparison with results from further surveys will allow a better measure of the effects of MDA.

Timor-Leste's elimination of lymphatic filariasis program includes the use of albendazole annually for all adults and six monthly for children aged two to sixteen years. Children under two receive pyrantel pamoate. These drugs treat the soil-transmitted helminths (STH) *Ascaris lumbricoides, Trichuris trichiura* and hookworm. These nematodes cause intestinal problems, contribute to malnutrition and hookworm and whipworm are associated with anaemia. Baseline and post-treatment surveys are necessary to determine the impact of the control program. Three villages, Buihomau, Suai Loro and Sika, were selected and, using local volunteers to approach every household, faecal samples were collected from village residents. The samples were transported back to James Cook University, Australia, preserved in sodium-acetic acid-formalin, concentrated and examined for parasitic protozoan cysts and helminth eggs, larvae or adults. Assessment of intestinal parasites in Timor-Leste revealed an overall prevalence among the three sites of 34.8%, 1.3% and 0.9% of hookworm, *Ascaris lumbricoides* and *Trichuris trichiura* respectively. Most hookworm infections were of light intensity (97.2%), which may be an artefact due to the delay in processing. Hookworm prevalence increased with age with the highest prevalence found in the ≥ 70

age group (83%). Also of importance were one case of *Strongyloides* infection and a 2.4% prevalence of taeniasis. The prevalence rate of non-pathogenic *Entamoeba coli* was 76.6% (95% CI: 72.1–81.1). Prevalence rates for STH in this survey will be used to compare with later surveys and determine the effect of mass drug administration. There have been no cases of *Strongyloides* spp. and only one case of *Taenia solium* in Timor-Leste reported in the literature. Free roaming pigs are the most common livestock and there are few latrines availabile to households in Buihomau and Suai Loro. Therefore, the environmental conditions for neurocysticercosis are present if cysticerci of *Taenia solium* is in the pig population. Improvements in water supply, sanitation and housing are needed in addition to MDA to reduce the parasite load in Timor-Leste.

Neurocysticercosis is one of the most common parasitic infections of the nervous system but has not been reported in PNG and Timor-Leste despite being present in nearby Indonesia. Testing blood for the presence of antibody can be a sensitive and specific method of determining neurocysticercosis. Serum samples from past parasitological surveys in PNG, Timor-Leste and Irian Jaya were tested by enzyme linked immunosorbent assay and immunoblot using glycoproteins from *T. solium* prepared by isoelectric-focusing and recombinant protein Ag1V1/Ag2. Using glycoproteins 1.7%, 2.1% and 2.0% of samples from Timor-Leste, PNG and Irian Jaya were repeatedly positive in the ELISA. There were two samples positive from each of Timor-Leste and PNG using purified glycoproteins and recombinant protein in the ELISA and immunoblot. Further surveys and testing is required to confirm this finding and if found, intervention measures should be put in place.

The absence of parasitological infection in humans has been suggested as the cause for the rise in some allergic and autoimmune diseases including Crohn's disease (CD). Although the cause of CD is not known, the yeast *Saccharomyces cerevisiae* has been

implicated. CD is a granulomatous disease that shows a Th1 cytokine profile. In contrast, immune responses to infection by *Necator americanus* shows a bias towards a Th2 cytokine pattern. Patients with CD given *Trichuris suis* orally have shown significant improvement. CD patients were inoculated with hookworm and the immune response to several crude antigens was measured. Compared to controls, CD patients had a greater lymphoproliferative response to crude antigen from *Saccharomyces cerevisiae* ($p<0.05$) and *Bacteroides fragilis* ($p<0.05$). Cytokine profiles were determined from whole blood cultures with crude antigen. Median interferon- γ production towards *B. fragilis* was lower ($p<0.05$) in CD patients with hookworm and taking methotrexate compared to controls. Net Interferon-γ/Net IL-10 ratios from whole blood stimulated with *S. cerevisiae* showed a step wise increase with CD patients with hookworm infection having lower ratios than CD patients without hookworm or control subjects. *S. cerevisiae* appears to have a role in the aetiology of CD while *N. americanus* may modify the immune response in CD patients. Consideration should therefore be given to the possible rise of allergic or autoimmune diseases when reducing parasite loads in populations.

This work has contributed to evaluating the comparative accuracy of diagnostic tests for lymphatic filariasis. The *BRUGIArapid* cassette was shown to be suitable for use in Brugian filariasis elimination programs. Conversely, the studies on the filter paper collection technique to determine TropBio antigenaemia demonstrated that the test was unsuitable. Investigation into the TropBio ELISA methodology resulted in the fast friendly field version of the TropBio ELISA. Baseline prevalence surveys in lymphatic filariasis and soil-transmitted helminths will now allow monitoring and evaluation of these programs to occur. Identification of *Taenia* spp. and serological evidence of neurocysticercosis suggests a comprehensive survey in PNG and Timor-

Leste is required to determine the extent of the problem. Finally, consideration should be given to the possible rise in autoimmune and allergic diseases as an unwanted effect of programs to reduce prevalence and intensity of infection of STH.

Abbreviations

ABTS	2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)
ASCA	anti-*Saccharomyces cerevisiae* antibodies
AU	arbitrary units
BCIP	5-Bromo-4 Chloro-3'Indoylphosphate p-Toluidine
CD	Crohn's Disease
CD/A	Crohn's Disease patients without hookworm infection and without methotrexate treatment
CD/HW	Crohn's Disease patients with *Necator americanus* infection and without methotrexate treatment
CD/HW/M	Crohn's Disease patients with *Necator americanus* infection and on methotrexate treatment
CD/M	Crohn's Disease patients without *Necator americanus* infection and with methotrexate treatment
CD-#	cluster of differentiation
CI	confidence interval
CO_2	carbon dioxide
CSF	cerebral spinal fluid
CT	computed tomography
DEC	diethylcarbamazine
DNA	deoxyribonucleic acid
ds	double strength

E/S	excretory/secretory
EDTA	ethylenediaminetetraacetic acid
EITB	enzyme-linked immunoelectrotransfer blot
ELISA	enzyme-linked immunosorbent assay
epg	eggs per gram
FDS	filarial dance sign
FFF	fast friendly field test
g	gram
H_2O	water
H_2SO_4	sulphuric acid
HCl	hydrochloric acid
HIV	human immunodeficiency virus
hr	hour
IBD	inflammatory bowel disease
ICT	immunochromatographic card test
IFN	interferon
Ig	immunoglobulin
IL	interleukin
kDa	kilodalton
l	litre
LF	lymphatic filariasis
LLGP	lentil-lectin affinity purified glycoproteins
LP	lamina propria
LPMC	lamina propria mononuclear cells
MDA	mass drug administration

mf	microfilariae
ml	millilitre
mm	millimetre
MOH	Ministry of Health
MRI	magnetic resonance imaging
mRNA	messenger ribonucleic acid
nm	nanometre
n	number
NaOH	sodium hydroxide
NBT	nitro blue tetrazolium
NCC	neurocysticercosis
NMF	nuclepore membrane filtration
OD	optical density
PAGE	polyacrylamide gel electrophoresis
PB	peripheral blood
PBMC	peripheral blood mononuclear cells
PBS	phosphate buffered saline
PCV	packed cell volume
PNG	Papua New Guinea
RNA	ribonucleic acid
SAF	sodium-acetic acid-formalin
SDS	sodium dodecyl sulphate
SST	serum separator tube
STH	soil-transmitted helminths
TBS	Thick Blood Smear

TrBS	tris-buffered saline
TEMED	tetramethylethylenediamine
TGF	transforming growth factor
Th(#)	T-helper
TMP	3,3´,5,5´-tetramethylbenzidine
TNF	tumour necrosis factor
UC	ulcerative colitis
USA	United States of America
µg	microgram
µm	micrometre
µl	microlitre
V	volt
W	watt
WHO	World Health Organization

Contents

Statement of Access ii
Declaration ii
Declaration on Ethics iii
Statement of Contribution of Others iv
Acknowledgements v
Abstract vi
Abbreviations xiii
Contents xvii
List of Tables xxii
List of Figures xxv
Introduction 1
Chapter One: Evaluation of the *BRUGIArapid* Cassette 4
1.1 Introduction 4
1.1.1 Requirements for the evaluation 4
1.1.2 Diagnostic tools 5
1.1.3 Indirect enzyme-linked immunosorbent assay (*Brugia*-ELISA) 6
1.1.4 *Brugia*-Rapid dipstick 6
1.1.5 *BRUGIArapid* cassette 7
1.1.6 Sensitivity, specificity and relationship to microfilaraemia 7
1.1.7 Development after exposure and response after treatment 14
1.1.8 Filariasis in Timor-Leste 16
1.2 Method 17
1.2.1 Site selection 17
1.2.2 Collection and test method 18
1.2.3 Assessment of the *BRUGIArapid* cassette test kit 19
1.3 Results 21
1.3.1 Prevalence of sero-positivity to *BRUGIArapid* cassette and microfilaraemia 21
1.3.2 Delivery and packaging 25
1.3.3 Instruction sheet 25
1.3.4 Usability 26

1.3.5 Readability 26
1.3.6 Robustness 33
1.3.7 Stability of reaction 34
1.4 Discussion 35
Chapter Two: Evaluation of Field Collection Methods of Diagnostic Tests used in Bancroftian Elimination of Lymphatic Filariasis Programs 38
2.1 Introduction 38
2.1.1 The need for antigen tests 38
2.1.2 ICT 47
2.1.3 Stability of the ICT result 50
2.1.4 Detecting the 200–kDa antigen in the ELISA format 51
2.1.5 TropBio ELISA 52
2.1.6 Persistence of antigen 53
2.1.7 Sensitivity and specificity of the ICT 54
2.1.8 Sensitivity and specificity of the TropBio ELISA 55
2.1.9 Comparison of Prevalence of the ICT and TropBio ELISA 56
2.1.10 Association of Og4C3 concentration with microfilaria concentration 57
2.1.11 Antigenaemia and age 57
2.1.12 Antigenaemia and gender 58
2.1.13 Clinical symptoms and antigen tests 59
2.1.14 Filarial dance sign in chronic filariasis and antigenaemia 63
2.1.15 TropBio antigen association with filarial antibodies 64
2.1.16 Antigen levels and treatment 65
2.1.17 Filter paper collection techniques and the TropBio ELISA 69
2.2 Method 73
2.2.1 Collection and handling of blood samples 73
2.2.2 Filter paper sample processing 73
2.2.3 Testing method 74
2.2.4 Testing precipitate by the TropBio ELISA in the field 75
2.2.5 Comparison of untreated and treated samples in the TropBio ELISA 75
2.2.6 Fast friendly field test 75
2.3 Results 76
2.3.1 Prevalence comparisons 76
2.3.2 Comparison of sensitivities and specificities 77

2.3.3 Comparison of titre groups 79
2.3.4 Effect of elution time on the filter paper technique 79
2.3.5 Filter paper results from specimens collected in Bougainville 80
2.3.6 Testing precipitate for antigen by the TropBio ELISA 82
2.3.7 Boiled supernatants compared to untreated specimens 82
2.3.8 Fast friendly field test 84
2.4 Discussion and Conclusion 84
Chapter Three: Baseline Monitoring of Lymphatic Filariasis Prevalence in Papua New Guinea 90
3.1 Introduction 90
3.1.1 Requirements for a successful control program 91
3.1.2 Lymphatic filariasis in PNG 92
3.2 Method 93
3.3 Results 96
3.3.1 Demographics of sentinel sites 96
3.3.2 Prevalence of antigenaemia and microfilaraemia 97
3.4 Discussion 111
Chapter Four: Baseline Monitoring of Soil-transmitted Helminths Prevalence in Timor-Leste 116
4.1 Introduction 116
4.1.1 Contribution of STH to disease 117
4.1.2 Age and gender profiles of STH infections 127
4.1.3 Environmental factors in STH transmission 128
4.1.4 Risk factors 129
4.1.5 STH interventions 133
4.1.6 Monitoring and evaluation by parasitological methods 136
4.1.7 STH in Timor-Leste 141
4.1.8 Conditions influenced by STH in Timor-Leste 142
4.2 Method 143
4.2.1 Study area 143
4.2.2 Questionnaire 143
4.2.3 Parasitology field procedures 144
4.2.4 Parasitology laboratory procedures 145
4.2.5 Analysis 146

4.3 Results 146
4.3.1 Basic demographic profile 146
4.3.2 Parasite examination for hookworm, *A. lumbricoides* and *T. trichiura*. 150
4.3.3 Parasitology examination for other cysts, eggs and larvae 153
4.3.4 Household survey 157
4.4 Discussion 158
Chapter Five: Immunodiagnosis of Neurocysticercosis in Papua New Guinea and Timor-Leste 166
5.1 Introduction 166
5.1.1 Clinical signs and symptoms 166
5.1.2 Diagnosis of neurocysticercosis 167
5.1.3 Antibody response in NCC 169
5.1.4 Antibody assays using whole crude antigen 170
5.1.5 Glycoproteins for diagnosing cysticercosis 172
5.1.6 Recombinant/synthesised glycoproteins 174
5.1.7 Antigen detection 178
5.1.8 Background of study 179
5.2 Method 180
5.2.1 Purification of *T. solium* glycoproteins from cyst fluid using preparative isoelectric-focusing 180
5.2.2 ELISA for NCC 180
5.2.3 SDS-PAGE and immunoblot 181
5.2.4 Samples 182
5.3 Results 182
5.3.1 ELISA results 182
5.3.2 Immunoblot 184
5.4 Discussion 187
Chapter Six: Immunological Responses of *Necator americanus* Infection in Subjects with Crohn's Disease and Healthy Volunteers 190
6.1 Introduction 190
6.1.1 Hygiene hypothesis 190
6.1.2 Crohn's disease 192
6.1.3 Immunology of Crohn's disease 194
6.1.4 Helminth immunology 194

6.1.5 Using parasites to cure disease 197
6.2 Methods.....198
6.2.1 Antigen preparation 198
6.2.2 Subjects.....200
6.2.3 Lymphocyte proliferation assay.....200
6.2.4 Whole blood cytokine assays.....201
6.2.5 IgG ELISA assay to E/S *N. americanus* antigen 203
6.2.6 Western blot.....203
6.2.7 Statistical analysis.....204
6.3 Results.....204
6.3.1 Serum IgG levels.....204
6.3.2 Western blot.....208
6.3.3 Lymphocyte stimulation to antigens and mitogen.....210
6.3.4 Whole blood stimulated cytokine assays 211
6.3.5 Serum cytokines.....216
6.4 Discussion.....217
Chapter Seven: Conclusion 224
References 229
Appendices 273
Appendix 1: Materials 273
Appendix 2: Checklist of Materials required for Baseline Survey.....281
Appendix 3: Survey Questions for Timor-Leste 282

List of Tables

Table 1–1 Sensitivity of tests using BmR1 antigen for the diagnosis of Brugian Filariasis compared to microfilaraemia 10
Table 1–2 Combined specificity of *Brugia*-ELISA and *Brugia*-Rapid Dipstick of individuals infected with other parasites 11
Table 1–3 Comparison of prevalence of microfilaraemia and seropositivity to BmR1 .. 12
Table 1–4 Change in prevalence of sero-positivity to BmR1 after treatment of Brugian filariasis 15
Table 1–5 Low endemic areas: demographic profile 22
Table 1–6 Low endemic areas: Results 22
Table 1–7 Demographic profile of positive samples in low endemic areas by *BRUGIArapid* 23
Table 1–8 Prevalence of seropositivity to *BRUGIArapid* by age in high endemic area (Rembor) 24
Table 1–9 Strength of reaction of positive results from Rembor 27
Table 1–10 Grading of the readability of results in Rembor 27
Table 1–11 Per cent positive of faint and clearly visible test line plasma spiked with red cells after a specified incubation time 32
Table 1–12 Effect of variation in *BRUGIArapid* cassette test protocol 34
Table 2–1 Comparison of ICT with detection of microfilaraemia 39
Table 2–2 Comparison of TropBio ELISA with detection of microfilaraemia 42
Table 2–3 Summary of reports where both TropBio ELISA and ICT are used 44
Table 2–4 Changes in Og4C3 antigenaemia after treatment with DEC or DEC/albendazole 67
Table 2–5 Studies comparing the TropBio ELISA filter paper with the TropBio ELISA or microfilaraemia 71
Table 2–6 Comparison of antigen diagnostic tests 78
Table 2–7 Comparison between filter paper and serum TropBio ELISA titre groups 78
Table 2–8 Comparison between elution of boiled antigen of filter paper overnight and standard filter paper technique 80

Table 2–9 Comparison of antigen detection techniques of samples collected in Bougainville 81
Table 2–10 Comparison between using precipitate and supernatant in the TropBio ELISA titre groups 82
Table 2–11 Comparison in titres between treated and untreated samples in the TropBio ELISA.....83
Table 2–12 Comparison of FFF with standard TropBio ELISA.....84
Table 3–1 Locations where blood collection occurred.....95
Table 3–2 Age and gender characteristics of sentinel sites.....98
Table 3–3 Prevalence of antigenaemia and microfilaraemia at sentinel sites.....99
(%, 95% CI [n=total number tested]) 99
Table 3–4 Prevalence of antigenaemia at Amfar and Oro Bay by self-reported place of residence % (n).....100
Table 3–5 Prevalence of titre group by location % (n) 101
Table 3–6 Microfilariae density by gender.....103
Table 3–7 Percentage of microfilaraemics by blood microfilariae density % (n).....104
Table 3–8 Prevalence of TropBio antigenaemia by age group % (n=total number of individual in each age group) 105
Table 3–9 Prevalence of antigenaemia and microfilaraemia by gender at each collection site % (n=total number of participants).....110
Table 4–1 Estimates of global prevalence of STH infections, absolute numbers (millions) and prevalence (%) 117
Table 4–2 Comparison between albendazole treated and placebo STH prevalence rates and changes in weight gain.....123
Table 4–3 Classes of intensity for STH (WHO, 2002).....133
Table 4–4 Classes of intensity and treatment strategy for STH (WHO, 2002).....134
Table 4–5 Community treatment strategies (WHO, 2006).....134
Table 4–6 Prevalence of conditions in Timor-Leste in children under five years of age (Ministry of Health and National Statistics Office et al., 2004).....142
Table 4–7 Prevalence of anaemia among children and women in Timor-Leste (Ministry of Health and National Statistics Office et al., 2004).....142
Table 4–8 Age and gender by village.....147
Table 4–9 Percentage of household livestock ownership (%).....148
Table 4–10 Characteristics of sub-population who had faecal samples examined 149

Table 4–11 Prevalence of hookworm, *A. lumbricoides*, *T. trichiura* and combined STH151
Table 4–12 Intensity of hookworm infection based on WHO criteria (2002).....153
Table 4–13 Prevalence's of additional cysts and eggs in Timor-Leste 155
Table 4–14 Demographics of *Taenia* infection 156
Table 5–1 Recent recombinant/synthesised proteins used for diagnosing NCC.....176
Table 5–2 Date of collection of samples used for testing for NCC.....182
Table 5–3 Prevalence of sero-positive individuals repeatedly positive by ELISA for NCC 183
Table 5–4 Results of further serological tests of specimens identified as positive in the ELISA screening test 186
Table 6–1 Percentage of subjects recognising 30 and 33 kDa E/S proteins of *N. americanus* by Western Blot after specified periods 209
Table 6–2 Serum cytokine levels in *N. americanus*-infected subjects 217

List of Figures

Figure 1–1 Relationship between prevalence of microfilaraemia and seropositivity to BmR1 13
Figure 1–2 Sites in Timor-Leste where the evaluation was conducted. The smaller arrows indicate the five locations (supplied by WHO, Timor-Leste) 18
Figure 1–3 Time course showing the clearing of the *BRUGIArapid* test pad over 30 minutes 28
Figure 1–4 Extent of blood clearing from the test area arrow indicates position of positive test line 28
Figure 1–5 Variability of cassettes in clearing with 35 µl of pipetted blood over time 29
Figure 1–6 Effect of varying blood volume (µL) and PCV on readability 30
Figure 1–7 Clearance of blood after 60 minutes after allowing liquid front to migrate to end of test strip 31
Figure 1–8 Test lines of two different plasma samples 31
Figure 1–9 Reactions of plasma with faint test line (1) and plasma with clearly visible test line (2) spiked with red cells 32
Figure 2–1 Comparison of prevalence of antigenaemia and microfilaraemia from multiple studies 56
Figure 2–2 Graph of OD treated *versus* untreated specimens in the TropBio ELISA 83
Figure 3–1 Age and gender characteristics of combined sentinel sites 96
Figure 3–2 Titre group percentage by location 102
Figure 3–3 Microfilariae blood density by location 102
Figure 3–4 Relationship between the antigen prevalence and the percentage titre group seven of all TropBio antigenaemics at each collection site 103
Figure 3–5 Total prevalence (excluding Rorovana) of Tropbio antigenaemia by age group 106
Figure 3–6 Relationship between TropBio antigenaemia prevalence and microfilaraemia prevalence 107
Figure 3–7 Ratio of TropBio antigenaemia prevalence/microfilaraemia prevalence+ 0.1 108
Figure 3–8 Prevalence of microfilaraemia by age range 109
Figure 4–1 Study characteristics 146

Figure 4–2 Demographic profile of responders to questionnaire 147
Figure 4–3 Prevalence of hookworm infection by age 152
Figure 4–4 Prevalence of *E. coli* infection by age 155
Figure 4–5 A rhabditoid larvae of *Strongyloides stercoralis* from a faecal sample from the village Sika 156
Figure 4–6 Percentage of individuals (n=333) with total number of intestinal parasites detected 157
Figure 4–7 Household size and the number of households not providing faecal samples157
Figure 4–8 Percentage of households, with more than one member and in which all members provided a sample, with varying number of members with hookworm 158
Figure 5–1 Graph of the optical density by ELISA using purified glycoproteins to detect antibody for NCC from samples originating from Timor-Leste, PNG and West Papua184
Figure 6–1 Concentration of serum IgG (µg/ml) to E/S antigens of *N. americanus* post-inoculation 205
Figure 6–2 Change in specific IgG concentration (µg/ml) over time in patients with CD (Number in bottom left corner patient identification) 206
Figure 6–3 Change in specific IgG concentration (µg/ml) over time in control subjects (Number in bottom left corner patient identification) 208
Figure 6–4 Immunoblot of ES products from adult worms of *N. americanus* probed with serum pre- and post-infection and identified for bound IgG 209
Figure 6–5 Lymphocyte stimulation to the mitogen, PHA (CD+HW, CD, Controls) ..210
Figure 6–6 Lymphocyte stimulation to various antigens (CD+HW, CD, Controls) 211
Figure 6–7 Net Interferon-γ production from whole blood stimulated with mitogen and various antigens 212
Figure 6–8 Net IL-6 production from diluted whole blood stimulated with PHA, crude *B. fragilis* antigen and whole dead *S. cerevisiae* 212
Figure 6–9 Net IL-10 production from diluted whole blood stimulated with PHA, crude *B. fragilis* antigen and whole dead *S. cerevisiae* 213
Figure 6–10 Net IL-13 production from diluted whole blood stimulated with PHA, crude *B. fragilis* antigen and whole dead *S. cerevisiae* 213
Figure 6–11 Net Interferon-γ/Net IL-10 ratio from diluted whole blood stimulated with *S. cerevisiae* 214
Figure 6–12 Gross production of IFN- γ after stimulation with *B fragilis* and *S. cerevisiae in* RPMI diluted blood after 48 hr incubation 216

Introduction

This thesis is predominately about the evaluation and use of diagnostic tests, one of the fundamental tools of public health. For the diagnosis of parasitic infections, the oldest tool, the microscope, is still used extensively. For many decades, it has been the only tool available to diagnosis parasitic infections and due to its apparent simplicity, the microscope is found in clinics and laboratories around the world. Apart from the initial cost of the microscope, consumables, such as glass slides and cover slips, are relatively inexpensive compared to diagnostic kits.

However, microscopy has severe limitations. It takes skill and expertise to identify what may be seen under the microscope. In the developing world, where most parasitic infections are found, there may be little training given to those responsible for identifying the organism or microscopes may be physically suboptimal resulting in poor accuracy. For example, in the course of my research, one small laboratory in a developing country assisted by examining thick blood smears for microfilariae. Despite a 10% prevalence found upon re-examination by the author, no smears were identified by this laboratory as having microfilariae present. Similar problems with false negatives and false positives have been reported for malaria and other parasites in developing countries (Durrheim et al., 2001). Another limitation to microscopy is the small sample size that can be examined. A drop of blood or piece of faecal material is stained and examined on a slide. If the density of the parasite is low, the technique may not be sufficiently sensitive to determine parasitism. The third limitation is the time consuming process to examine a slide compared to rapid diagnostic techniques. There has been a notable failure to automate the process.

With the advent of the enzyme-linked immunosorbent assay and its automation, diagnosis of some parasitic infections has allowed large-scale testing of populations. The

technique is often more sensitive in diagnosis and quicker to perform when testing a large number of samples compared to microscopy. Additionally, the ELISA technique could be reformatted so that the methodology was simpler and discrimination between positive and negative easier to determine.

Lymphatic filariasis related morbidity includes both acute inflammatory episodes and chronic manifestations including lymphoedema exacting physical and psychological burdens (Addiss and Brady, 2007). Lymphatic filariasis was acknowledged to be potentially eradicable by the WHO which has urged member states to develop national plans for its elimination (WHO, 1997). At a forum in Philadelphia, United States of American (USA) in 2003, the scientific lymphatic filariasis community determined a strategic plan for research to support the global program to eliminate transmission of lymphatic filariasis. One of these needs was to establish the tools and measures of program success by evaluating comparatively the diagnostics and sampling strategies. Comparative effectiveness including ease-of-use, cost and reliability of all monitoring tools available through field-monitoring of sentinel sites in countries with active LF elimination programs is needed (Gyapong, 2004).

Principally, this thesis is concerned with diagnostic tests for lymphatic filariasis and intestinal helminths and the application of these tests in baseline surveys in Timor-Leste and Papua New Guinea (PNG) in their Elimination of Filariasis Programs. Reduction in prevalence and intensity of soil-transmitted helminths may be an expected consequence of using anthelmintics in the filariasis elimination programs. Additionally, this thesis also considers diagnostic testing for neurocysticercosis in these countries. The final chapter considers if there are any potential negative consequences in eliminating intestinal helminths from populations by determining the immunological effect hookworm has on Crohn's disease patients. Due to the emphasis on diagnostic testing, this thesis contains an extensive literature review on the current knowledge of the performance of diagnostic tests for lymphatic

filariasis, intestinal helminths and neurocysticercosis. This literature review is presented in the relevant chapters.

Chapter One: Evaluation of the *BRUGIArapid* Cassette

1.1 Introduction

The *BRUGIArapid*-cassette is a new reformatted diagnostic test to determine infection of *Brugia* spp. The literature describing BmR1, the antigen used to detect IgG4 antibody to *Brugia* spp., was summarised. This test was evaluated to determine its suitability in field surveys in settings of low and high prevalence in Timor-Leste.

1.1.1 Requirements for the evaluation

As the *BRUGIArapid* cassette was a new product, the World Health Organization (WHO) requested that the WHO Collaborating Centre for Control of Lymphatic Filariasis, within the Anton Breinl Centre at James Cook University, evaluate the test. The requirements were:

1. Test the field friendliness of the test and report on packaging and clarity of instructions.
2. Compare microfilaraemia and sero-positivity to BmR1 by the *BRUGIArapid* cassette in high and low prevalence settings.
3. Summarise the literature comparing microfilaraemia and BmR1 in its various test formats.
4. Describe the relationship between microfilaraemia prevalence and prevalence of sero-positivity to BmR1 to determine if there is a target prevalence of sero-positivity for starting or stopping MDA.

1.1.2 Diagnostic tools

Brugian filariasis is caused by the filarial parasites *Brugia malayi* and *Brugia timori* infecting about 13 million individuals predominantly in India, Indonesia, South China, Vietnam, Thailand, the Philippines and Malaysia (Rahmah et al., 2001b). The transmission of *B. malayi* and *B. timori* is targeted for interruption as part of the Global Program to Eliminate Lymphatic Filariasis.

Traditional diagnosis of lymphatic filariasis requires collection of blood samples, staining and examination by microscopy for the presence of microfilariae. Detection of *W. bancrofti* microfilariae in peripheral blood has poor sensitivity and will not detect low numbers, single sex infections and amicrofilaraemic infection thereby missing many positive cases (Turner et al., 1993). In addition, collecting night blood samples is inconvenient for both subjects and collectors. Smear examination for microfilariae can be considered a laborious and time consuming process (Denham, 1975).

Within the last 20 years, highly specific and sensitive *W. bancrofti* antigen tests have become available. The ICT antigen card test is simple to perform and can be used with capillary blood samples taken any time of the day. It is used for mapping endemic areas, monitoring progress of elimination programs and surveying potential transmission once elimination programs are complete (PacELF Monitoring and Analysis Network, 2004). However, the ICT does not detect antigen from *Brugia* spp. (Weil et al., 1997).

Immunological testing relies on detection of anti-filarial IgG4 antibody. Anti-filarial IgG4 is elevated in active filariasis infection (Ottesen et al., 1985; Kwan-Lim et al., 1990; Rahmah et al., 1998) and decreases after treatment (Rahmah et al., 2001a).

BmR1 is an antigen related to the presence of *B. malayi* adult worms (Lim et al., 2004) that was first described in 2001 and used to detect IgG4 antibody to *Brugia* spp. (Rahmah et

al., 2001a). It is related to the presence of the gene that codes for BmR1. The gene is cloned into *Escherichia coli*, which is induced to express the protein. It has also been found in soluble antigens from microfilariae and adult worms. The antigen is a common protein found in the female adult worm's uterine and male adult worm's vas deferens epithelial membranes (Noordin et al., 2004a).

BmR1 has been incorporated into several ELISA formats. These are the *Brugia*-ELISA, *Brugia*-Rapid dipstick and *BRUGIArapid*-cassette.

1.1.3 Indirect enzyme-linked immunosorbent assay (*Brugia*-ELISA)

Microtitre wells are coated with BmR1 followed by the addition of diluted sera. After washing, anti-human IgG4-HRP detects IgG4 bound to BmR1, which is read by a spectrophotometer. A cut-off optical density (e.g. 0.300), based on the mean OD of negative controls discriminates between samples that are positive and samples that are negative. The OD is proportional to the amount of antibody present in the original sample (Rahmah et al., 2001a).

1.1.4 *Brugia*-Rapid dipstick

Brugia-Rapid dipstick is an indirect solid-phase immunochromatographic assay. A membrane card is prepared with goat anti-mouse antibody as the top control line and BmR1 as the second test line. The lined card is dried and treated with a blocking solution. An absorbent pad is pasted on top of the card and a serum filter pasted at the bottom and the card cut into 3-8 mm strips (dipstick). Monoclonal anti-human IgG4 antibody is gold conjugated and dried into plastic wells. Paired wells, one with dried gold-conjugate (well B) and one without (well A), and the dipstick are then packaged with a desiccant into an individual pouch.

When performing the test, phosphate buffered saline is first placed in both wells. Serum is added to well A, mixed with buffer and the dipstick is then added so the sample flows up the strip. When the sample reaches near the top of the strip, the filter is cut off and the dipstick is placed in well B and left for 10 minutes. The control line must become positive for a valid test and the appearance of a second line indicates a positive result (Rahmah et al., 2001b).

1.1.5 *BRUGIArapid* cassette

BRUGIArapid is a cassette based version of the *Brugia*-Rapid dipstick produced by Malaysian Bio-Diagnostics Research SDN. BHD., Bangi, Selangor, Malaysia. The dipstick and an absorbent pad are enclosed within a plastic casing.

The kit instruction sheet indicates 35 µl of whole blood, serum or plasma is added with buffer to the bottom well. This migrates upwards and complexes with the immobilised antigen if antibodies are present. The antigen-antibody complex is detected after addition of a buffer in the top well where a dye conjugated with anti-human IgG4 flows back down the strip resulting in a pink-purplish line if positive. A control line containing goat-anti-mouse IgG antibody binds to the mouse anti-human IgG4 indicating proper migration and addition of the buffer.

1.1.6 Sensitivity, specificity and relationship to microfilaraemia

Table 1–1 details the reported sensitivity and specificity of assays using BmR1 compared to the presence of microfilaraemia.

Compared to microscopy, *Brugia*-ELISA and *Brugia*-Rapid dipstick have sensitivities of 95.5% and 96.7% respectively with a range of 87%-100% (see Table 1–1). In comparison the ICT sensitivity compared to Bancroftian microfilaraemia ranges from 95–100%

(Bhumiratana et al., 1999; Njenga and Wamae, 2001; Chandrasena et al., 2002; Braga et al., 2003). Samples from both *B. malayi* and *B. timori* microfilaraemic individuals show similar sensitivities. It has been previously reported that 10% of *W. bancrofti* microfilaraemic individuals have low levels of anti-filarial IgG4 antibody (Marley et al., 1995). This may also apply to *Brugia* spp. microfilaraemic individuals, which will contribute to false negative results and sensitivities less than 100%.

The lowest reported sensitivity (see Table 1–1) was 87% (CI: 66.4–97.2) using *Brugia*-Rapid dipstick with capillary blood samples (Jamail et al., 2005). It is logical to assume that using whole blood would have a slightly reduced sensitivity compared to using serum as whole blood has approximately half the amount of serum for the same volume. The lower sensitivity using whole blood as the sample could be of concern if the true sensitivity is nearer the lower end of the confidence interval. The numbers of microfilaraemics in this study were few, but it would be prudent to perform sensitivity and specificity studies using a larger sample size with whole blood to clarify this observation. The specificity in all studies is 100% when samples are taken from individuals with no known infections and who reside in non-endemic areas.

Crude preparations from whole organisms contain a wide range of antigens and sometimes cross-react with samples from individuals with different infections, therefore reducing the specificity of a test. Since a recombinant antigen is a single protein, it is less likely cross-reactions will occur unless the antigen is conserved among species.

Table 1–2 combines the results of several papers where the antigen BmR1 has been used in individuals with a known parasitic infection but no Brugian filariasis infection. Approximately 50% of patients with *W. bancrofti* microfilaraemia demonstrate seropositivity. However, there is wide variation depending on location (Noordin et al., 2004a). Apart from

the additional cost of testing, this should be of no concern as the two infections are easily discriminated by antigen tests.

There is cross-reaction with individuals infected with the closely related species *Loa loa*. However, this is not co-endemic with Brugian filariasis. The cDNA sequences of BmR1 have been identified in *W. bancrofti* where it is identical to *Brugia* spp. The BmR1 cDNA sequences in *Onchocerca volvulus* and *L. loa* are identical and share 99.7% homology with *Brugia* spp. (Noordin et al., 2004a). False positive results have been recorded in those with *Entamoeba histolytica* infections, hookworm, *Toxocara* infection (Rahmah et al., 2001b), serologic evidence of toxoplasmosis and amoebiasis (Rahmah et al., 2001a) and one suspected *Dirofilaria repens* infection (Fischer et al., 2005).

Table 1–1 Sensitivity of tests using BmR1 antigen for the diagnosis of Brugian Filariasis compared to microfilaraemia

Format	Sample Numbers	Sample origins	Sensitivity	Sample numbers	Specificity	Reference
Brugia-ELISA	147 sera from microfilaraemic individuals obtained from various institutions	Malaysia, India, Thailand	99.3%	929 sera from non-endemic areas excluding those with parasitic or known bacterial infections	100%	Rahmah et al. (2001a)
Brugia-ELISA	28 sera from *B. malayi* microfilaraemic individuals	Pasir Mas (Malaysia)	100%			Lammie et al. (2004)
Brugia-ELISA	156 sera from microfilaraemic individuals	India, Malaysia	91.0%	50 sera samples from individuals of non-endemic areas	100%	Noordin et al. (2003)
Brugia-ELISA	3 sera from *B. malayi* microfilaria-infected individuals	Terengganu (Malaysia)	100%	1131 sera from individuals negative by microscopy	97.8%	Jamail et al. (2005)
Brugia-Rapid Dipstick	207 sera from microfilaraemic individuals	Malaysia, Indonesia, India	96.6%	546 sera from individuals of non-endemic areas including individuals with other parasitic infections	99%	Rahmah et al. (2001b)
Brugia-Rapid Dipstick	23 capillary blood samples performed in field	Sarawak (Malaysia)	87%	512 capillary blood samples from individuals of an area with no history of filariasis for 10 years	100%	Jamail et al. (2005)
Brugia-Rapid Dipstick	27 sera from *B. malayi* microfilariae-infected individuals	Pasir Mas (Malaysia)	100%			Lammie et al. (2004)
Brugia-Rapid dipstick	109 sera. 97 individuals with *B. timori*, 12 with *B. malayi*	Uganda, Indonesia	100%	10 sera from Europe	100%	Fischer et al., 2005
Brugia-Rapid dipstick	156 sera from microfilaraemic individuals	India, Malaysia	96.8%	50 sera samples from individuals from non-endemic area	100%	Noordin et al. (2003)
Brugia-Rapid dipstick	269 sera from microfilaraemic individuals	India, Indonesia, six samples from the sera bank at the Swiss Tropical Institute	93.3%	266 samples from non-endemic individuals excluding patients with *W. bancrofti*, *O volvulus* and *L. loa* infections	100%	Rahmah et al. (2003b)
Brugia-Rapid dipstick	196 sera from *B. timori*-infected individuals	Alor island (Indonesia)	100%	50 sera from western Uganda	100%	Supali et al. (2004)
ELISA Total	**334**		**95.5%**	**2110**	**98.8%**	
Rapid dipstick Total	**987**		**96.7%**	**1434**	**99.6%**	

Table 1–2 Combined specificity of *Brugia*-ELISA and *Brugia*-Rapid Dipstick of individuals infected with other parasites

Parasitic Infection	Total Number Tested	Specimens Positive	References
W. bancrofti microfilaraemic	86	39.5 % by *Brugia*-ELISA	Rahmah et al. (2001a); Lammie et al. (2004); Noordin et al. (2004a)
W. bancrofti microfilaraemic	108	54.6 % by *Brugia*-Rapid Dipstick	Rahmah et al. (2003b); Lammie et al. (2004); Fischer et al. (2005)
O. volvulus	287	1.0 % by either *Brugia*-Rapid Dipstick or *Brugia*-ELISA	Rahmah et al. (2001a); Rahmah et al. (2001b); Rahmah et al. (2003b); Lammie et al. (2004); Noordin et al. (2004a); Fischer et al. (2005)
L. loa	77	23.4 % by either *Brugia*-Rapid Dipstick or *Brugia*-ELISA	Rahmah et al. (2003b); Lammie et al. (2004); Noordin et al. (2004a)

Table 1–3 Comparison of prevalence of microfilaraemia and seropositivity to BmR1

Location	Sample Numbers	Microfilariae Detection Method	Prevalence of microfilaraemia %	BmR1 Method	Prevalence by BmR1 %	Prevalence of microfilaraemia/ Prevalence by BmR1	Reference
Setiu, Terengganu, Malaysia	1134	60 µl Smear	0.26	*Brugia*-ELISA	2.47	9.5	Lim et al. (2001)
Southern Sarawak, Malaysia	688	60 µl Smear	1.89	*Brugia*-Rapid Dipstick	18.6	9.8	Jamail et al. (2005)
Northern Sarawak, Malaysia	1095		0.55		6.3	11.5	
Central Sarawak, Malaysia	762		0.52		5.4	10.4	
Alor Island, Indonesia	759	1ml filtration	26	*Brugia*-Rapid Dipstick	80.5	3.1	Supali et al. (2004)
Alor Island post-1 year MDA	66	1ml filtration	12.1	*Brugia*-Rapid Dipstick using sera from eluted blood spots	71.2	5.9	Fischer et al. (2005)
Gual Periok, Malaysia	737	Smear	0.27	*Brugia*-ELISA	1.36	5.0	Rahmah et al. (2003a)

Figure 1–1 Relationship between prevalence of microfilaraemia and seropositivity to BmR1

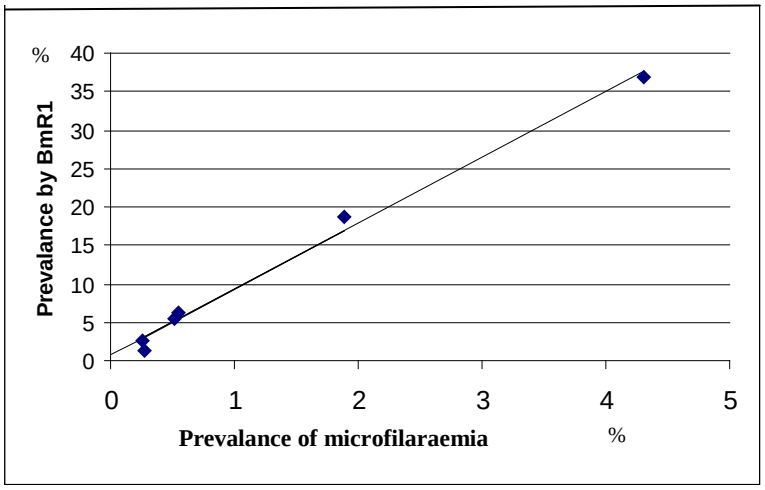


Figure 1–1 was generated using data from Table 1–3 and from the prevalence at Rembor shown in Table 1–8. Data from Alor Island (see Table 1–3) was excluded as the prevalence of microfilaraemia was determined with one millilitre filtrated blood. This has increased sensitivity compared to examination of blood smears (Moulia-Pelat et al., 1992). Additionally, one result from Alor Island (Fischer et al., 2005), followed a mass drug administration (MDA) program where it might be expected that the prevalence of microfilaraemia would be further reduced compared to the prevalence of seropositivity to BmR1 (Tisch et al., 2008). The data was analysed using Prism 3 GraphPad Software (San Diego, CA) and demonstrates a linear relationship between the prevalence of microfilaraemia and prevalence of seropositivity to BmR1 ($r^2 = 0.992$) when the microfilaraemia prevalence is less than 5%.

The linear equation has a slope of 8.6 (95% CI, 7.5–9.6) and intercept of 0.7 (95% CI, -0.3–0.1). The equation predicts that an area with 1% microfilaraemia will have a 9.3% (95% PI, 5.2 –13.3) seropositivity to BmR1.

The decision whether to use the *BRUGIArapid* cassette is analogous to the decision that was taken to use the ICT for Bancroftian filariasis instead of microfilaraemia. In both cases, the exact relationship between microfilaraemia prevalence and antigen or antibody prevalence is unknown and after treatment, both take several years before returning negative results. While a positive ICT indicates the presence of adult worms, a positive *BRUGIArapid* cassette test may indicate only exposure to the parasite. Notably, the ICT is being used for mapping Bancroftian filariasis without the exact relationship to microfilaraemia being determined or understood.

1.1.7 Development after exposure and response after treatment

In a transmigrant population relocated to a *B. malayi* endemic area in South-Sulawesi, the earliest detection of IgG4 by *Brugia*-Rapid dipstick was three years after arrival. After six years, the prevalence in the transmigrant population was similar to long-term residents (Noordin et al., 2004b). *Brugia*-Rapid dipstick has also been found positive in children as young as three years old (Supali et al., 2004).

Table 1-4 details the percentage of patients still positive after time periods following treatment. Reinfection, continued exposure or treatment failure may have influenced the length of time the test remained positive following treatment in the above studies.

In two trials, shown in Table 1–4, all patients became negative two years after treatment. There is a decrease in OD over time until it is below the cut-off absorbance. In comparison, *Brugia*-Rapid Dipstick shows a high percentage of patients still positive two years and longer after treatment.

Table 1–4 Change in prevalence of sero-positivity to BmR1 after treatment of Brugian filariasis

Location	Test	Number Treated	Treatment	Time since Treatment	% Positive	Reference
Malaysia	*Brugia*-ELISA	15	6 days DEC (6 mg/kg bodyweight)	6 m	87 %	Rahmah et al. (2001a)
				1–1.5 years	60 %	
				2 years	0 %	
India	*Brugia*-ELISA	30	DEC (details unspecified)	1 year	70 %	Rahmah et al. (2001a)
Thailand	*Brugia*-ELISA	15	4 courses (details unspecified) DEC over 2 years	Following end of treatment	0 %	Rahmah et al. (2001a)
Central Sulawesi, Malaysia	*Brugia*-Rapid Dipstick	18	100 mg DEC per week for 1 year	2 years post-treatment	53 %	Braga et al. (2003)
Sumatra, Indonesia	*Brugia*-Rapid Dipstick	19	100 mg DEC per week for 1 year	1 year post-treatment (9 samples)	78 %	Braga et al. (2003)
				2 years post-treatment (10 samples)	60 %	
South Sulawesi, Malaysia	*Brugia*-Rapid Dipstick	21	6 mg/kg DEC for 3 consecutive 12 day courses, with 2 week intervals between courses where DEC was administered once a week	3 years post-treatment	71 %	Braga et al. (2003)

The graph of OD, from *Brugia*-ELISA, *versus* months after treatment in the study by Rahmah and Lim (2001a) shows OD of all treated individuals decreasing below the cut-off level of 0.300, but there remains some individuals with ODs midway between 0 and 0.300. Is it possible that *Brugia*-Rapid dipstick could be positive in some of these individuals?

Comparing sixteen samples from microfilaraemic individuals that were negative by one or both *Brugia*-ELISA or *Brugia*-Rapid Dipstick, *Brugia*-Rapid Dipstick was positive in 62.5% while Brugia-ELISA was positive in 6.25% (Noordin et al., 2003) indicating that *Brugia*-Rapid dipstick may be more sensitive with low IgG4 responders. Since antibody concentration to BmR1 decreases after treatment the high positive rate with *Brugia*-Rapid dipstick may be due to its increased sensitivity to low IgG4. If so, this could have implications for using the *BRUGIArapid* cassette to monitor an elimination program and to survey an area after the completion of a MDA program as it may be many years before individuals become negative.

To allow comparison across surveys, it would be interesting to equate an OD value in *Brugia*-ELISA with the lowest detectable level that is positive with the *BRUGIArapid* cassette.

1.1.8 Filariasis in Timor-Leste

Brugia timori was first reported in East Timor in 1964 (David and Edeson, 1964). A survey conducted in 1962–1964 showed a prevalence rate of 2.6% in and around Dili among army recruits aged 18–22 years using 20 µl Giemsa stained blood smears taken at night. Another survey performed in April–June 1964 in five suburbs of Dili showed a prevalence rate of 5.4% with *B. timori*, 0.7% with *W. bancrofti* and 0.3% with a mixed infection. The microfilarial carrier rate ranged from 1.5 to 11.3%. In the district of Manatuto, 15.3% had

B. timori microfilaraemia, 0.8% *W. bancrofti* microfilaraemia and 1.2% had a mixed infection (David and Edeson, 1965).

A more recent survey found 419 military recruits from around Timor-Leste tested by *Brugia*-Rapid dipstick, ICT and TropBio Og4C3 ELISA showed 9.8% prevalence for Brugian filariasis and 0.8 % for Bancroftian filariasis. Sixty-eight samples tested from outpatients at the Dili Hospital showed a 7.3% prevalence for Brugian filariasis and 2.9% for Bancroftian filariasis. The village Rembor, in the Manatuto district had a prevalence of 34% and 1.7% for Brugian and Bancroftian filariasis respectively (Melrose, 2002).

1.2 Method

1.2.1 Site selection

A field evaluation of the *BRUGIArapid* cassette was undertaken, by myself and WHO staff in Timor-Leste, in November 2004 and April 2005. The intention was to evaluate the test in both low and high endemic areas and include patients infected with *B. malayi* and *B. timori.* A high endemic *B. malayi* site in Indonesia had been identified to evaluate the cassette. However, due to the tsunami on the 26 December 2004, this was changed to evaluate the cassette in a low and high endemic area in Timor-Leste. Although only *B. timori* is present, this was not expected to influence the outcome of the evaluation as the sensitivity of tests using BmR1 is similar for both species (Rahmah et al., 2001; Supali et al., 2004).

Based on previous studies, Dili city and its district were chosen for the low endemic area site and the village of Rembor, in the Manatuto district, for the high endemic area. It was intended to spend two nights testing at Rembor but a river en route to the village was in flood so an alternate village, Aitehen, was chosen after an attempt to cross the river failed. Testing occurred at Rembor the following night. Aitehen had a low prevalence of filariasis, the results of which are included with areas of low endemicity.

Figure 1–2 shows the locations where the evaluation took place. Samples were selected from self-selecting residents from the five locations.

Figure 1–2 Sites in Timor-Leste where the evaluation was conducted. The smaller arrows indicate the five locations (supplied by WHO, Timor-Leste)

1.2.2 Collection and test method

Boxes of *BRUGIArapid* cassette were transported from Malaysia to Australia and then to Timor-Leste. The first shipment of boxes, except for one box, was stored at 4 ºC several weeks before transport to Timor-Leste. The second shipment of boxes was kept for several days at room temperature before being transported. In Timor-Leste, the boxes were kept in an air-conditioned office up to a week before being used.

Capillary samples were collected and tested between 19:00 and 23:00 hours. Finger prick blood was collected into either a heparinised 100 µl capillary tube or two 70 µl heparinised

capillary tubes. Capillary tubes had been marked so the appropriate volumes could be delivered.

Thirty-five microlitres of heparinised blood were added to the lower sample well of the *BRUGIArapid* cassette with one drop of Chase buffer. This was allowed to migrate up the strip until liquid reached the blue line. Two drops of buffer were added to the top well, the plastic strip was pulled out and one drop was added to the bottom well. The tests were read at 10 minutes, in accordance with the test instructions, on tests performed in Dili and the surrounding district. The instructions had been modified by April 2005 so tests performed in Rembor and Aitehen were read after 15 minutes.

Three 20 µl blood strips were placed on a glass slide for subsequent Giemsa staining and examination for microfilariae. The slides were stained the following day with 3% Giemsa stain for 30 minutes, dried and examined for microfilariae.

1.2.3 Assessment of the *BRUGIArapid* cassette test kit

The test kit, cassette and instruction sheet and the experience using them within Timor-Leste were recorded. Usability, readability, robustness of the methodology and stability of the reaction were considered.

Usability was determined by demonstrating the test to three WHO staff who had never used the test before and asking them to read the instructions. They were then asked to perform the test themselves.

Readability of the test cassette was evaluated by grading the colour intensity of the test line and the ease of reading the test line. Three grades were used for the colour intensity: very faint, faint, and strong. As blood in some cases tended to obscure the test line on the cartridge, tests performed at Rembor were graded on whether red blood cells had totally

cleared from the reading area, partially cleared or had not cleared therefore obscuring the test line. Five cassettes from different survey subjects were photographed at 5, 10, 15, 20, 25 and 30 minutes to record the clearing of red blood cells from the test area. Photographs are shown in Figure 1-3.

It was found at Aitehen large number of cassettes was unreadable due to a failure of the red cells to clear from the test area. This obscured any positive line that may have been present. This may have been due to any of the following conditions:

- Too much blood added to the cassette.
- Exact test protocol was not followed and the liquid flow line migrated significantly past the blue line before buffer was added to well two. This would allow a greater volume of blood to flow up the strip.
- Sample population had a high packed cell volume (PCV) compared to the other areas such that there was comparatively more red cells per volume of blood added that could not be absorbed away.
- Variability inherent within the cassette.

In the laboratory, the factors that may have contributed to the poor clearance of red blood cells were investigated. Thirty-five microlitres of the same heparinised blood were pipetted onto 10 cassettes and the method protocol followed exactly. Blood clearance from the testing area was recorded at 15, 30 and 60 min. Volumes of the same blood sample (PCV=0.46), 15 to 50 µl in steps of 5 µl, were added to cassettes and red blood cell clearance recorded at 15 and 30 min. The PCV of the same whole blood specimen was adjusted by mixing packed red cells and plasma. These samples, PCV ranging from 0.10 to 0.53, were added to the cassettes and red cell clearance noted at 15 and 30 min. To see whether variation in the protocol influenced red cell clearance, ten replicates using a single blood sample (PCV =0.46) were

performed allowing the liquid line to migrate to the end of the visible strip and beyond rather than as the instructions indicated to the required position on the test cartridge.

In order to see the effect of blood clearance on reading a positive result, two plasma samples were spiked with red blood cells (PCV=0.34). One of these plasma samples gave a strong intensity test line, the other a weak intensity test line. Seven replicates were performed on both spiked red cell samples and results recorded at 15, 30 and 60 minutes.

To the test robustness in small variations in test procedure, the test was performed with a weak reacting plasma sample (plasma titre with *BRUGIArapid* cassette: 1/8) spiked with red cells (PCV =0.34). The effect of using 25 μl and 30 μl of this blood sample, adding two or three drops of buffer, adding two drops after pulling the plastic tab on the cassette instead of before pulling the tab, a short delay in adding sample buffer and allowing the liquid line to migrate too far before adding buffer were investigated on the positive reaction of this plasma sample. Results were read at 30 minutes.

1.3 Results

1.3.1 Prevalence of sero-positivity to *BRUGIArapid* cassette and microfilaraemia

The demographic profiles and the results of the testing of low endemic areas are shown in Table 1–5, Table 1–6 and Table 1–7. Table 1–8 details the demographic profile of those tested positive in the high endemic areas.

Table 1–5 Low endemic areas: demographic profile

	Location			
	Nazare, Dili City	**Sao Francisco, Dili City**	**Hera, Dili District**	**Aitehen, Manatuto District**
Number Tested	59	94	52	103
Male: Female Ratio	1:0.79	1:1.35	1:1.60	1:0.63
Age Range in Years	%	%	%	%
0–4	1.7	14.9	13.5	8.7
5–9	1.7	20.2	23.8	17.5
10–14	8.5	11.7	9.6	15.5
15–19	8.5	9.6	0.0	21.4
20–24	20.4	9.6	19.2	5.8
25–29	8.5	10.6	5.8	5.8
30–34	10.2	4.3	7.7	6.8
35–39	6.8	8.5	1.9	2.9
40–44	11.9	8.5	5.8	6.8
45–49	6.8	0.0	3.8	2.9
50–54	6.8	1.1	9.6	3.9
55–59	1.7	0.0	0.0	1.0
60–64	5.1	1.1	0.0	2.9
65–69	0.0	0.0	0.0	0.0
70–74	0.0	1.1	0.0	0.0
75–79	1.7	0.0	0.0	0.0

Table 1–6 Low endemic areas: Results

	Nazare, Dili City	**Sao Francisco, Dili City**	**Hera, Dili District**	**Aitehen, Manatuto District**
% BmR1 positive	1.7 %	2.1 %	3.8%	1.0 %
% Microfilariae positive	0.0 %	0.0 %	0.0 %	0.0 %

Table 1–7 Demographic profile of positive samples in low endemic areas by *BRUGIArapid*

Location	Sex	Age	Strength of reaction
Sao Francisco, Dili	Male	60	Very weak
Sao Francisco, Dili	Female	22	Very weak
Nazare, Dili	Male	22	Very weak
Hera, Dili District	Female	23	Weak
Hera, Dili District	Female	13	Weak
Aitehen, Manatuto District	Male	60	Weak

Table 1–8 Prevalence of seropositivity to *BRUGIArapid* by age in high endemic area (Rembor)

Rembor, Manatuto			
Grouping (Age/Gender) n=117	% of Sample	% of Age Group/Gender *BRUGIArapid* positive	% of Age Group/Gender Mf positive
0–4 y	18.8	0.0	0.0
5–9 y	19.7	47.8	4.3
10–14 y	14.5	35.3	0.0
15–19 y	3.4	50.0	0.0
20–24 y	6.8	37.5	11.1
25–29 y	7.7	33.3	0.0
30–34 y	3.4	75.0	25.0
35–39 y	7.7	33.3	0.0
40–44 y	4.3	60.0	20.0
45–49 y	5.1	66.7	0.0
50–54 y	2.6	0.0	0.0
55–59 y	0.9	100.0	0.0
60–64 y	3.4	75.0	25.0
65–69 y	1.7	50.0	0.0
5–14 y	34.1	42.5	2.5
20–69 y	47.1	51.0	7.8
Male	52.1	41.0	4.9
Female	47.9	30.9	3.6
Total		36.8	4.3

There were five cases of Brugian microfilaraemia with one, from an eight year old child, negative by the *BRUGIArapid* cassette. At Rembor, the prevalence with *BRUGIArapid* cassette was 8.6 times greater than the prevalence of microfilaraemia. Eleven of the 23 children, aged five to nine years of age, were positive by the test but only one child was microfilaraemic. In the low endemic areas no cases of microfilaraemia were seen.

1.3.2 Delivery and packaging

The contents are packaged in a sturdy cardboard box. Fifty test cassettes are individually enclosed in a pouch containing a desiccant. Two five millilitre buffer bottles are enclosed within the box.

Two shipments of tests cassettes were received from Malaysia and then transported to Timor-Leste. Each box contains 50 cassettes and two bottles of buffer. All 26 bottles of buffer received had leaked to some extent but the bottles were enclosed in a plastic bag so the contents were retrievable. During the first field study, when the bags were opened at night in poor lighting, buffer was lost such that only 200 out of 250 tests could be performed.

Leaking appeared to be from around the nipple of the bottle that sits within the bottle's neck. This fitting was too loose so buffer leaks between the nipple and neck. Some bottles also had cracks in the plastic in the neck of the bottle that resulted in additional leaking. Buffer was also lost from the insecure fitting when performing the test.

The second shipment of eight boxes of test cartridges that should have had 16 bottles of buffer contained only eight bottles. The company was contacted and another eight bottles were promptly sent to Timor-Leste.

1.3.3 Instruction sheet

The instruction sheet was concise and contained all the required information. There was a diagram of the cassette alongside instructions on how to perform the method. Although step one, step two and step three were written in bold, there was a potential for a person with English as their second language to start at the first step listed (step two). This was

demonstrated when a East Timorese laboratory worker was given the sheet to see if he could follow the instructions and he started with the first step listed, step two.

In step three, the instruction was to read the result in five minutes. A note below the diagram then informs to read serum/plasma/within 10 minutes and blood within 15 minutes.

1.3.4 Usability

With a person recording patient's details, another person collecting the capillary blood sample and another performing testing, an individual could be processed every two minutes.

Two tests (0.4 %) of all tests performed were invalid as evidenced by the lack of a control line. Possibly, this was due to insufficient buffer being added to well two.

1.3.5 Readability

Samples from the low endemic areas gave faint positive lines. Careful examination is required otherwise the test was easily misinterpreted. In contrast, most of the tests performed in Rembor gave easily read test lines. This could be related to intensity of infection. The positive reactions from Rembor were graded as shown in Table 1–9. There were equal numbers of children and adults giving faint or very faint reactions.

Despite the instructions changing the incubation time to 15 minutes for the Rembor survey, the majority of tests could not be read at this time due to inadequate clearing of the blood from the test area. Most tests could be read within 30 minutes with some needing up to one hour.

Figure 1–3 shows five tests performed at Hera and the clearing off blood from the test area over 30 minutes.

Of the tests performed in Dili and at Hera, three samples (1.4%) had not cleared adequately for the test to be read. This situation was amplified at Aitehen where 33 (24%) of the tests could not be read. At Rembor, five (3.4%) samples did not clear. One of these samples had cleared by the following morning to reveal a positive result. The tests at Rembor were graded on how well the blood cleared from the strip as shown in Table 1–10. Tests that did not clear were not included (see Table 1–6) except for the test that revealed a positive result the next morning.

Figure 1–4 shows a negative result with partial clearing (specimen 203), a weak positive result (specimen 190) and a cassette where the blood did not clear from the viewing area (specimen 175). This blood could obscure reading a positive result.

Table 1–9 Strength of reaction of positive results from Rembor

Strength of reaction	% of Positive results (n=43)
Very faint	4.7
Faint	14.0
Strong	81.3

Table 1–10 Grading of the readability of results in Rembor

Clearance	% (n=121)
No clearance	3.4
Partial clearance	28.9
Total clearance	67.7

Figure 1–3 Time course showing the clearing of the *BRUGIArapid* test pad over 30 minutes

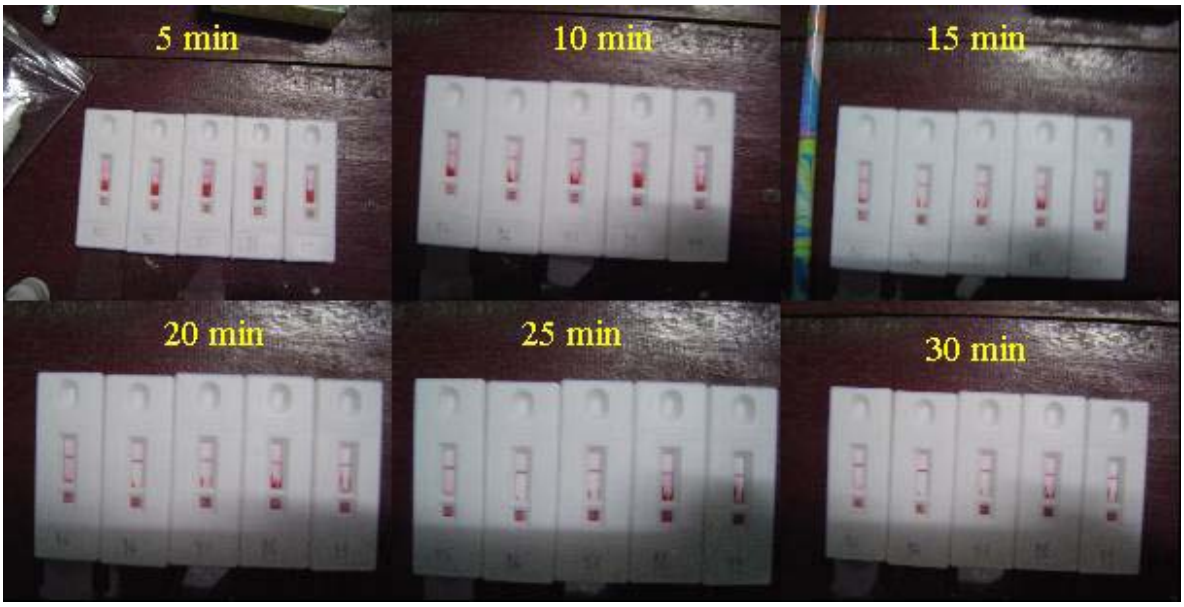


Figure 1–4 Extent of blood clearing from the test area arrow indicates position of positive test line

arrow indicates position of positive test line

Figure 1–5 shows the readability up to 60 minutes of 35 µl of a heparinised sample (PCV =0.46) pipetted onto 10 cassettes. Two (20%) of the tests could not be read at 60 minutes. Note that the far right cassette in Figure 1–5 has completely cleared of blood from the viewing area compared to the other cassettes.

Figure 1–5 Variability of cassettes in clearing with 35 µl of pipetted blood over time

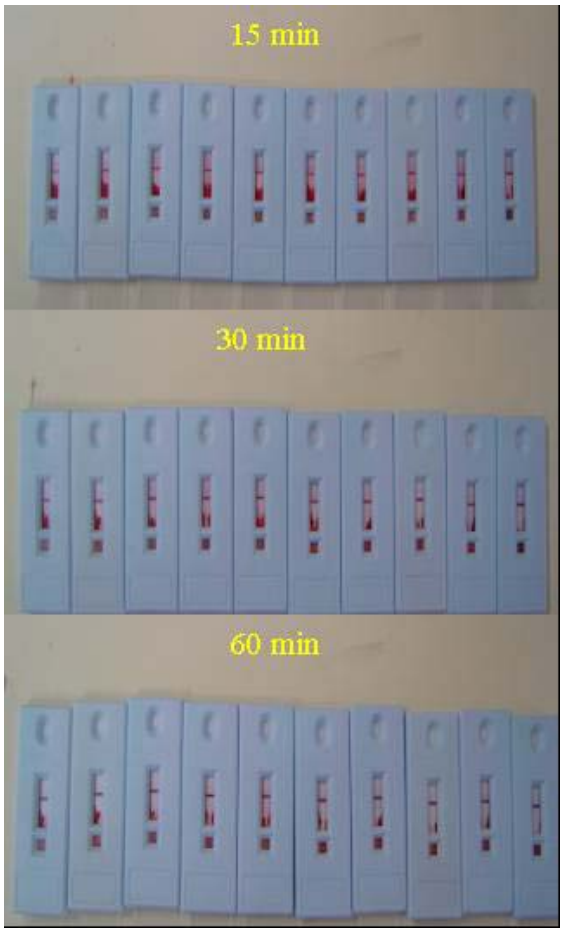
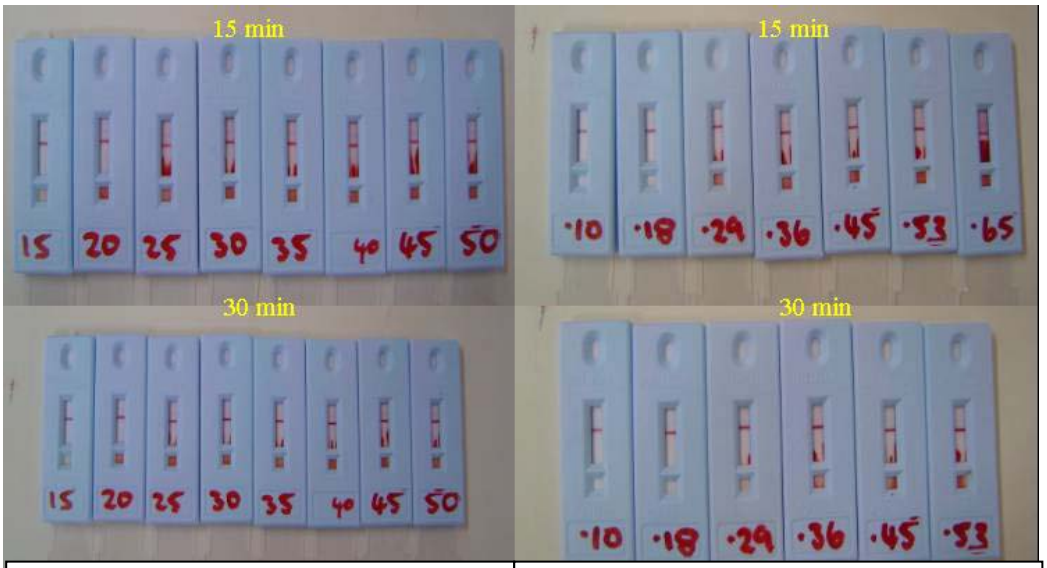


Figure 1–6 shows the test results when performed using varying volumes of blood, 15 to 50 µl in steps of 5ul (PCV = 0.46). A population of healthy individuals ranging in age from young children to adults would have a PCV ranging from 0.34–0.54. This means in a 35 µl blood sample the actually volume of plasma containing antibody to BmR1 will range from 16–23 µl. Clearance of the blood from the test area was related to volume and PCV with increasing volume or PCV equating to reduced readability. At 15 minutes, both the tests that had 15 µl and 20 µl added had cleared totally. By 30 minutes, the test could generally be examined though becoming more difficult with a higher PCV.

Figure 1–6 Effect of varying blood volume (µL) and PCV on readability



Cassettes with varying volumes of whole blood (µL shown in red on cassette) at 15 and 30 minutes	Cassettes with varying PCVof whole blood (PCV shown in red on cassette) at 15 and 30 minutes

At 60 minutes, one sample (10%) had cleared completely, six samples (60%) had partially cleared, though a positive line would be partially obscured, and three samples (30%) were unreadable. This is shown in Figure 1–7.

Figure 1–7 Clearance of blood after 60 minutes after allowing liquid front to migrate to end of test strip

In order to see the effect of blood clearance on reading a positive result two plasma samples were spiked with red blood cells to a PCV of 0.34. Figure 1–8 shows the reaction of the plasma samples without red blood cells added after the recommended incubation time of 10 minutes. The test line is faint with sample on the left, (1) and clearly visible with the second sample (2) on the right.

Figure 1–8 Test lines of two different plasma samples

Figure 1–9 Reactions of plasma with faint test line (1) and plasma with clearly visible test line (2) spiked with red cells

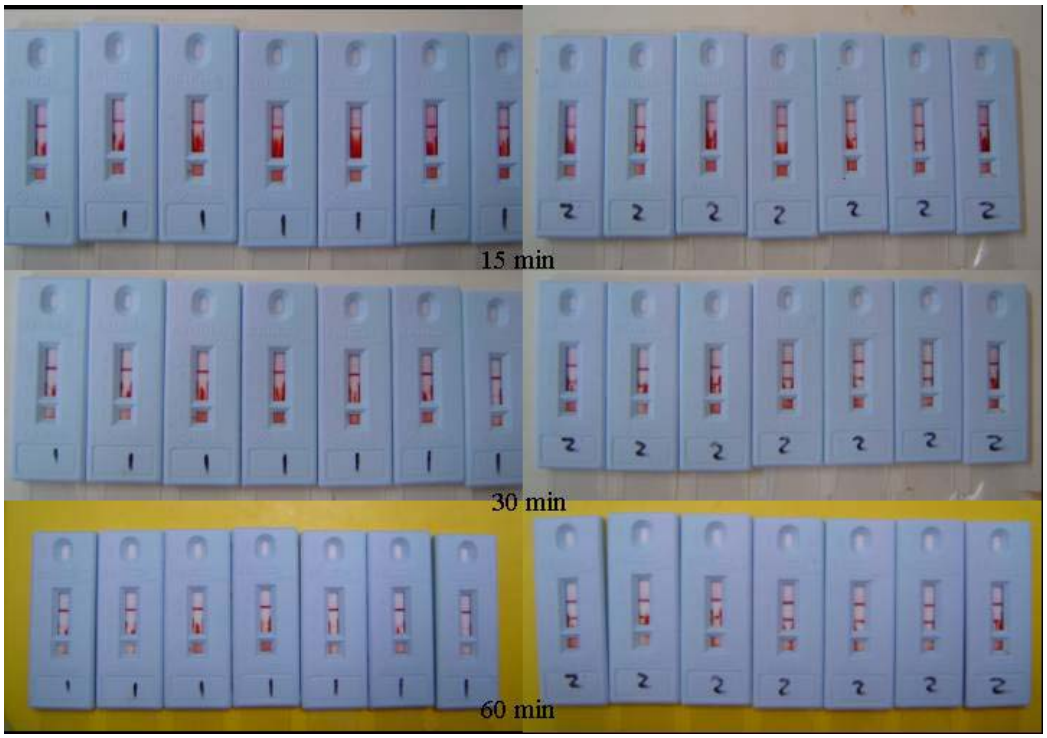


Table 1–11 Per cent positive of faint and clearly visible test line plasma spiked with red cells after a specified incubation time

Incubation time	Plasma with faint test line	Plasma with clearly visible test line
15 min	0 %	71.4 %
30 min	71.4 %	100 %
60 min	100 %	100%

Figure 1–9 shows the reactions of the spiked blood sample at 15, 30 and 60 minutes. There is clearly a positive result at 15 minutes in the plasma that had a clearly visible test line in the cassette second from the right. Blood is obscuring the reaction in the other cassettes.

The percentage of positive samples is shown in Table 1–11. No plasma samples that had a faint test line were positive after 15 minutes incubation (recommended time) due to red blood cells obscuring the reading area. All samples could be determined positive after one hour incubation. In four cassettes (two of the plasma with faint lines and two of the plasma with clearly visible lines) blood obscured the line making it difficult to read.

1.3.6 Robustness

As shown in Table 1–12, the test appears quite robust. Variations that influenced results were adding an extra drop of buffer to the sample that diluted a weak result sufficiently to turn it negative and, as previously discussed, a delay in adding buffer to the blood sample or allowing the sample to flow to the end of the visible strip, which increases the amount of blood around the test area and inhibits reading.

Table 1–12 Effect of variation in *BRUGIArapid* cassette test protocol

Parameter	Actual Instructions	Reaction	How method deviance could occur in the field
25 µl blood sample added	35 µl blood	Positive result noticeably fainter than original sample	Under filling capillary tube Capillary tube contains bubbles Not all blood released by capillary action into sample well
30 µl blood sample added	35 µl blood	Positive result, no noticeable effect	
2 drops of buffer added with blood into sample well	1 drop of buffer should be added	First result negative, repeat test showed a very faint positive	Too much pressure placed on sample bottle Leaking sample bottle
3 drops of buffer added to top well	2 drops of buffer added to top well	Positive result, no noticeable effect	
2 drops buffer added to sample well after pulling plastic tab	1 drop of sample buffer added to sample well at end	Positive result, no noticeable effect	
Delay in adding sample buffer following 35ul blood	Buffer should be added immediately after blood sample	Red cells covering greater area of test strip resulting in reduced clearance of blood and inability to read results.	Completing other tasks while waiting for blue line to be reached Inattention to migration of flow line
Allowing liquid front line to go end of the sample strip before adding buffer to top well	Buffer should be added when liquid flow line reached the blue line	Red cells covering greater area of test strip resulting in reduced clearance of blood and inability to read results.	

1.3.7 Stability of reaction

Positive test lines and negative tests were stable and positive lines remained visible for hours including overnight. By the next morning, in many instances, the test area was discoloured and obscured by haemolysed blood. This was more pronounced with samples that had partially cleared.

1.4 Discussion

The *BRUGIArapid* cassette prevalence (36.8%) showed excellent agreement with the survey previously performed at Rembor (34%) in 2002 with the *Brugia*-Rapid dipstick (Melrose, 2002). At Rembor, the prevalence with *BRUGIArapid* cassette was 8.6 times greater than the prevalence of microfilaraemia. This agrees with the published data shown in Table 1–1.

The buffer bottle leaked during transport and use. The company has since modified the bottle design to prevent this occurring. It could be advantageous to include capillary tubes calibrated to deliver the correct volume and a small volume of liquid quality control material. Although a control line is present, it is only ensuring that buffer has been added to well two and the reaction between conjugate and anti-mouse IgG has not been compromised. A positive and negative control sample would ensure the integrity of the test line and that the whole process including manufacture, transport, storage of the cassette and the correct test procedure has followed good practice. This would alert users of problems similar to ones experienced with the ICT card test (Rajgor et al., 2002; Braga et al., 2003).

An error in the instructions was noted regarding the time to read to the result. This has since been updated. WHO staff who were shown the testing method had no problem in performing the test. At Aitehen there were a large number (24%) of unreadable cassettes. A combination of cassette variability, operator error allowing the liquid flow to migrate too far past the blue line and possibly a relatively higher PCV compared to the other populations tested may have contributed to the high unreadability. The incomplete clearing of red cells from the test area was the main concern with the cassette. Possibilities to reduce this problem in testing whole blood may involve the use of an alternative design or absorbent pad so red cells can be absorbed away more effectively, reducing the sample volume, moving the test

line higher up the test strip so that the buffer/plasma mix crosses the line but the red cells do not, and/or increasing the incubation time.

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage (International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use, 1995). Unlike the controlled setting of the laboratory, performing a test in the field introduces variables that cause small variations in the test procedure. This can be due to distractions, multi-tasking, being too hurried, poor lighting and variation in technique by and between operators. This test may be used by healthcare workers who are not familiar with testing devices. Once a volunteer has been finger pricked it can be difficult to find the individual again and request another sample if the test has been improperly performed. The individual is also unlikely to subject themselves to another finger prick. The test appeared quite robust to small changes in procedure and results very stable for several hours.

Assays using BmR1 antigen show good sensitivity and high specificity compared to *B. malayi* and *B. timori* microfilaraemia. There is some cross-reaction with samples from individuals infected with *W. bancrofti*, therefore specificity for only Brugian filariasis will be lower where *Brugia* spp. and *W. bancrofti* coexist. However, as the same MDA program is used for both Brugian and Bancroftian filariasis elimination programs, this should be of no consequence. If discrimination is required antigen testing is available to determine prevalence of *W. bancrofti* infection.

There is only one published study (Jamail et al., 2005) using capillary blood, the specimen of choice in large-scale testing. Since this study showed a comparatively reduced

sensitivity compared to studies using sera, it would be prudent to perform a larger study to determine sensitivity with this sample type.

This test appears acceptable for mapping of *Brugian* spp. endemic areas prior to elimination. In order to confirm there is no more *W. bancrofti* transmission it is recommended a transmission assessment survey targeting children born in the first year of the program be tested by the ICT (PacELF Monitoring and Analysis Network, 2004). The *BRUGIArapid* cassette could also be used to determine if transmission is occurring in this group. Due to the long delay before the test becomes negative, it is less certain what its role is in monitoring an elimination program.

As the exact relationship between prevalence of microfilaraemia and seropositivity to BmR1 is unknown further data is needed. Although there is no direct evidence, discussion at the Lymphatic Filariasis Scientific Working Group, Geneva, May 2005, suggested that an elimination program would not be required with a prevalence of seropositivity to BmR1 below 1% and would be indicated above 10%. Between 1 and 10%, microfilaraemia prevalence should be determined to indicate whether a program should be started. This additional data can be used to refine the correlation equation.

The *BRUGIArapid* cassette is user friendly and can be easily used in the field by those unfamiliar with point of care diagnostic tests. The readability of the test line is obscured by red blood cells when using whole blood. Increasing the test pad area or amount of absorbent material within the cassette is needed to remove red cells from the testing area. The buffer bottle was redesigned to have a tight and secure fitting lid to prevent leakage. The *BRUGIArapid* cassette is a tool that is quick to perform, easy to use, displays a result on site and is transported easily. The cassette's convenience should allow large-scale population testing in *Brugian* spp. elimination programs to be conducted with minimal difficulty.

Chapter Two: Evaluation of Field Collection Methods of Diagnostic Tests used in Bancroftian Elimination of Lymphatic Filariasis Programs

2.1 Introduction

The filter paper collection technique has shown poor sensitivity when used with the TropBio assay in lymphatic filariasis infection (Gyapong et al., 1998). This technique and a field version of the TropBio assay were evaluated using specimens collected in PNG as part of the baseline evaluation for filariasis elimination program.

2.1.1 The need for antigen tests

Historically, lymphatic filariasis infection has been determined by the detection of microfilariae in blood samples by microscopy. Microscopy is a relatively difficult to perform well and consistently in the field (Weil et al., 1997). Additionally, for many subspecies of *W. bancrofti* the optimum time for sampling is between 10 pm and 2 am when the concentration of microfilariae is greatest in the peripheral blood, therefore requiring the need for night blood surveys. These create problems for surveyed populations and survey staff.

Sensitivity is dependent on the blood volume examined (see Tables 2–1 and 2–2). A 60 µl blood smear may only detect about half of all patients with low density microfilaraemia (1–30 mf/ml). The prevalence of microfilaraemia in a population is dependent on the method. An apparent increase in prevalence in a community can be due simply to the technique used and the volume of blood tested (Southgate, 1974; Dreyer et al., 1996). Sensitivity also depends on the skill of the examiner (Weil et al., 1987).

Table 2–1 Comparison of ICT with detection of microfilaraemia

Details	ICT	Microfilaraemia	Reference
▪ Well characterised sera from a reference laboratory in St. Louis (USA)	▪ 96.2% (51/53) sensitivity compared to microfilaraemia ▪ 4% (1/23) positive with other parasitic infections. The one specimen testing positive likely coinfection with *W. bancrofti* ▪ 0% (0/70) non-endemic individuals negative		Weil et al. (1997)
▪ Well characterised sera from a reference laboratory in Basle (Switzerland)	▪ 100% (48/48) sensitivity compared to microfilaraemia ▪ Negative with sera from individuals infected with onchocerciasis (n=20), *M. perstans* (n=5), *L. loa* (n=5), strongyloidiasis (n=5), echinococcosis (n=5) and protozoan infections (n=10) ▪ 0% (0/20) non-endemic individuals negative		
▪ Well characterised sera from CDC (USA)	▪ 100% (32/32) sensitivity compared to microfilaraemia ▪ 0% (0/11) non-endemic individuals negative		
▪ Egypt ▪ Sampling 22:00–02:00 hr ▪ Read after 15 minutes	▪ 98.0% (50/51) sensitivity compared to TBS (50 µl) ▪ 95.3% (61/64) sensitivity compared to membrane filtration (1 ml) ▪ 94.0% (173/184) sensitivity of serum samples compared to whole blood samples ▪ 9.0% (163/1813) prevalence ▪ 102 non-endemic controls negative	▪ 2.8% (51/1813) prevalence (50 µl TBS) ▪ 3.5% (63/1813) prevalence (1 ml membrane filtration)	Ramzy et al. (1999)
▪ Thailand ▪ Sampling 20:00–24:00 hr	▪ 100% (56/56) sensitivity compared to microfilaraemia (60 µl TBS) ▪ 12.3% (56/454) prevalence ▪ Negative reactions with sera from individuals infected with soil-transmitted helminths (n=14) and *B. malayi* (n=2) ▪ 10 non-endemic controls negative	▪ 9.03% (41/454) prevalence (60 µl TBS) ▪ 7.71% (35/454) prevalence (60 µl capillary tube technique) ▪ 9.03% (41/454) prevalence (1 ml membrane filtration)	Phantana et al. (1999)

Details	ICT	Microfilaraemia	Reference
▪ Thailand ▪ Sampling 18:00–24:00 hr	▪ 20% (45/225) prevalence ▪ 100% (14/14) sensitivity compared to TBS (60 µl) ▪ 84 % (17/20) sensitivity compared to recall or physical examination ▪ Negative with subjects with Brugian microfilaraemia (n=10), *O. viverrini* (n=5), and hookworm (n=10) infected subjects and non-endemic control sera (n=20)	▪ 5.8% (13/225) prevalence (TBS 60 µl) ▪ 5.3% (12/225) by capillary technique (60 µl)	Bhumiratana et al. (1999)
▪ India ▪ Sampling 20:00–22:00 hr for microfilaraemia ▪ Sampling 10:00–16:00 hr for ICT	▪ 98.5% (64/65) sensitivity compared to microfilaraemia (60ul TBS) ▪ 71.9% (64/89) sensitivity compared to microfilaraemia (1ml membrane filtration) ▪ 189 non-endemic controls negative		Pani et al. (2000)
▪ Kenya ▪ Sampling 21:00–24:00 hr	▪ 100 % (96/96) sensitivity compared to microfilaraemic individuals positive by both counting chamber and Knott's concentrations (1 ml) ▪ 37.3% prevalence estimated from 24/97 amicrofilaraemic individuals that were ICT positive ▪ 100 non-endemic controls negative	▪ 16.7 % (117/701) prevalence (counting chamber method 100 µl) ▪ 14.6 % (102/697) prevalence (Knott's concentration 1 ml)	Njenga and Wamae (2001)
▪ Uganda ▪ Sampling 21:00–02:00 hr ▪ ICT read after 2 minutes	▪ 100% (238/238) compared to microfilaraemia (TBS, volume used not stated) ▪ Prevalence of 29.1% (259/890), 18.3% (164/896) and 30.1% (271/900) at Albetong, Lwala and Obalanga communities ▪ 14 *M. perstans*-infected individuals negative	▪ Prevalence of 25.5% (82/322), 9.7% (41/422) and 22.4% (115/513) at Albetong, Lwala and Obalanga communities (TBS, volume not stated)	Onapa et al. (2001)
▪ India ▪ Time of collection not stated	▪ 100% (428/428) sensitivity compared to microfilaraemia (TBS 60 µl) ▪ 23.7 % (831/3505) prevalence	▪ 12.2% (428/3505) prevalence (TBS 60 µl)	Sunish et al. (2001)

Details	ICT	Microfilaraemia	Reference
▪ Sri Lanka ▪ Sampling 21:00–24:00 hr	▪ 100% (67/67) sensitivity compared to microfilaraemic by NMF (1 ml) ▪ 37.1 % (79/213) prevalence ▪ 29 non-endemic controls negative	▪ 94.0% (63/67) sensitivity (60 µl TBS) compared to membrane filtration (1 ml) ▪ 29.6 % (63/213) prevalence by TBS (60 µl) ▪ 31.5 % (67/213) prevalence (1ml membrane filtration) ▪ 29 non-endemic controls negative	Chandrasena et al. (2002)

Table 2–2 Comparison of TropBio ELISA with detection of microfilaraemia

Details	TropBio ELISA	Microfilaraemia	Reference
▪ Sera from filariasis serum bank of the WHO from Philippines, Sri Lanka, PNG, Tahiti ▪ Positive > 100 AU/ml	▪ 100% (49/49) sensitivity compared to asymptomatic microfilaraemic individuals ▪ 98.5% (67/68) sensitivity compared to clinically or parasitologically confirmed individuals ▪ Mean antigen units (Number); AU/ml; Philippines (n=16) 10724; Sri Lanka n=(23,) 10388; PNG (n=6) 5448, Tahiti (n=11), 10672 ▪ Non-reactive with sera from individuals infected with *B. malayi* (10), *O. volvulus* (28), *L. loa* (7), *B. timori* (6), non-endemic controls (20)		More and Copeman (1990)
▪ Papua New Guinea ▪ Sampling after 22:00 hr	▪ 100 % (175/175) sensitivity compared to 1 ml Knott's concentration. (Positive mean + 3SD of negative control) ▪ 57.0 % (219/384) prevalence	▪ 26.2 % (46/175) prevalence (1ml Knott's concentration)	Turner et al. (1993)
▪ French Polynesia	▪ 94.5% (259/274) sensitivity compared to microfilaraemia (1ml membrane filtration). Positive > 100 AU/ml ▪ Geometric titre of microfilaraemic individuals 1676 AU/ml		Chanteau et al. (1994b)
▪ Haiti ▪ Sampling 20:00–22:00	▪ 100% (121/121) sensitivity compared to microfilaraemia (TBS 20 µl) ▪ 49.4% (207/419) prevalence ▪ Samples diluted 1:10. 100 µl aliquot collected by finger prick ▪	▪ 28.9% (121/419) microfilaraemia prevalence by TBS	Lammie et al. (1994)
▪ French Polynesia	▪ 94.5% (191/202) compared to1 ml membrane filtration ▪ Prevalence of 1.1% (2/196), 20.4% (210/1027) and 41.3% (259/627) at Moerai, Afareaitu and Opoa villages ▪ Geometric mean of 223, 643 and 1958 AU/ml at the three villages.	▪ Prevalence of 1% (2/196), 6.6% (68/1027) and 21.5% 9135/627) at Moerai, Afareaitu and Opoa villages by membrane filtration (1ml) ▪ Geometric mean of 5, 142 and 361 mf/ml at the three villages.	Chanteau et al. (1995)

Details	TropBio ELISA	Microfilaraemia	Reference
▪ Brazil	▪ 97.9% (276/282) sensitivity. Positive compared > titre 2 (OD=0.033) compared to 1,5 and 10 ml membrane filtration ▪ 1.6% (1/63) individual positive from endemic area		Rocha et al. (1996)
▪ Tanzania ▪ Sampling from 20:00–24:00	▪ 100% (93/93) sensitivity compared to microfilaraemia (50 µl counting chamber). Positive > titre 2 ▪ 54.6% (160/293) prevalence	▪ 31.7% (93/293) prevalence (50 µl counting chamber	Simonsen et al. (1996)
▪ French Polynesia	▪ 98.9% (183/185) sensitivity, Positive > 100 AU/ml compared to 1ml membrane filtration		Nicolas et al. (1997)
▪ Sri Lanka ▪ Sampling 22:00–01:00	▪ 77.5% (31/40) sensitivity compared to 1ml membrane filtration. Positive > titre 2 ▪ 20.7 % (73/353) prevalence	▪ 11.3% (40/353) prevalence (1 ml membrane filtration) ▪ 7.9% (28/353) prevalence (60 µl TBS)	Itoh et al. (1999)
▪ India	▪ 90.9% (40/44) sensitivity compared to microfilaraemia (TBS 60 µl)		Ravindran et al. (2000)
▪ Thailand ▪ Sampling from 20:00 to 24:00	▪ 100% (20/20) compared to microfilaraemia (60 µl TBS). Positive > 100 AU/ml ▪ 22.4% (44/196) prevalence	▪ 10.2% (20/196) prevalence (60 µl TBS).	Nuchprayoon et al., 2001
▪ Maupiti ▪ 34 years of intermittent DEC treatment	▪ 4.6% (46/997) prevalence ▪ Mean 714 AU/ml	▪ 0.4% (4/999) prevalence (1 ml membrane filtration)	Esterre et al. (2001)
▪ India ▪ Sampling 20:00–22:00	▪ 96.8 % (61/63) sensitivity compared to microfilaraemia (1ml membrane filtration .Positive> Titre 2) ▪ Geometric mean of microfilaraemic = 601 AU/ml ▪ 0% (0/40) non-endemic normal		Hoti et al. (2002)
▪ India ▪ Sampling 20:30–23:30	▪ 100% (47/47) sensitivity compared to microfilaraemia 50 µl TBS. Positive > titre 3. ▪ Negative in *Ascaris* (20). Malaria (~10), leprosy (10) infected individuals and non-endemic controls (20)		Bal et al. (2003b)

Table 2–3 Summary of reports where both TropBio ELISA and ICT are used

Details	TropBio ELISA	ICT	Microfilaraemia	Reference
▪ Brazil		▪ 95.6% (43/45) sensitivity compared to TropBio ELISA		Freedman et al. (1997)
▪ Serum collected from individuals after 4 years of DEC and Ivermectin on Tabaa ▪ Serum collected from individuals after six years of semi annual Ivermectin on Opoa ▪ Serum collected from individuals after 30 years of intermittent DEC treatment on Maupiti	▪ 37.1% (148/399), 26.0% (52/200)and 4.1% (41/996) prevalence's on Tabaa, Opoa, Maupiti islands	▪ 51.2% (104/203) sensitivity compared to TropBio ELISA. ▪ Agreement 92.4%, κ test= 0.60 ▪ 1.6% (22/1392) positive ICT but negative TropBio ELISA ▪ 19.0%, (76/399) 13.0% (26/200) and 2.4% (24/996) prevalence's on Tabaa, Opoa, Maupiti islands	▪ 12.7% (51/399), 3.5% (7/200) and 0.4% (4/996) prevalence's on Tabaa, Opoa, Maupiti islands (1 ml membrane filtration(	Nguyen et al. (1999)
▪ Ghana ▪ Sampling 21:00–24:00 hr	▪ 100% (24/24) sensitivity compared to microfilaraemia (100ul capillary tube). Positive > titre group 2 ▪ 38.5% (10/27) amicrofilaraemic positive ▪ 10 non-endemic controls negative	▪ 100% (24/24) sensitivity compared to microfilaraemia (100ul capillary tube) ▪ 29.6 % (8/27) amicrofilaraemic individuals positive ▪ 10 non-endemic controls negative		Simonsen and Dunyo (1999)
• Indian migrant workers in Saudi Arabia	▪ 200 non-endemic individuals negative and those with schistosomiasis (n=31), echinococcosis and one mixed infection were negative	▪ 100% (32/32) sensitivity compared to TropBio ELISA		Omar et al. (2000)

Details	TropBio ELISA	ICT	Microfilaraemia	Reference
▪ Thailand ▪ Sampling from 20:00 to 24:00	▪ 100% (11/11) sensitivity compared to microfilaraemia (60 µl TBS). Positive> 100 AU/ml. ▪ 19.5% (53/272) prevalence	▪ 100% (11/11) sensitivity compared to microfilaraemia (60 µl TBS) ▪ 12.7% (28/252) prevalence	▪ 3.3% (11/337) prevalence (60 µl TBS)	Nuchprayoon et al. (2003)
▪ Brazil ▪ Sampling 21:00–24:00 hr ▪ Non-endemic controls sampling 07:00–13:00 hr	▪ 0% (0/100) positive in non-endemic area. Positive > titre group 2	▪ 94.7 % (36/38) sensitivity compared to TBS (60 µl) ▪ 31.7 % (198/625) prevalence ▪ 72.4%– 100% estimated specificity ▪ 29.6% (32/110) weak positive in non-endemic area	▪ 6.1 % (38/625) prevalence (60 µl TBS)	Braga et al. (2003)
▪ India ▪ Sampling 20:00–22:00	▪ 91.2% (31/34) compared to microfilaraemia (TBS 60 µl) ▪ 24% (48/200) prevalence	▪ 100% (34/34) sensitivity compared to TBS (60 µl) ▪ 37 % (74/200) prevalence	▪ 17% (34/200) prevalence (TBS 60 µl)	Pani et al. (2004)
▪ Myanmar migrant workers in Thailand ▪ Sampling 30 min after DEC provocation	▪ 5.9% (17/287) prevalence. Positive > 120 AU/ml	▪ 70.6 % (12/17) sensitivity compared to TropBio ELISA ▪ 98.2% (270/275) specificity compared to TropBio ELISA ▪ 4.2% (12/287) prevalence ▪ κ test= 0.82 with TropBio ELISA	▪ 0.3% (3/860) prevalence (1ml Knott's concentration)	Bhumiratana et al. (2004)
▪ Haiti		▪ 90.9% (100/110) compared to TropBio ELISA (positive > 250 AU/ml) ▪ 2.0% (2/102) positive by ICT but negative by TropBio ELSIA		Fox et al. (2005)

Details	TropBio ELISA	ICT	Microfilaraemia	Reference
▪ Immigrant workers entering Kuwait ▪ Sampling 08:00–10:00	▪ 20.3 % (213/1050) prevalence. Positive > titre 2 ▪ All non-endemic individuals (50) and those infected with malaria (50), hydatids (30), amoeba (20) and schistosomes (60) negative	▪ 93.8% (30/32) compared to microfilaraemia (1ml membrane filtration) ▪ 90.1% (192/213) sensitivity compared to TropBio ELISA (Positive > 32 AU/ml) ▪ Specificity calculated as 84.1–100% ▪ All non-endemic individuals (50) and those infected with malaria (50), hydatids (30), amoeba (20) and schistosomes (60) negative ▪ 18.3% (192/1050) prevalence	▪ 3.0% (32/1050) prevalence (1ml membrane filtration)	Iqbal and Sher (2006)

Microfilariae also appear to be unevenly distributed in the circulatory system. More are found in venous blood, up to 30 fold, than might be expected based on capillary sampling (Eberhard et al., 1988; Dickerson et al., 1989; Dreyer et al., 1996) though this has not been found with the subperiodic *W. bancrofti* var. *pacifica* in the Marquesas Islands (Moulia-Pelat et al., 1993).

Since the introduction of antigen tests many amicrofilaraemic individuals have found to be antigenaemic. Antigenaemia with amicrofilaraemia may be explained due to either a pre-patent stage where only juvenile worms are present, a low number of adult worms with few female worms, single sex infections or effete worms (Lammie et al., 1994; Itoh et al., 1998; Itoh et al., 1999; Chandrasena et al., 2002). The thick blood smear is insensitive to low level infections of individuals who may still contribute to transmission (Weil and Ramzy, 2007).

The two commercial antigen tests used in Bancroftian Filariasis Elimination Programs are the ICT and the TropBio ELISA.

2.1.2 ICT

A 200-kDa antigen released by *Dirofilaria immitis* and *W. bancrofti* binds two monoclonal antibodies AD 12.1 and DH6.5 (epitope AD-DH). The monoclonal antibody AD 12.1 is used in the ICT (Weil et al., 1997; Simonsen and Magesa, 2004). In *D. immitis,* binding of the antibodies is strongest in the cuticle of both sexes. The epitope is present in many filarial and non-filarial nematodes. However, it is only present in the sera of individuals infected with *W. bancrofti* and has not been shown to be present in sera from patients infected with *B. malayi*, *L. loa*, *Ascaris lumbricoides*, *Strongyloides stercoralis* or *Necator americanus*. A single serum positive for

microfilariae of *O. volvulus* was reported though this may have been due to a double infection (Weil and Liftis, 1987; Weil et al., 1987). The antigen does not react with samples with high titres of rheumatoid factor or antinuclear antibodies (Vanamail et al., 1996).

In animal models, the presence of antigenaemia occurs four to eight weeks after infection (Weil et al., 1996) and there is a good correlation between antigen levels and the number of worms (Weil et al., 1985; Weil et al., 1990). The antigen is also very stable and can be detected for a week or more in blood stored at ambient temperatures (Weil and Liftis, 1987; Weil et al., 1987).

The ICT uses polyclonal antifilarial antibodies coupled to colloidal gold in solution and a monoclonal antibody bound to a nitrocellulose strip. If filarial antigen is present in a sample, it binds firstly to the colloidal gold bound antibody that then moves along the nitrocellulose strip. The secondary antibody captures this complex on the strip forming a visible line (Weil et al., 1997). The test was originally developed by ICT diagnostics (Australia) but is now marketed by Binax Inc, Portland, (USA) (Anonymous, 2003; Simonsen and Magesa, 2004).

The first ICT version used only plasma and serum samples. However, the second generation test allows whole blood to be used as well. No differences were seen in results with duplicate serum testing between the first and second generation tests (Nguyen et al., 1999). In another comparison study, the whole blood version showed higher sensitivity than the serum version. It was suggested that this could be due to the greater volume of serum used in the whole blood assay, 65 µl of serum in 100 µl volume of whole blood *versus* 50 µl in the serum version. However, of 11 samples positive using whole blood only four tested positive by the ELISA (Ramzy et al., 1999),

indicating a potential problem with the new generation test or using whole blood as a sample.

The ICT was chosen in 2000 for mapping lymphatic filariasis (Weil and Ramzy, 2007). It offers many advantages including its ease of performance, needing no specialised technicians, the quickness of results, its long shelf life and its ease in transport and storage. Communities used to finger prick collection for malarial diagnosis are likely to find the ICT more acceptable than methods requiring venous collection; therefore increasing participation (Santhanam et al., 1989; Phantana et al., 1999; Ramzy et al., 1999; Njenga and Wamae, 2001). Compared to venous collection, fingers lancets are less expensive and more easily transported. The main advantage is the ability to use blood taken during daylight hours. The requirement for day time testing is not new (Dennis et al., 1976) and this point was noted in a summary of the Pacific Program where it was considered the development of the ICT as particularly noteworthy by eliminating the need for night time surveys (Burkot and Ichimori, 2002).

Based on recurrent expenditure, including labour and consumables, the ICT was calculated as being 9.2 times more expensive than the thick blood film (Chandrasena et al., 2002). Pani et al. (2000) found that the cost of a night blood smear was US$0.50 inclusive of all overheads compared to the cost of an ICT card alone at US$1.05. Weil (2000) considered that not everyone in a village or district needs to be tested to identify endemic areas and intelligent sampling methods can decrease the number of people tested. However, even with intelligent sampling this cost can still be prohibitive for some countries and should be reduced (Phantana et al., 1999; Eigege et al., 2003).

2.1.3 Stability of the ICT result

Prior to Binax Inc. taking over the manufacturing of the ICT, test cards had shown good stability. Using serum, reactions could be read after five minutes but reactions read after 45 minutes increased sensitivity and specificity readings (Bhumiratana et al., 1999). Using whole blood, reactions read at 15 minutes then re-examined at one hour for confirmation had the same results (Njenga and Wamae, 2001). Cards reread within six months of testing by three independent readers showed an overall agreement of 87% with improved agreement, presumably with mostly positive ICT cards, among microfilaraemic individuals (93% agreement) *versus* 88% for amicrofilaraemic individuals from a non-endemic area (Pani et al., 2000). Weil et al. (1997) stated that the sensitivity was slightly improved by reading at 15 minutes compared to five minutes and cards could be stored as a permanent record.

After Binax took over manufacturer of the cards in 2001, it was noted by program managers that the test appeared to be providing false positives. Previously, card results were stable until the next day but with the change of manufacturer this was no longer the case. A multicentre trial showed that the test was sensitive and specific at 10 minutes, but variability was introduced when reading at 30 minutes (Anonymous, 2003). It was reported that 21.5 % of 172 cards that were negative after reading at 10 minutes were noted to be positive the next day. Fourteen of these were from individuals who were asymptomatic and amicrofilaraemic and who were previously negative (Rajgor et al., 2002). The presence of faint pink lines was also recorded in 26.4% of individuals in a non-endemic area that were negative by the TropBio ELISA (Braga et al., 2003).

In an evaluation of the effect of reading time at 10 minutes, 50% tested positive (48/96) at 10 minutes with test lines developing in 31 of the 48 negative individuals by 60 minutes. Development of these later test lines appeared to be age dependant with all those above 15 years of age and negative at 10 minutes, recording positive at 60 minutes. When further samples were compared to the TropBio ELISA, 90.9% (10/11) that were negative at 10 minutes, but positive at 60 minutes were negative by the TropBio ELSIA. It was observed that the lines that developed between 10 and 60 minutes were different in appearance, being broader and more faint, compared to those that were truly positive (Simonsen and Magesa, 2004). It was hoped in 2003 that this could be eliminated by 2004 (Anonymous, 2003), but this has not yet been achieved.

Although the main problem with the current ICT card is the appearance of a false test line, cards read the next day have shown a reduction in sensitivity with the disappearance of the test line being associated with those who were amicrofilaraemic (Pani et al., 2004). This could be due to the concentration of the antigen.

The manufacturer now requires strict reading at 10 minutes, but this requirement can be problematic to follow in field conditions where it can be difficult to collect specimens from many patients and examine the cards at the same time (Rajgor et al., 2002; Simonsen and Magesa, 2004).

2.1.4 Detecting the 200–kDa antigen in the ELISA format

In the ELISA format, using AD12.1, the assay has shown better than 97% sensitivity compared to one millilitre membrane filtration or 20 µl TBS and shown no reaction with serum specimens from individuals with a variety of parasitic infections from non-endemic areas in Egypt. Antigen titres are significantly correlated with the

concentration of *W. bancrofti* microfilariae (Weil et al., 1987; Ramzy et al., 1991; Faris et al., 1993).

Performance was poorer in an evaluation comparing the ICT with the AD12.1 ELISA. The ELISA showed sensitivity compared to 50 µl TBS and one millilitre membrane filtration of 86.3% and 86.0% respectively. The ICT, using whole blood, detected only 50.5% (52/103) of ELISA positive amicrofilaraemic subjects while recording 3.3% (54/1646) positive amicrofilaraemic subjects that the ELISA determined were negative. Although the prevalence was similar, 8.8% by ELISA and 9.0% by ICT, they were not detecting the same individuals. This was suggested to be due to the different detection systems employed (Ramzy et al., 1999). However, the poor sensitivity compared to a format using the same antibody should be of concern.

The 200-kDa antigen binds to several other monoclonal antibodies, Gib-13 and $CA_{101.}$ Monoclonal antibody CA_{101} binds to the 200-kDa antigen as well as a 160-kDa and 78-kDa antigens and has shown 93% sensitivity compared to microfilaraemia (Lal et al., 1987). Monoclonal antibody Gib-13 has shown 93% sensitivity compared to microfilaraemia. Antigen levels using this antibody show a rapid increase in concentration in those less than 20 years of age suggesting worms burdens plateau once adulthood is reached due to worm mortality or individual resistance if biting rates are similar among age groups (Forsyth et al., 1985; Weil et al., 1987; Day et al., 1991).

2.1.5 TropBio ELISA

The TropBio ELISA detects the Og4C3 antigen. The monoclonal antibody that detects this antigen was produced from stimulating mice with homogenised male *Onchocerca gibsoni.* This antibody binds to a range of filarial and non-filarial nematodes including *O. volvulus, D. immitis, Ancylostoma caninum* and

Toxocara canis. It binds to antigens greater than 130-kDa and between 50–60 kDa. These antigens are constitutional antigens located at the junction of the cuticle and hypodermis, in the intestinal cells of adults and in intra and extrauterine *O. gibsoni* microfilariae (More and Copeman, 1990). Only a small amount of antigen can be extracted from microfilariae (28 AU/1000 mf) (Chanteau et al., 1994b).

Since antigen levels are similar in both day and night time samples, time of collection is not an issue (Moulia-Pelat et al., 1993; Lammie et al., 1994; Meyrowitsch et al., 1995; Lalitha et al., 1998). However, it has been reported that specimens collected onto filter paper and tested using the TropBio ELISA had a greater proportion of positive samples collected before noon compared to those collected in the afternoon (Hoti et al., 2002).

The antigen is found in hydrocele fluid with concentrations about half those found in serum though showing good correlation (r=0.73) in individuals (Rocha et al., 2004). It is not detectable in urine either due to its high molecular mass or because it is degraded (Chanteau et al., 1994b).

Currently, the TropBio ELISA is not a field test because it requires a reader, and it has a cumbersome labour intensive format and an expensive infrastructure (Weil et al., 1997; Njenga and Wamae, 2001). In cross-checks for the public health reference laboratory, the TropBio ELISA is appropriate for evaluating and monitoring the effects of MDA (Bhumiratana et al., 2004).

2.1.6 Persistence of antigen

Individual antigen levels can remain stable over some years without variation related to seasonal differences (Jaoko et al., 2001). Over a period of 13 years,

microfilaraemia prevalence among 59 carriers decreased to 37% (n=16) and microfilaraemia concentration decreased to 10% of initial levels. However, Og4C3 antigenaemia was still present in 95% of individuals with no difference between those who remained microfilaraemic and those who became amicrofilaraemic, although the geometric mean was five times higher in microfilaraemic subjects. This could be due to female worms losing fecundity but worms still being viable (Sahoo et al., 2002). Fecund life span of *W. bancrofti* has been estimated at 5 years (Vanamail et al., 1996).

Rocha et al. (2004) discussed two amicrofilaraemic individuals who had hydroceles for longer than 10 years and without the filarial dance sign who were antigen positive using serum, but not in the hydrocele fluid. They speculated that this could be antigen from dead worms. This could imply that after drug treatment an individual with a positive antigen test may still record a positive result quite sometime later despite having no live worms.

2.1.7 Sensitivity and specificity of the ICT

Table 2–1 and Table 2–3 compare the ICT with detection of microfilaraemia and the TropBio ELISA. Combining these studies the ICT has a sensitivity of 99.5% (955/959) compared to TBS, 88.1% (222/252) compared to microfilaraemia by membrane filtration and 77.9% compared (483/620) compared to the TropBio ELISA. The test shows excellent specificity with those from non-endemic areas.

Compared to the ELISA AD12.1 assay, the ICT has a sensitivity of 86% (19/22) for amicrofilaraemic individuals with greater than > 10 ng/ml antigen and 46% (11/24) with antigen levels 2–9 ng/ml (Weil et al., 1997). Sensitivity has been reported as low as 33% with microfilaraemic counts less than 10 mf/ml (Pani et al., 2000). Weil (2000) states that the observed sensitivity of a test for a set of samples will depend on the

distribution of antigen levels among subjects. Many people with low microfilarial density and antigen levels will show a lower sensitivity than those more heavily infected. Sensitivity is also affected by the amount of blood used and the packed cell volume. Weil (2000) believes that people with counts less than 10 mf/ml may not contribute significantly to transmission and therefore it may not be important if the test does not detect them.

Poor sensitivity (51.2%) was shown by Nguyen et al. (1999) with the ICT when compared to the TropBio ELISA. Sensitivity was noted to decrease when microfilaraemia prevalence was lower and it has been suggested that the ICT is less useful in this situation with endpoints for ceasing mass treatment being difficult to define. In another post-treatment study after 36 months with 12 weekly doses of DEC, the ICT was positive in 79% (11/14) compared to 100% (14/14) using the TropBio ELISA (Schuetz et al., 2000). Samples that are ICT negative but TropBio ELISA positive show significantly lower Og4C3 antigen concentration compared to ICT positive samples (Nuchprayoon et al., 2003; Bhumiratana et al., 2004). Therefore, the difference between the two tests may either be due to the sensitivity of the tests detecting low levels of their respective antigens or the antigen detected by antibody AD 12.1 is produced at a much lower concentration in the blood.

2.1.8 Sensitivity and specificity of the TropBio ELISA

Combining the data from Table 2–2 and Table 2–3 shows the TropBio ELISA has a sensitivity of 98.0% (343/350) compared to microfilaraemia by TBS and 95.7% (1001/1046) by membrane filtration. Like the ICT the test also shows excellent specificity.

The sensitivity has been reported to be only 75% in individuals with low numbers (< 50 mf/ml) of microfilaraemia (Chanteau et al., 1994b) and only 10% in individuals with less than three microfilariae/ml (Itoh et al., 1999). From graphical data presented by Tisch et al. (2001), sensitivity is estimated to be 70–75%, compared to microfilaraemia with a concentration between 1–10 mf/ml. Rocha et al. (1996) showed sensitivity increased from 60% to 76.9% to 97.6% to 100% with microfilarial concentrations of 1 mf/10 ml, 1 mf/5 ml, 1–30 mf/ml, and > 31 mf/ml respectively. The low sensitivity (78%) of the TropBio assay compared to microfilaraemia within a population has been attributed to the low density of infection (McCarthy et al., 1995).

2.1.9 Comparison of Prevalence of the ICT and TropBio ELISA

Figure 2–1 Comparison of prevalence of antigenaemia and microfilaraemia from multiple studies

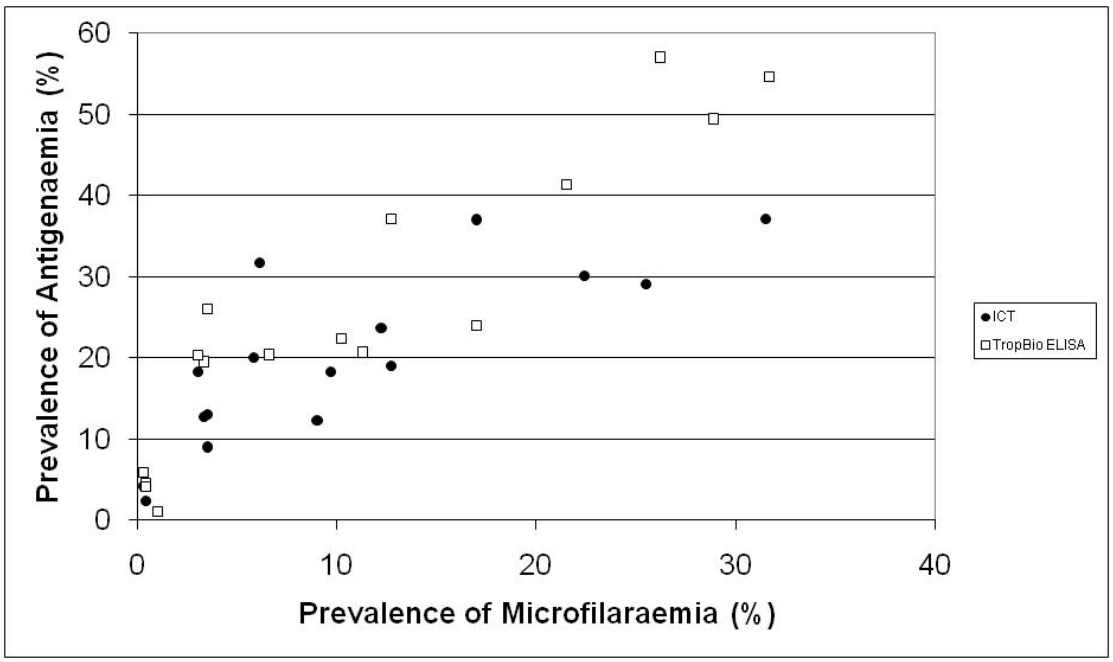


Figure 2–1 shows the relationship between the prevalence of antigenaemia by the TropBio ELISA and ICT and prevalence of microfilaraemia taken from the data presented in Tables 2–1, 2–2 and 2–3. Where multiple methods were used for detecting microfilariae, the method using the greatest blood volume was used. There was a

significant correlation between the prevalence of antigenaemia determined by the ICT and microfilaraemia (r= 0.80, p<0.001) and the prevalence determined by the TropBio ELISA and microfilaraemia. (r=0.93, p< 0.001).

Five studies in Table 2–3 tested all individuals both by ICT and TropBio ELISA. The prevalence of antigenaemia combining these studies was 16.6% (519/3132) by the TropBio ELISA and 12.9% (404/3132) by the ICT. The difference in prevalence was significant (p<0.001). There was one additional study that reported different sample sizes. Five of these six studies showed a greater prevalence by the TropBio ELSIA compared to the ICT. Using the ICT alone may miss a significant number of individuals who have filariasis.

2.1.10 Association of Og4C3 concentration with microfilaria concentration

Antigen concentration is significantly correlated with microfilaraemia density with amicrofilaraemic individuals having lower antigen concentration compared to microfilaraemic individuals (More and Copeman, 1990; Chanteau et al., 1994a; Chanteau et al., 1994b; Lammie et al., 1994; Meyrowitsch et al., 1995; Moulia-Pelat et al., 1995; Rocha et al., 1996; Nicolas et al., 1997; Itoh et al., 1999; Steel et al., 2001; Tisch et al., 2001; Hoti et al., 2002). This correlation was not observed in a Tanzanian study, possibly due to very high antigen levels (Simonsen et al., 1996). The range of antigen concentrations appears greater in populations with low microfilariae densities compared to those with high densities (Tisch et al., 2001).

2.1.11 Antigenaemia and age

No significant difference in prevalence according to age group has been reported in adults only (Ngwira et al., 2002; Sherchand et al., 2003) or among all age groups (Ramzy et al., 1999; Braga et al., 2003; Weerasooriya et al., 2003). In other studies, ICT

prevalence has shown to increase with age (Onapa et al., 2001; Engelbrecht et al., 2003).

Very high prevalence, up to 82%, has been reported among children under 15 years of age, with those as young as one year being reported as positive (Onapa et al., 2001; Nielsen et al., 2002). In Southern India, 92.3% of two to five year old children were amicrofilaraemic but the ICT was positive, with the prevalence of children antigenaemic doubling from age two to five (Sunish et al., 2001).

TropBio antigen prevalence is positively correlated with age (Chanteau et al., 1994b; Chanteau et al., 1995; Gyapong et al., 1998) and associated with an early rapid acquisition in childhood and a slower gain in adults (Lammie et al., 1994). Children with proportionally more amicrofilaraemic antigenaemic individuals could be due to low worm loads (Itoh et al., 1999). Age has shown to be a significant variable for TropBio antigen concentration (Simonsen et al., 1996; Steel et al., 2001; Das et al., 2005).

The increase in antigen prevalence with age could be due to exposure to infective larvae being age dependant, increasing susceptibility to adult worms with age, or antigenaemia from resolved infections. The absence of a decrease in prevalence and intensity of antigenaemia in older adults may indicate lack of protective immunity (Lammie et al., 1994; Tisch et al., 2001).

2.1.12 Antigenaemia and gender

The prevalence of a positive ICT has been reported as higher in males (Onapa et al., 2001; Ngwira et al., 2002; Braga et al., 2003) with other reports showing no difference

between the genders (Ramzy et al., 1999; Nielsen et al., 2002; Sherchand et al., 2003; Weerasooriya et al., 2003).

Males have also shown higher TropBio antigen prevalence rates than females (Chanteau et al., 1995; Gyapong et al., 1998; Itoh et al., 1999; Weerasooriya et al., 2002) though the prevalence was only higher in men over 60 years of age compared to women in Mauke (Steel et al., 2001). Bockarie et al. (2000) reported no difference between the genders in the Madang province of PNG.

Where there is no difference between prevalence rates between genders, this might be due to very high infection rates (Simonsen et al., 1996; Tisch et al., 2001). Where there are differences in gender, this might be due to males being exposed at an earlier age or due to a lower worm burden in females and therefore a lower sensitivity of the TBS in diagnosis (Braga et al., 2005). Chanteau et al. (1995) suggested males had greater prevalence rate in villages in French Polynesia possibly due to a longer time spent in outdoor activities.

2.1.13 Clinical symptoms and antigen tests

There are several reports of an absence of correlation between a positive ICT or AD12.1 ELISA and chronic filariasis. Men with hydrocele in Nigerian villages were no more likely to be positive than men without hydrocele although village hydrocele prevalence correlated with ICT prevalence (Eigege et al., 2003).

Of clinical filariasis cases who were amicrofilaraemic, only 10% (4/40) were antigen positive using AD12.1 and DH 6.2 (Santhanam et al., 1989). This compares to 50% (3/6) amicrofilaraemic clinical cases positive by ICT and 100% (7/7) of microfilaraemic clinical cases (Bhumiratana et al., 1999). Of 20 individuals with clinical

filariasis, lymphoedema (n=11), hydrocele (n=6), and elephantiasis (n=3), 90% were amicrofilaraemic and ICT and AD12.1 ELISA negative (Ramzy et al., 1999).

Many reports detail that the majority of people with chronic symptoms are antigen negative using the TropBio ELISA. Clinical signs give no consistent trends when compared to the TropBio ELSIA or detection of microfilaraemia though fever cases appear more strongly associated with antigenaemia (Turner et al., 1993; Itoh et al., 1999; Jaoko et al., 2006). Subclinical hydrocele is also not associated with antigenaemia (Simonsen et al., 2002). However, subclinical hydrocele has been observed in 10% (2/20) of men from non-endemic areas (Faris et al., 1998). In Haiti, in those with lymphoedema and elephantiasis only, 9% (1/11) were antigen positive (Lammie et al., 1994) while no (0/10) individuals with chronic lymphatic obstruction were antigen positive in a survey in Madas, India (Lalitha et al., 1998). In French Polynesia, among chronic filariasis patients only 23% (11/48) were antigenaemic (Chanteau et al., 1994b). Bal et al. (2003b) report that of those with hydrocele, 39.5% (17/43) were antigenaemic and 12% (3/25) of those had elephantiasis. There were very similar percentages reported in two Indian villages where antigen was detected in 49.4% (40/81) with hydrocele and 10% (2/20) with elephantiasis. In the Panna district of Madhya Pradesh, 30.4% (7/23) of those with acute lymphangitis were antigenaemic (Das et al., 2005) and as reported by Ravindran et al. (2000), 34.1% (15/44) of hydrocele cases were antigen positive. In the Cook Islands, antigenaemia was recorded in 70.6% (12/17) of hydrocele cases and in 42.8% (3/7) with elephantiasis. Of 29 microfilaraemic individuals, 62.1% (18/29) cleared both microfilariae and antigen after 17 years with one dose of DEC given five years previously. Five (27.8%) developed lymphoedema or a hydrocele. Eleven individuals (37.9%) remained microfilaraemic after 17 years with 36% (4/11) developing lymphoedema or hydrocele. Thirteen initially microfilaraemic individuals,

after 17 years, had no antigen and microfilariae and no evidence of infection of this time interval despite continual exposure (Steel and Ottesen, 2001; Steel et al., 2001).

No difference in the antigen level was found between those with hydrocele or leg lymphoedema compared to controls though those with leg lymphoedema were more likely to be antigen negative in PNG (Tisch et al., 2001). Five elephantiasis cases in a study in Tanzania were reported as antigen negative while 40% (2/5) of amicrofilaraemic hydrocele cases were reported as antigen negative (Simonsen et al., 1996). In 104 patients with testicular hydrocele living in Brazil, only 38% (39/104) were antigen positive (Rocha et al., 2004).

Antigen positive children are more likely to have interdigital lesions within web spaces, 74.6 % (82/110), than antigen negative children, 56.1% (46/82). These can provide a break in the skin for bacteria to enter leading to acute dermato-lymphangioadenitis (Fox et al., 2005).

In some populations, the development of chronic symptoms is rare. In Sri Lanka, the incidence and development of adenolymphangitis and lymphoedema over six to 12 years in microfilaraemic individuals was only 0.9% (1/110) although this study excluded hydrocele formation, a more common occurrence (Dissanayake, 2001). In populations who are followed over time, after 13 years only 6.7% (2/43) of asymptomatic microfilaraemic individuals in India developed hydroceles, both of who were antigenaemic. This compared to 14.0% (12/73) amicrofilaraemic individuals who developed hydrocele with 25% (3/12) antigen positive (Sahoo et al., 2002). This absence of association between adenolymphangitis and microfilaraemia and a negative association between microfilaraemia and elephantiasis has also been reported in Ghana (Gyapong, 1998).

In a review article, Rajan (2005) compared the development of acute filariasis in American servicemen during World War II who were amicrofilaraemic and returned home and did not develop later sequelae. Indonesians relocated from non-endemic Irian Jaya to the endemic island of Flores developed acute symptoms with a large percentage going on to develop lymphoedema and elephantiasis. In this population, only 5% developed microfilaraemia, suggesting that those who develop disease have been exposed to large burdens of the parasite. Individuals mount an immune response eliminating the parasite or an ineffective response allowing it to survive. Those who eliminate it show no evidence of infection or develop chronic disease.

Alternatively, individuals may be genetically predetermined whether they will develop disease or not. In Tanzania after 16 years of follow-up, 81.9% of microfilaraemics were still microfilaraemic and 81.3% of amicrofilaraemic individuals remained amicrofilaraemic indicating innate susceptibility (Meyrowitsch et al., 1995). Ten years after DEC (12 days, 6 mg/kg) treatment in 44 asymptomatic microfilaraemic individuals, 82% were TropBio antigenaemic indicating they had reacquired infection or some surviving adult worms. In the same village, 67.5% (27/40) of IgG4 negative amicrofilaraemic individuals remained amicrofilaraemic with no development symptoms of filariasis over the same period. Only one person developed microfilaraemia and another hydrocele or acute symptoms (Beuria et al., 2001; Beuria et al., 2002).

Ravindran (2003) outlines two models relating infection to disease. A static immunological model is one in which an infection in an individual can take two routes, either a patent infection with pathology, or pathology without microfilaraemia with differing immune responses determining the pathway. The dynamic model considers

that there is a sequential progression from infection to microfilaraemia or amicrofilaraemia to obstructive disease with adult worms mediating disease.

2.1.14 Filarial dance sign in chronic filariasis and antigenaemia

As described previously, most people with chronic filariasis pathology are antigen negative. Is this because they harbour no adult worms or due to another reason? Ultrasound can be used to detect adult worms in the lymphatic system where their movement can be identified. This has been coined the 'filarial dance sign' (Amaral et al., 1994). Rocha et al. (1996) reported that of ten amicrofilaraemic individuals with FDS only 60% (6/10) were positive for Og4C3 while of eight amicrofilaraemic individuals who had worms identified after a biopsy, only 75% (6/8) were positive by the TropBio ELISA. In a later study by Rocha et al. (2004), among men with testicular hydrocele, 10 men who were amicrofilaraemic, and had FDS, only 30% (3/10) were antigen positive by the TropBio ELISA, with an additional individual positive in hydrocele fluid only. In two East African villages, 13.1% (18/61) and 44.4% (8/18) of those with FDS were negative for antigen by the TropBio ELISA (Simonsen et al., 2002).

The above findings would suggest that in some cases antigen does not make it into the circulation. Possibly, the majority of antigen is produced during parturition by the worm with the secretion of products from the uterus of adult female worms. This would mean limited or no reproduction due to few adult worms or single sex infections would cause no detectable antigen but worms are still present (Rocha et al., 2004). This would also agree with the observations previously mentioned of antigen correlation with microfilariae density.

2.1.15 TropBio antigen association with filarial antibodies

Filarial antibodies, particularly IgG4, are a potential diagnostic tool, (Ottesen et al., 1985) but they cannot detect between past and present infections and are not correlated to worm intensity. Although sensitive, they have low specificity (Turner et al., 1993; Weil et al., 1997). This could be due to exposure to infective mosquitoes, persistence of antibodies to past infections, low worm burdens or cross-reactions with other helminths (Chanteau et al., 1994a; Muck et al., 2003).

IgG1 concentration to *B. pahangi* adult worms has been shown to be higher in younger age groups (< 15 years of age) who were TropBio antigen positive compared to antigen negative individuals. In those older than 15 years, the mean filarial specific IgG1 level is higher in antigen negative individuals. Filarial specific IgG1 status appears more related to microfilaraemia status than antigen status. The mean filarial specific IgG4 is higher in antigen positive individuals compared to antigen negative individuals. This difference is greater than that observed when microfilaraemia is compared to filarial specific IgG4 indicating that filarial specific IgG4 is more related to infection rather than microfilaraemia status (Simonsen et al., 1996). Worms may enhance their survival by causing production of IgG4, which blocks effector mechanisms (Jaoko et al., 2006).

Antigenaemia and antibody to filarial protease show significant association (Bal et al., 2003b) while the presence of antigen also shows a strong inverse relationship with the presence of anti-sheath antibodies where these antibodies could eliminate adult worms or inhibit their growth and development (Ravindran et al., 2000). Antigen status is also strongly associated with the presence of IgG antibody to DssdI, a filarial surface glycoprotein localised on the sheaths of *W. bancrofti* microfilariae, with 98% (98/100)

of Og4C3 negative individuals in an endemic area having the presence of this antibody compared to 18.8% of Og4C3 positive individuals (Bal et al., 2003a).

2.1.16 Antigen levels and treatment

DEC and albendazole are used in the PNG Filariasis Elimination Program. Table 2–4 summarises studies using DEC or albendazole and the changes on Og4C3 concentration over time. Combination DEC and albendazole appears more effective against adult worms as measured by Og4C3 concentration than DEC alone though interestingly not against microfilaraemia density. Those with lower levels of antigen have a greater percentage converting to negative than those with high levels do (Bockarie et al., 2007). As shown in the table, even after multiple doses of DEC and/or albendazole, the percentage of people not converting from positive to negative can be quite substantial. Simonsen P.E. et al. (2005) relate how 11 individuals with greater than 32,000 AU/ml, even after eight half-yearly doses, still had antigen levels above this amount. In PNG after four MDA rounds of either DEC or DEC and Ivermectin microfilaraemia reduced from 70% to 4% while antigenaemia had only reduced from 84% to 78% (Tisch et al., 2008).

Freedman et al. (2001) suggest that factors that lead to increased inflammation and clinical filariasis may potentiate actions of DEC. In this study, total clearance of antigen occurred after 24 months of multiple doses in 12% (3/26) of asymptomatic patients, but in 58% (7/12) of those with chronic symptoms.

Where the ICT has been used, it has also taken a long time for populations to return negative results. After 10 rounds of mass treatment of DEC, with an average number of 7.2 doses per person, 45.5% (10/22) of those that were microfilaraemic before the first MDA, were still positive by the ICT with those with high microfilariae concentration

more likely to be positive. Of those with high pre-MDA microfilariae concentrations (n=4, range 408–935 mf/ml) two still remained microfilaraemia with all four individuals still ICT positive. Again, this indicates that clearance of antigen is related to pre-treatment adult worm burden (Ramaiah et al., 2007). In Tanzanian communities with antigen prevalence between 41–47%, given either 12 daily DEC, semi-annually over one year or 12 monthly doses, still had antigen prevalence, by ICT, of 42–45% ten years later (Meyrowitsch et al., 2004). It is possible that the slow disappearance of antigen could be due to natural worm death rather than the action of DEC killing the adult worms (Simonsen et al., 2005).

Table 2–4 Changes in Og4C3 antigenaemia after treatment with DEC or DEC/albendazole

Location	Medication and Dosage	Change in Antigenaemia	Change in Microfilaraemia	Reference
▪ French Polynesia ▪ n=19	▪ One dose DEC 6 mg/kg	▪ Og4C3 ▪ Geometric mean 5812 AU/ml ▪ Residual geometric mean antigen level 78%, 62%, 54%, 50% at 1, 6, 9, 12 months respectively	▪ Geometric mean 266 mf/ml at baseline ▪ Residual mf concentration 11.3%, 6.3%, 7.9%, 14.7 % at 1, 6, 9, 12 months respectively	Moulia-Pelat et al. (1995)
		▪ 200 kDa assay ▪ Geometric mean 20 ng/ml ▪ Residual geometric mean antigen level 42%, 22%, 15%, 18% at 1, 6, 9, 12 months respectively		
▪ Tahaa Island, French Polynesia	▪ One dose DEC 6 mg/kg	▪ Microfilaraemic (n=46) ▪ Geometric mean 5045 AU/ml ▪ Residual geometric mean antigen level 55.5% and 37.6% at 12 and 24 months ▪ No percentage reduction in antigen carriers	▪ Geometric mean 645 mf/ml ▪ Residual mean microfilaraemia concentration 9.4% and 2.8% at 12 and 24 months ▪ 11.1% and 35.7% still microfilaraemic at 12 and 24 months	Nicolas et al. (1997)
		▪ Amicrofilaraemic (n=28) ▪ Geometric mean 736 AU/ml ▪ Residual mean antigen level 22.3% and 16.4% at 12 and 24 months ▪ 79.6% and 75.0% still antigenaemic at 12 and 24 months		
▪ Haiti	▪ 12 weekly doses of 6 mg/kg DEC ▪ n=14	▪ Median residual antigen levels 56% and 25% after 6 months and 3 years ▪ 100% still antigenaemic after 3 years	▪ Median residual mf concentration 0 % after 6 months and 3 years	Eberhard et al. (1997)
	▪ 1 dose DEC 6 mg/kg ▪ n=8	▪ Median residual antigen levels 68%, 62% and 58% at 6 months, 1 year and 2 years post-treatment ▪ 100% still antigenaemic after 3 years	▪ Median residual mf concentration 10%, 12% and 14% at 6 months, 1 year and 2 years post-treatment	

Location	Medication and Dosage	Change in Antigenaemia	Change in Microfilaraemia	Reference
▪ 6 villages in Milne Bay province, PNG	▪ Mean 878 kg 0.2% w/v DEC salt per household	▪ Prevalence of antigenaemia declined from 55% to 36% after 6 months		Sapak et al. (2000)
	▪ Single dose DEC 6 mg/kg	▪ Prevalence of antigenaemia declined from 71% to 66% after 6 months		
▪ Brazil ▪ Positive > titre 32 AU/ml	▪ 6 mg/kg/day for 12 days at 0, 6, 12 and 18 months	▪ 94.3% and 72% antigenaemic at 6 and 24 months ▪ Residual median antigen level 53%, 32% and 21% at 6, 12 and 24 months		Freedman et al. (2001)
▪ Matara, Sri Lanka	▪ 12 daily doses DEC (Dose not stated)	▪ Of those microfilaraemics who turned amicrofilaraemic after treatment. 76.1% were antigen positive 17 months later	▪ 22% still microfilaraemic after 17 months	Weerasooriya et al. (2002)
▪ Myanmar ▪ Positive > 120 AU/ml	▪ 300 mg DEC given at 0, 6, 12 and 18 months ▪ Blood sampling just prior to dose ▪ Minimum detection for positive ICT = 7500 Og4C3 AU/ml	▪ Individuals with strong ICT results (n=4), residual antigen levels of 27%, 34%, 10% and 54% after 18 months. Initial range of antigen concentration 75–181 x 10^3 AU/ml ▪ Individuals with weak ICT results (n=6) no residual antigen after 6 months. Initial range of antigen concentration 15–37 x 10^3 AU/ml ▪ Individuals with negative ICT results (n=5) no residual antigen after 6 months. Initials range of antigen concentration 127–4700 AU/ml		Koyadun et al. (2003)
▪ Papua New Guinea	▪ One dose DEC 6 mg/kg	▪ 89.6% (60/67) still antigenaemic after 24 months	▪ 91.3%, 74.2%, 50% still microfilaraemic at 6, 12 and 24 months (n=62)	Bockarie et al. (2007)
	▪ One dose DEC 6 mg/kg and albendazole 400 mg/kg	▪ 83.3% (80/96) antigenaemic after 24 months	▪ 72.9%, 65.7%, 34.3% still microfilaraemic at 6, 12 and 24 months (n=70)	

Ivermectin was shown to have a poor effect on clearing antigen, although this may depend on the individual (Moulia-Pelat et al., 1995; Nicolas et al., 1997). Eberhard et al. (1997) showed it was comparable to DEC. Interestingly, antigen concentrations after 400 mg/kg ivermectin reduced to 96%, 80%, 72% after one, six and nine months, but then rebounded to 94% at 12 months using the TropBio ELISA. This rise was not shown in the 200 kDa ELISA assay (Moulia-Pelat et al., 1995). Why would the antigen level rise after treatment? Is ivermectin only having a partial macrocidal effect or an effect on fecundity that worms can recover from?

Using the AD12.1 ELISA assay, 84.6 % (22/26) patients cleared their antigenaemia after 18 months after receiving DEC 8/mg/kg daily for seven days or DEC 8 mg/kg weekly for five months. After only four months, the geometric mean had decreased to 10% of initial values. Although this was attributed to low levels of initial antigenaemia and previous treatment five years earlier (McCarthy et al., 1995), it raises the question of whether assays based on AD 12.1 monoclonal antibody show greater reduction of antigen compared to the TropBio assay. As the ICT uses the same monoclonal antibody as the AD 12.1 ELSIA assay, this could indicate that the end points for MDA may depend on which antigen assay is being used.

2.1.17 Filter paper collection techniques and the TropBio ELISA

The filter paper technique refers to the collection of capillary blood onto filter paper and then testing this blood for the constituent of interest. It has been used with AD 12.1 and DH 6.5 monoclonal antibodies (Santhanam et al., 1989) and showed good correlation with serum for filarial antibodies (Chanteau et al., 1991; Terhell et al., 1996). Table 2–5 summarises papers that have used the filter paper technique with the TropBio assay. Itoh (1998) used the filter paper technique due to difficulty in obtaining

and storing serum samples. There was no deterioration storing dried filter paper samples at 4 ºC in a refrigerator for 12 months. Weerasooriya et al. (2002) stored specimens for up to 23 months at 4 ºC without effecting antigen levels. It requires minimum facilities and is less troublesome than venipuncture (Lalitha et al., 1998).

However, it is still an invasive and slightly painful technique that is disliked by subjects (Weerasooriya et al., 2003). The TropBio filter paper test is comparatively cheaper than the ICT with about a threefold difference in price. For a large community surveillance, the test could be performed in a centralised laboratory by a skilled technician, to avoid the errors of an unskilled worker (Hoti et al., 2002).

Good correlation has been found between antigen units (r=0.97) and titre groups between the TropBio filter paper and TropBio serum ELISA (r=0.98). Among 60 paired samples only one serum ELISA classified in titre group one was classified in titre group three using the filter paper technique (Itoh et al., 1998). Lalitha et al. (1998) also found a positive correlation (r=0.83) between filter paper and serum samples. Simonsen and Dunyo (1999) recorded significant correlation between antigen concentrations of serum and filter paper results (r=0.679) and between titre groups (r=0.925) More recently, Wattal et al. (2007) showed good correlation between concentration obtained by the filter paper technique and serum for both microfilaraemic individuals (r=0.959) and individuals with no evidence of infection (r=0.942). Similar results were obtained by using 50 µl or 25 µl of blood collected onto the filter paper.

Table 2–5 Studies comparing the TropBio ELISA filter paper with the TropBio ELISA or microfilaraemia

Details	TropBio Filter	TropBio ELISA	Microfilaraemia	Reference
▪ Ghana ▪ 15 µl collected onto filter paper ▪ Stored at -20ºC	▪ 50.3% (163/324) sensitivity (Positive > Titre Group 3) compared to microfilaraemia (20 µl TBS). ▪ Prevalence 12.0% (217/1808) ▪ Geometric mean OD 0.58		▪ Prevalence 17.9% (324/1808) (20 µl TBS)	Gyapong et al. (1998)
▪ India ▪ Sampling 21:00–02:00 ▪ 20 µl collected filter paper ▪ Stored room temperature ▪ Eluted 4 ºC overnight	▪ 100 % (40/40) sensitivity compared to microfilaraemic individuals. (1ml membrane filtration) Positive > 80 AU/ml.			Lalitha et al. (1998)
▪ Sri Lanka ▪ Sampling 22:00–01:00 ▪ 100 µl collected onto filter paper ▪ Stored 12 months at 4 ºC before assayed	▪ 97.4% (38/39) sensitivity compared to TropBio serum ELISA. (Positive: Titre >3) ▪ 100% (33/33) sensitivity compared to microfilaraemia (TBS 60ul) ▪ Prevalence 22.5% (139/619)	▪ 100% sensitivity compared to microfilaraemia (39/39) Positive: Titre >3 (TBS 60ul)	▪ Prevalence 7.3% (45/619) 60 µl TBS	Itoh et al. (1998)
▪ Ghana ▪ Sampling 21:00–24:00 hr ▪ 30 µl collected onto filter paper and tested ▪ Stored at -80ºC	▪ 100% (24/24) sensitivity (Positive > Titre group 2) compared to microfilaraemia (100 µl capillary tube). ▪ 33.3% (9/27) amicrofilaraemic individuals positive ▪ 10 non-endemic controls negative	▪ 100% (24/24) sensitivity compared to microfilaraemia (100ul capillary tube). Positive > titre group 2 ▪ 38.5% (10/27) amicrofilaraemic individuals ▪ 10 non-endemic controls negative		Simonsen and Dunyo (1999)
▪ Sri Lanka ▪ Sampling 22:00–01:00 ▪ Treated group 12 daily doses DEC ▪ Method of Itoh	▪ 11.7 % (295/2520) prevalence non-treated ▪ Geometric titre non-1718 AU/ml treated ▪ 45.7% (101/221) prevalence treated ▪ Geometric titre treated 4248 AU/ml		▪ 2.7% (68/2520) prevalence non-treated ▪ 11.8 % (26/221) prevalence treated	Weerasooriya et al. (2002)

Details	TropBio Filter	TropBio ELISA	Microfilaraemia	Reference
▪ India ▪ Sampling different times during day ▪ 20 µl collected onto filter paper ▪ Using 30 microfilaraemic individuals ▪ Eluted 4 ºC overnight	▪ 06:00–08:00 hr 93.3% (28/30) Geometric titre =69.2 AU/ml ▪ 10:00–12:00 hr 93.3% (28/30) Geometric titre =61.2 AU/ml ▪ 14:00–16:00 hr 76.7% (23/30) Geometric titre =67.6 AU/ml ▪ 20:00–22:00 hr 86.7% (26/30) Geometric titre =57.7 AU/ml	▪ 10:00–12:00 hr 100% (30/30) geometric titre =612.4 au/ml ▪ 20:00–22:00 hr 100% (30/30) geometric titre =628.1 au/ml		Hoti et al. (2002)
▪ India ▪ Sampling 08:30–24:00 ▪ 50 µl collected onto filter paper	▪ 100% (29/29) sensitivity (Positive > Titre Group 3) compared to microfilaraemia (3 X 20 µl TBF). ▪ 96.7% (88/91) sensitivity (Positive > Titre Group 3) compared to microfilaraemia (3 X 20 µl TBF). ▪ 0% (0/27) positive in acute cases ▪ 0% (0/51) positive in chronic cases	▪ 100 % sensitivity (Positive > Titre Group 3) compared to microfilaraemia (3 X 20 µl TBF). ▪ 94.5% (86/91) sensitivity (Positive > Titre Group 3) compared to microfilaraemia (3 X 20 µl TBF). ▪ 11.1% (3/27) positive in acute cases ▪ 7.8% (4/51) positive in chronic cases		Wattal et al. (2007)

2.2 Method

2.2.1 Collection and handling of blood samples

As part of the baseline survey for the PNG Filariasis Elimination Program, venous blood was collected from self-selecting residents, aged five years or older, into EDTA and SST II BD Vacutainers between 19:00 and 01:00 hours. This was performed in the PNG provinces of New Ireland in May 2006 and Bougainville in November 2006.

One hundred microlitres of blood was extracted from the SST II tube using a heparinised capillary tube and an ICT card test (Binax NOW Filariasis Test) was performed. The next morning whole blood from the EDTA sample was added to three ears of the filter paper, supplied with the Trop-Ag *W. bancrofti* test kit (TropBio, Townsville, Australia) and dried. A 60 µl thick smear was also prepared from the EDTA tube. The SST II tubes were centrifuged 1–3 days post-collection then stored at 4 ºC until analysis. The thick films were stained with 3% Giemsa for 30 minutes and examined for microfilariae.

Samples were transported to Townsville, Australia by air freight and stored at 4 ºC for up to two weeks before analysis. One hundred microlitres of serum was diluted with 300 µl of diluent then placed in a boiling water bath for five minutes then centrifuged at 2000 g for 15 minutes. A comparison between untreated serum samples and treated samples was performed.

2.2.2 Filter paper sample processing

The blood soaked filter paper was transferred back to Townsville, Australia for further analysis. The three ears of the filter paper were cut in half and all ears placed in a microtitre tube. Two hundred microlitres of diluent was added to paper, the tubes boiled for five minutes, centrifuged at 2000 g for 15 minutes and supernatant then used in the TropBio

assay. Some boiled filter paper samples in diluent were stored at 4 ºC and retested after sitting overnight.

Filter paper samples collected in Bougainville were soaked overnight in diluent at 4 ºC and boiled, centrifuged and tested as described.

The ICT card was opened and the blood soaked filter paper removed and placed into a microtitre tube with 400 µl of diluent, boiled and supernatant then tested.

2.2.3 Testing method

All materials are supplied in the Trop-Ag *W. bancrofti* ELISA kit. Polystyrene plates are supplied pre-coated with Og4C3 monoclonal antibody. Fifty microlitres of serum or supernatant was added to a well. Seven standards ranging in concentration from < 10 to 32,000 antigen units (AU) were added in duplicate to each plate. Plates were incubated overnight at room temperature then washed three times with the supplied wash buffer. Wells were blocked by adding 50 µl of hydrogen peroxide and incubating at room temperature for ten minutes followed by three more washes in the wash buffer. Rabbit anti-onchocerca antibody was added and plates incubated for one hour followed by another three washes. Anti-rabbit horse radish peroxidase conjugate was added with another one hour incubation followed by three washes. 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) was added and then incubated for one hour. These were read with a spectrophotometer with dual wavelengths at 405 and 492 nm.

The absorbance value of the sample was then compared to the average absorbance values of seven standards run in duplicate to allocate the sample to a titre group. Samples with more than 128 AU (titre group 3) were classified as positive. Although there are seven standards

supplied with the kit there was often no discrimination based on absorbance between the high concentration standards, six and seven.

Results were entered into Microsoft Excel (2003) and analysed using SPSS version 13 (SPSS Inc. Chicago, IL).

2.2.4 Testing precipitate by the TropBio ELISA in the field

In an effort to create a field friendly version of the TropBio ELISA, the assay was trialled in the field without the centrifugation step. Serum samples from clotted blood collected by venipuncture in New Ireland were added to three parts diluent and boiled for five minutes. These specimens were not centrifuged to obtain supernatant but instead the precipitate was added to a coated ELISA plate and incubated at room temperature for four hours. The procedure was continued as described for the standard TropBio ELISA assay except the results were read by visual inspection.

2.2.5 Comparison of untreated and treated samples in the TropBio ELISA

Treated and untreated serum specimens were compared using samples collected from West New Britain, PNG. Treated specimens were treated as in the standard procedure by diluting 1:3 with diluent, boiled for five minutes then centrifuged. Fifty microlitres of supernatant was then added to a coated ELISA plate. For the untreated comparison, 50 µl of serum was added to the ELISA plate. Both treated and untreated specimens were tested in duplicate and both treated and untreated samples from the same individual were placed in the same ELISA plate.

2.2.6 Fast friendly field test

With the information found by the investigations, a fast friendly field (FFF) kit was developed and field tested with whole blood. The FFF kit contains 12 strips, with eight wells

per strip, in sealed pouches and reagents supplied in dropper bottles. Wash solution is provided as a concentrate and diluted with bottled water. A negative, weak positive and strong positive control are included with the kit.

One drop of diluent and approximately 50 µl of whole blood was added to each well and kept overnight at room temperature. The next morning the plates were tested using reagents supplied with the FFF kit. The technique was then the same as described for the serum TropBio test except incubation times for the secondary antibody and conjugate were 30 minutes and results were read by visual inspection by examining wells for colour development.

2.3 Results

The demographic profile of the tested population is described in Chapter Three.

2.3.1 Prevalence comparisons

The prevalence for each technique is shown in Table 2–6. Not all individuals were tested by all techniques due to the difficulty in obtaining venous blood samples and errors in processing. There was a significant difference (χ^2=65.1, df=2, p<0.001) between the prevalence obtained by the various techniques. There was no difference between the prevalence obtained by ICT and the filter paper TropBio ELISA (χ^2=0.18, df=1, p=0.67) while the serum TropBio ELISA showed a significantly greater prevalence with the ICT (χ^2=48.9, df=1, p<0.001) and the filter paper technique (χ^2=41.0, df=1, p<0.001). The prevalence of microfilaraemia was 16.3% (n=808).

2.3.2 Comparison of sensitivities and specificities

Table 2–6 also shows the sensitivity and specificity compared to the TropBio ELISA and sensitivity compared to TBS. There was no difference in the sensitivity or specificity, using a cut-off of 128 AU/ml, compared to the TropBio ELISA between the two techniques. Sensitivity, compared to TBS, ranged from 88.6–96.9%. The serum TropBio ELISA had the highest sensitivity of the four techniques.

There were 54 samples within titre group three of the TropBio ELISA but only two of these were ICT positive, both of which were amicrofilaraemic. Reducing the cut-off for the filter test from 128 AU/ml to 32 AU/ml increases the sensitivity from 67.2% to 91.5% (CI, 88.1–94.2). However, the specificity (n=389) decreases to 84.8% (CI, 80.9–88.3). Sensitivity, using a cut-off of 32 AU/ml, compared to microfilaraemia (n=121) was 95.0% (CI, 89.5–98.2).

There were 252 specimens tested by all four techniques. The proportion of positive specimens of this sample was 42.5% (CI: 36.5–48.6), 35.3% (CI: 29.7–41.4), 32.9% (CI: 27.4–39.0) and 32.1% (CI: 26.7–38.1), for the serum TropBio, TropBio using filter paper from the ICT, TropBio filter paper and ICT respectively. For these samples, the sensitivity for the ICT, TropBio filter paper and the TropBio using filter paper from the ICT were 74.8% (CI: 65.5–82.7), 77.6% (CI: 68.5–81.7) and 83.2% (CI: 74.7–89.7) compared to the standard TropBio ELISA test. Of these specimens, 44 were microfilaraemic. The sensitivity of the TropBio using the filter paper from the ICT was 93.2% (CI, 81.3–98.6), ICT 93.2% (CI, 81.3–98.6), TropBio filter paper 90.9% (CI, 78.3–97.5) and TropBio ELISA 97.7% (CI, 88.0–99.9). The specificity of the TropBio ELISA using the filter paper from the ICT (n= 147) was 100% (CI: 97.5–100).

Table 2–6 Comparison of antigen diagnostic tests

Technique	Antigen Prevalence	Sensitivity compared to TropBio ELISA.	Specificity compared to TropBio ELISA	Positive Predictive value	Negative Predictive Value	Sensitivity in comparison with TBS
ICT	31.5% (n=863) CI: 28.4–34.6	63.6% (n=393) CI: 58.6–68.4	97.6% (n=423) CI: 95.7–98.9	96.2% CI: 93.0–98.1	74.3% CI: 70.4–77.9	88.6% (n=132) CI: 82.0–93.5
TropBio filter paper	32.5% (n=766) CI: 29.2–35.8	67.2% (n=354) CI: 62.1–72.1	99.2% (n=391) CI: 97.8–99.8	98.8% CI: 96.4–99.7	77.0% CI: 70.4–77.9	88.4% (n=121) CI: 81.4–93.5
TropBio using filter paper from the ICT		83.2% (n=107) CI: 74.7–89.7)	100.0% (n=147) CI: 98.0–100.0	100.0% CI: 96.7–100.0	89.1% CI: 83.3–93.4	93.2% (n=44) CI: 81.3–98.6
TropBio ELISA	48.2% (n=817) CI:44.8–51.6					96.9% (n=128) CI: 92.2–99.1

Table 2–7 Comparison between filter paper and serum TropBio ELISA titre groups

		Titre groups by TropBio ELISA						
Titre groups by Filter Paper		**1**	**2**	**3**	**4**	**5**	**6**	**7**
	1	30	8	5	1			
	2	194	59	34	20	6		3
	3	14	35	9	6	24	24	32
	4					4	17	83
	5			1	1		3	55
	6							29
	7						1	45

2.3.3 Comparison of titre groups

The TropBio filter paper test uses about 10 µl of blood per ear. This method uses three ears approximately 12 µl of serum, which is diluted in 200 µl of diluent. There is a 4.4 fold reduction in serum concentration tested compared to the TropBio ELISA, which is equivalent to the difference between one titre group.

As seen in Table 2–7, most samples that recorded a titre group of seven by the standard serum technique recorded a lower titre using the filter paper. While there was only one discordant sample positive by the filter paper technique but negative by the standard assay, there were 116 samples negative by the filter paper but positive by the TropBio assay. There is a significant correlation between the two techniques ($r= 0.79$, $p < 0.001$) with a slope of 1.31 (CI, 1.24–1.39).

2.3.4 Effect of elution time on the filter paper technique

Samples (n=155) were tested after the boiled filter paper and diluent were left overnight at 4 ºC. The sensitivity leaving the filter paper to elute at 4 ºC was significantly increased compared to a minimal elution time ($\chi^2=4.62$, df=1, $p= 0.03$). The specificity was similar between these two techniques while the sensitivity compared to TBS was lower when the filter paper was immediately boiled.

There was a significant correlation between the titres of the two filter paper testing regimes ($r=0.82$, $p<0.001$) with a slope of 1.46 (CI, 1.20–1.62) and intercept of -1.69 (CI, -2.26–-1.13).

Table 2–8 Comparison between elution of boiled antigen of filter paper overnight and standard filter paper technique

Technique	Sensitivity compared to TropBio serum. Cut-off 128 AU/ml (n=82)	Specificity compared to TropBio ELISA (n=73)	Sensitivity in comparison with TBS (n=20)
Standard Filter paper technique immediate boiling	58.5% CI: 47.1–69.3	100% CI: 96.0–100.0	90.0% CI: 68.3–98.8
Filter paper with elution of filter paper at 4 °C overnight	74.3% CI: 63.6–83.4	98.6% CI: 92.6–100.0	100.0% CI: 86.1–100.0
ICT	57.3% CI: 45.9–68.2	97.3% CI: 90.5–99.7	90.0% CI: 68.3–98.8
TropBio using filter paper from the ICT			100.0% CI: 86.1–100.0

2.3.5 Filter paper results from specimens collected in Bougainville

Filer paper samples collected in Bougainville were incubated overnight at 4 °C in diluent then boiled, centrifuged and the supernatant tested by the TropBio ELISA. The sensitivity of samples collected onto the supplied filter paper (χ^2 =9.44, df=1, p=0.001) and using filter paper from the ICT (χ^2 =7.22, df=1, p=0.007) were significantly lower than the sensitivity of the ICT. The specificity and sensitivity compared to microfilaraemia of the three techniques were similar.

There was a significant difference (χ^2=8.99, df=1, p=0.003) between the sensitivity of the filter paper technique between New Ireland (41.7%) and Bougainville (74.3%). There was a significantly (p< 0.001) lower average titre (2.58) in the serum TropBio of the filer paper subset from Bougainville compared to samples from New Ireland (3.86).

There was also a difference (χ^2=5.89, df=1, p=0.015) between the sensitivity of the ICT between New Ireland (57.3%) and Bougainville (84.0%). Five samples that were positive by TropBio ELISA but were negative by the ICT were retested using the same ICT lot that had been used in New Ireland as well as a new lot used in Bougainville. These five samples were again negative by the old lot but tested positive with the new lot

Table 2–9 Comparison of antigen detection techniques of samples collected in Bougainville

Technique	**Sensitivity compared to TropBio ELISA Cut-off 128 AU**	**Specificity compared to TropBio ELISA**	**Sensitivity in comparison with TBS**
ICT	84.0% (n=25) CI: 63.9–95.5	100.0% (n=121) CI: 97.6–100.0	85.7 % (n=7) CI: 42.1–99.6
TropBio filter paper	41.7% (n=24) CI: 22.1–63.4	98.0% (n=50) CI: 89.4–100.0	85.7 % (n=7) CI: 42.1–99.6
TropBio using filter paper from the ICT	48.0% (n=25) CI: 27.8–68.7	100.0% (n=50) CI: 94.2–100.0	85.7 % (n=7) CI: 42.1–99.6
TropBio serum			100% (n=7) CI: 59.0–100.0

2.3.6 Testing precipitate for antigen by the TropBio ELISA

The sensitivity using precipitate compared to the standard test was 93.2% (CI, 83.8–98.1) and the specificity 87.7% (CI, 69.3–96.2). Compared to microfilaraemia (n=20), sensitivity was 100% (CI, 86.1–100.0).

Table 2–10 Comparison between using precipitate and supernatant in the TropBio ELISA titre groups

		Result by standard TropBio ELISA	
Result using precipitate		Negative	Positive
	Negative	26	4
	Positive	4	55

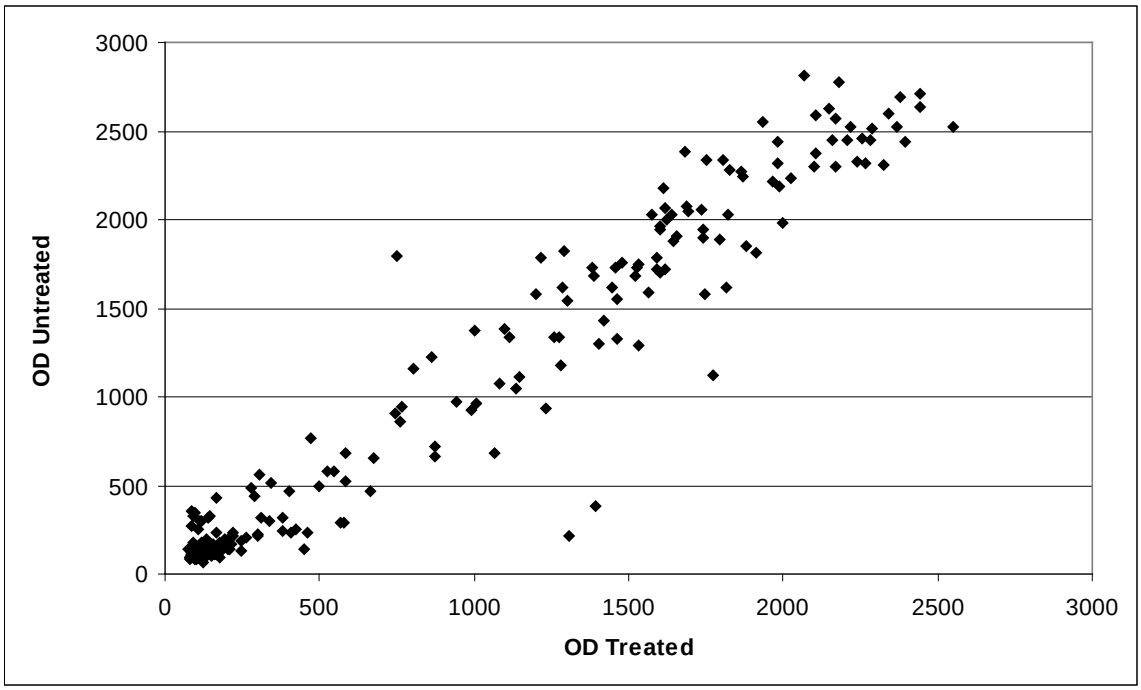
2.3.7 Boiled supernatants compared to untreated specimens

Two hundred and fifty boiled supernatants and untreated serum specimens collected from West New Britain were compared in the TropBio assay. The absorbance readings between the two measurements were significantly correlated ($r=0.97$, $p<0.001$). A plot of the absorbance readings is shown in Figure 2–2. There was a significant ($p<0.001$) difference of the paired mean difference of 80 absorbance units (CI, 52–108) between untreated and treated OD (untreated OD > treated OD). The positive percentage agreement was 94.6% (CI: 88.7–98.0) and negative percentage agreement 97.1% (CI: 92.8–99.2).

Table 2–11 Comparison in titres between treated and untreated samples in the TropBio ELISA

		Titre groups by standard TropBio ELISA						
Titre groups using untreated specimens		**1**	**2**	**3**	**4**	**5**	**6**	**7**
	1	73	15					
	2	17	8	6	2			
	3	8	1	6	3	1		
	4			4	13	4		
	5				2	14	1	1
	6					5	2	1
	7				1	4	13	45

Figure 2–2 Graph of OD treated *versus* untreated specimens in the TropBio ELISA



2.3.8 Fast friendly field test

The FFF was developed in response to the realisation that pre-treatment did not influence specimens collected in the field. The comparison with the standard assay is shown in Table 2–12.

Table 2–12 Comparison of FFF with standard TropBio ELISA

		Standard TropBio ELISA	
FFF		Negative	Positive
	Negative	119	1
	Positive	2	24

The sensitivity compared to the TropBio ELISA was 96.0% (CI, 79.7–99.9) and specificity 98.4% (CI, 94.2–99.8). Sensitivity compared to microfilaraemia (n=7) was 100% (59.0–100.0).

2.4 Discussion and Conclusion

Currently, there is only one accepted field test used for the mapping of lymphatic filariasis: the ICT. This test only uses 100 µl of capillary blood, gives a result in 10 minutes and can be performed with minimal training. It is useful for initial mapping and is more sensitive than the TBS. However, the manufacturer has problems with supply of the test and quality of the results. Consequently, in the PNG Filariasis Elimination Program, the TropBio ELISA was the standard test for baseline surveys.

The purpose of this study was to evaluate whether blood collected via fingerpick onto filter paper and tested by the TropBio ELISA filariasis test would be a suitable alternative to the ICT. Previous studies have showed that the filter paper test and serum version are comparable (Itoh et al., 1998; Lalitha et al., 1998; Simonsen and Dunyo, 1999; Hoti et al., 2002; Wattal et al., 2007). It has also been used successfully to determine post-treatment

filariasis infection rates (Bockarie et al., 2000; Sapak et al., 2000). This is in contrast to Gyapong (1998) who only reported a sensitivity of 50.3% compared to microfilaraemia (20 µl TBS). This was not attributed to storage conditions or errors reading the TBS. and thought not due to antigenic differences in *W. bancrofti* between Ghana and PNG. It was considered that possibly the cut-off point was set too high, though this was only titre group three, or that 15 µl of whole blood was not sufficient to reliably detect antigen. Gyapong noted that other investigators also had experienced low sensitivity with this method. The poor sensitivity of the filter paper test was also reported by several researchers at a filariasis workshop in 1997 (Simonsen et al., 1997), which was attributed to in part a defective batch of antiserum (Simonsen and Dunyo, 1999). It would be expected that the filter paper technique would have a lower sensitivity compared to the serum version due to a dilution effect when eluting the blood from the paper. Indeed, a tenfold lower geometric mean titre has been reported (Hoti et al., 2002).

During the course of the evaluation, it was observed that the blood soaked filter paper from the ICT card might be able to be used to recheck the ICT result. The ICT, TropBio filter paper technique and the filter paper from the ICT were compared to the serum TropBio and TBS. The sensitivity of the all techniques compared to the serum TropBio was poor, but were better when compared to the TBS. The sensitivity of the TropBio filter paper test (84%, 81.4–93.5%) compared to TBS was similar to that reported by Hoti et al. (2002) although lower than the 100% sensitivity reported elsewhere (Itoh et al., 1998; Lalitha et al., 1998; Simonsen and Dunyo, 1999; Wattal et al., 2007). This might be partly explained by the lower cut-off value used in several studies or difference in techniques determining microfilaraemia.

The sensitivity was improved after allowing the filter paper and boiled supernatant to stand overnight but conversely the sensitivity was reduced after soaking the paper overnight

and then boiling. The difference in sensitivity of the filter paper technique between samples collected in New Ireland (41.7%) and Bougainville (74.3%) could be due a lower antigen concentration in the Bougainville sample.

Testing of the filter pad from the ICT card was shown to be comparable to the TropBio filter paper test and with the ICT test itself. Of the 27 specimens from New Ireland that were tested by the TropBio ELSIA using the filter paper from the ICT and that were positive by the serum TropBio ELISA but negative by the ICT, 33% (n=9) were positive. Testing of eluted blood from the ICT could be an additional quality control procedure when antigen prevalence and concentrations become low after multiple MDA.

The sensitivity of the ICT, compared to the TropBio ELISA, was better with samples collected from Bougainville. This was a new lot of ICT cards and five samples collected from New Ireland that were repeatedly negative by the lot used in New Ireland and positive by the TropBio were positive with a new lot of ICT cards. The older lot had one week left before it passed its expiry date so some degradation of the cards may have occurred or the lot was of poor quality.

The good performance of the assay in the field using precipitate raised the question whether the boiling step was necessary. Serum in the standard TropBio assay is heat-treated using the method of Weil and Liftis (1987) where one part serum is boiled with three parts sodium EDTA for five minutes. This was to release antigen from immune complexes and to inactivate rheumatoid factor. More and Copeland (1990) noted that the sensitivity of the assay was improved by heat pre-treatment and was able to detect less than three antigen units from a minimum detectable antigen level of 256 AU/ml. However, they also noted that this level of sensitivity was not needed since large amounts of antigen was present in most cases

and that modification for field use would not adversely affect final results. This was shown to be correct with 40.8% (102/250) positive by the standard method using boiled supernatant and 40.0% (100/250) using untreated serum. The untreated specimens showed a higher average absorbance compared to their matched treated sample. Although boiling with EDTA appears to release more antigen, this effect is nullified by the dilution effect. The pre-treatment introduces an extra level of complexity to the procedure that precluded the assay from being introduced into the field. Showing that this requirement in field settings was not required allowed a trial of a TropBio ELISA field test, the FFF.

The FFF was trialled in Bougainville and showed good sensitivity 96.0% (24/25) compared to the standard test and was positive with all detected cases of microfilaraemia. There were two samples (1.7%) that were positive by the FFF but negative by the standard TropBio. The development of the reaction for one of these specimens was closely observed and it was noted that the colour formation occurred along in a single line down the ELISA well, which may have indicated poor washing of this well.

The requirements for a field test for mass testing are that it uses whole capillary blood, is easy to perform, gives rapid results, is relatively inexpensive and has good sensitivity and specificity. The ICT meets those requirements although there appears to be significant lot-to-lot variation in sensitivity and specificity. The ICT also has an advantage in baseline surveys where immediate results informs the Elimination Program as to its suitability for a sentinel site while there is an initial three hours incubation using the FFF and filter paper strips must be transported back to a laboratory.

The TropBio filter paper technique is cumbersome as it involves detaching the filter ears and placing them into a tube for further processing. The ears are too large to fit into a standard one millilitre microtitre tube and therefore must be further cut. It also shows a poor

sensitivity compared to the standard TropBio assay. The technique could be improved by changing the shape of the filter paper from a round ear to a single strip. This strip could be then put straight into a microtitre tube containing diluent.

The FFF can be used on whole blood and, although capillary blood was not used in this evaluation, there should be no reason why it could not be suitable. Using whole blood in the ELISA format and reading plated by eye using monoclonal antibody AD 12.1 has shown a good sensitivity 97.4 % (38/39) compared to microfilaraemia (40 µl TBS) (Santhanam et al., 1989).

Although the FFF takes longer to perform and is more complex, several hundred samples can easily be analysed in half a day and the results available in the field. Users with basic laboratory skills could be quickly taught its methodology.

It had also been considered whether it was possible to add capillary blood to the ELISA wells, incubate overnight, wash and then send the plate back to a reference laboratory for further processing. This was done with a week delay between addition of specimen and processing. No positive results were obtained indicating the antigen antibody complex on the ELISA plate was not stable.

A capillary finger prick sample is considered more suitable for mass screening compared to venous collection. It is less invasive, and the collection technique easily learned. However, venous night blood collections occur more efficiently as venous blood can be collected more quickly than capillary blood and the requirement of testing and reading cards at 10 minutes is eliminated. The extra cost of collection materials such as collection tubes, storage tubes and needles is offset by the reduced cost of the TropBio ELISA test. Although a rapid test is useful for quickly identifying a sentinel site, the actual base line survey and additional

surveys during the elimination program can quite easily be conducted with venous blood collection and the TropBio ELISA.

This study has shown that the filter paper collection method is unsuitable for lymphatic filariasis prevalence studies. Additionally the ICT was shown to record a significantly lower antigen prevalence than the serum TropBio ELISA. This study has shown that venous blood collection is quite acceptable to populations that are traditionally been used to finger prick collection. Therefore, among the techniques evaluated, the serum TropBio should be the preferred method for antigen prevalence studies for lymphatic filariasis.

Chapter Three: Baseline Monitoring of Lymphatic Filariasis Prevalence in Papua New Guinea

3.1 Introduction

In 2006, PNG initiated MDA in six provinces containing 1.2 million people and an estimated coverage of 48% (WHO, 2007). As part of the Roll Back Malaria campaign, treated bed nets have also been handed out to villagers, which is likely to assist in reducing transmission of filariasis (Odermatt et al., 2008). Baseline filariasis surveys are needed as part of the monitoring and evaluation of filariasis elimination program to monitor the effects of MDA (PacELF Monitoring and Analysis Network, 2004). Baseline monitoring was conducted in five provinces of PNG.

LF signs and symptoms include lymphoedema, hydrocele, chyluria, adenopathy, haematuria with the infected suffering from acute inflammatory episodes and chronic manifestations (Addiss and Brady, 2007). LF is a disease of poverty and a contributor to poverty (Perera et al., 2007; Streit and Lafontant, 2008) with infected individuals showing a loss of productivity and greater individual health costs with physical and psychological burdens (Addiss and Brady, 2007; Wynd et al., 2007c).

In 1997, the Fiftieth World Health Assembly issued WHA50.29 acknowledging lymphatic filariasis as one of six eradicable diseases and urged member states to develop national plans leading towards its elimination as a public health problem by 2020 (WHO, 1997). By the end of 2006, the at-risk population was estimated to be 1254 million in 83 endemic countries (WHO, 2007). By 2008, 51 countries had started MDA (WHO, 2009). From 2000 to 2007, it is estimated that the global eradication program prevented 6.6 million babies acquiring the disease and protected 9.5 million from either hydrocele or lymphoedema

(Ottesen et al., 2008).With the aim of eradicating transmission globally by 2020, low coverage experienced in some endemic areas combined with insufficient resources in low income countries means this goal may not be achieved (Gyapong and Twum-Danso, 2006).

3.1.1 Requirements for a successful control program

Elimination requires an efficient drug distribution system and high compliance (Burkot and Ichimori, 2002). Successful control programs also emphasise community involvement (Prybylski et al., 1994) with community participation in selecting drug distributors, an important factor in motivating health workers to interact with the community and leaders resulting in better coverage (Babu and Satyanarayana, 2003). There needs to be national importance placed on a program for success (Cao et al., 1997).

In China, elimination was achieved using both chemotherapy and vector control (Cao et al., 1997). Although four to six rounds of MDA can greatly reduce microfilaraemia prevalence, a greater number of rounds and additional control measures are likely to be needed in high transmission areas (Bockarie et al., 2002) with baseline transmission intensity and prevalence strongly influencing the number of rounds of MDA (Grady et al., 2007). It is believed that targeted treatment will also be needed at the end of the MDA (Dean, 2003).

There are many factors that may lead to elimination programs extending many years or failing to stop transmission. The long duration of control programs, repeated surveys and treatments can reduce enthusiasm among workers and communities (Jihui, 2005). Remote, economically disadvantaged communities with poor or no roads make the annual distribution of drugs a financial and logistical problem that can put the whole program at risk (Fischer et al., 2004). Reduced coverage in country programs has been due to distributors not visiting their required houses or the householder not in their homes when distributors have called. Poor compliance has been noted due to forgetfulness in taking the drugs, the fear of side

effects and the perception that taking drugs is unnecessary (Babu and Satyanarayana, 2003; Babu and Mishra, 2008). The large number of pills that must be swallowed has also been identified as a significant barrier to compliance in Haiti's national programs (Talbot et al., 2008).

3.1.2 Lymphatic filariasis in PNG

In PNG, over one million of the five million residents are infected (Kazura and Bockarie, 2003). Microfilaraemia, chronic and acute disease prevalence is higher in PNG than all other filariasis endemic countries (Reeder, 2003) probably due to the very high transmission intensity compared to other parts of the world (Tisch et al., 2001). Published surveys from PNG show the prevalence of lymphoedema and hydrocele varies widely in different communities (Knight et al., 1979; Kazura et al., 1984; Turner et al., 1993; Hii et al., 2000) with leg lymphoedema even being recorded in those less than 10 years of age (Kazura et al., 1997).

PNG recognises lymphatic filariasis as a major health problem and priorities include an integrated vector control program, diagnostic and treatment services and expanding community wide treatment (Kazura and Bockarie, 2003). Pilot DEC control programs in PNG have shown good reduction in microfilaraemia prevalence and density (Prybylski et al., 1994; Schuurkamp et al., 1994; Bockarie et al., 2000). Filariasis eradication has been attempted in certain areas with private partnerships such as in Lihir, New Ireland Province with the support Lihir Management Company (Hemer, 2005) and the Samari Murua District in the Milne Bay Province supported by Misima Mines Limited (Selve et al., 2000).

With the highest estimated prevalence in the Pacific, PNG is likely to have the greatest success in eliminating transmission. There is a population keen to participate due to high rates of observable pathology and the inefficiency of the primary vector *An. punctulatus* in

spreading the disease (Burkot et al., 2002). There have been high hopes that both vector control and MDA will eliminate transmission of filariasis (Bockarie, 1994) with an expectation this could be achieved by 2010 (Bockarie and Kazura, 2003).

A successful eradication campaign in Milne Bay was attributed to community ownership, commitment and involvement using familiar resources in local languages (Selve et al., 2000). The large number of pills that need to be taken and the perception that LF is not an important issue has been noted as a problem in focus groups in PNG who have undergone MDA (Wynd et al., 2007a), which may lead to the younger generation not compiling with the MDA program (Wynd et al., 2007b). Further socio-cultural research is needed to guide strategies in elimination programs tackling causes, consequences and means of prevention (Wynd et al., 2007c). DEC and albendazole are used in tablet form in the elimination program. However, due to high labour and transport costs, where less frequent visits to villages are needed, DEC salt may be the ideal drug distribution mode (Sapak et al., 2000). In communities that use salt from local sources, it is possible to add DEC salt to other products to obtain the same effect (Jihui, 2005).

3.2 Method

Two sentinel sites in the PNG provinces of New Ireland, West New Britain, East New Britain, Oro and the autonomous governing island of Bougainville were selected as recommended by the Ministry of Health, PNG. West New Britain had completed their first MDA one to two months prior to the samples being collected. All materials, as shown in the Appendix 2, were transported from Townsville, Australia to the required location in PNG. ICT kits were supplied by the MOH. Other items including generators, electrical lighting and fuel were acquired before travelling to the remote locations. Some locations were on the mainland and others on nearby islands requiring travel in small boats. Communication about

the requirements of the baseline study had been sent to the health posts at the villages, which then communicated this to the local community. On arrival at the location, discussions about the survey's requirements were held with local leaders who informed the community of the procedures. Local community members were employed to assist in tasks such as recording names and details of the participants. The location where collection occurred is shown in Table 3–1.

Convenience sampling (PacELF Monitoring and Analysis Network, 2004) was used to collect whole blood via venipuncture into Vacutainer SST II tubes between the hours of 7 pm and 1 am on residents aged five years and above. The early collection time was due to reducing the burden on participants as some participants lived a long walking distance from the collection site. Before the blood clotted, it was added to ICT cards and 60 µl thick films made using heparinised capillary syringes or using a 20 µl pipette from the SST tube. The whole blood was centrifuged within two days of collection then transported back at 4 °C to Townsville where the TropBio filariasis test, as described in 2.2.3: TropBio ELISA, was performed within two weeks. The ICT test was performed at the site in New Ireland and was also used in East and West New Britain with expired ICT cards. Due to a supplier problem in manufacturing the ICT cards there were no or insufficient ICT cards available for the other sites.

Results were entered into Microsoft Excel (2003) and analysed using SPSS version 13 (SPSS Inc. Chicago, IL).

Table 3–1 Locations where blood collection occurred

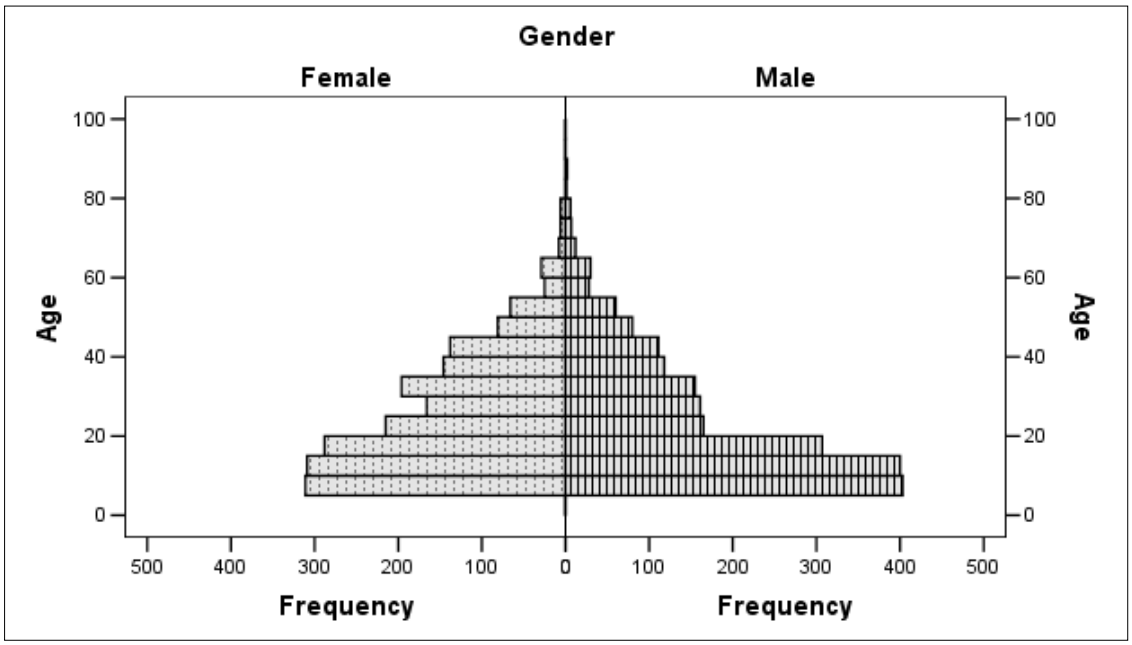
Province	Locality	Sub-locality
Bougainville	Rorovana	Rorovana
	Sipai	Keakara
		Sipai
		Sisiapai
East New Britain	Kokopo	Kerawera Island
		Utuan Island
	Pomio	Gar
		Lat
		Mango Station
New Ireland	Lovangai	Lovangai Mission
		Metvoe
		Ungat
		Vaikeb
	Tanga	Amfar
		Lif
		Malesak-Put
		Tefa
Oro	Oro Bay	Oro Bay
	Saiho	Awala
West New Britain	Gloucester	Giurisi
		Kokopo
		Malasongo
		Siamatai
	Vituhu	Vituhu

3.3 Results

3.3.1 Demographics of sentinel sites

Figure 3–1 and Table 3–3 detail the age and gender characteristics of the population at the sentinel sites. The total number of participants ranged from 331 in Sipai to 484 in Saiho. Interestingly, as Figure 3–1 shows, overall there were many more males under 15 years old (n=803) who were tested compared to females (n=620). There were significant differences in both the gender balance (χ^2 = 21.03, df=9, p=0.01) and the numbers of participants in each age group (χ^2 = 266.90, df=99, p<0.01) between all ten sites. In Sipai, only 43.2% of respondents were female while 55.8% of participants were female in Tanga.

Figure 3–1 Age and gender characteristics of combined sentinel sites



3.3.2 Prevalence of antigenaemia and microfilaraemia

Table 3–3 and Table 3–4 show the prevalence of antigenaemia and microfilaraemia. There were large differences between antigen prevalence of differing bleeding sites within the same sentinel site. For instance, at the Sipai site in Bougainville, 28.9% was recorded at Sipai while 81.1% antigenaemia was found at Sisiapai. At Tanga, in New Ireland, 5.8% antigenaemia was recorded on the main island while 80% was found on the nearby island of Lif.

At the collection sites of Amfar and Oro Bay, participants from nearby localities walked from their place of residence to the survey site. Of the 11 individuals at Amfar with antigenaemia, five of these (83%) were from Sumniul. In Oro Bay, all three people from Rebai were antigenaemic.

As described in Chapter Two, there was a significant difference between the prevalence of antigenaemia recorded by the ICT and TropBio at New Ireland. Six individuals were excluded from the prevalence TropBio data in Table 3–4, as an age was not recorded. Recently expired ICT cards were also used in East and West New Britain and showed the prevalence of 40.3% (n=419) and 25.2% (n=365) at the Kokopo and Pomio sites and 23.0% (n=405) and 6.1% (n=377) at the Gloucester and Vituhu sites.

Table 3–6 and Figure 3–2 show the distribution of titre groups between the sites. Similarities in distribution can be seen at the locations of Lif, Malesak-Put and Tefa, three small islands in the Tanga Group in New Ireland. At Keakara, there was a combined prevalence of 50.7% in titre groups four, five and six compared to 8% for titre group seven. This is in contrast to other sites where titre group seven tended to have a much higher prevalence compared to titre groups four to six.

Table 3–2 Age and gender characteristics of sentinel sites

Location	5–9 y	10–14 y	15–19 y	20–24 y	25–29 y	30–34 y	35–39 y	40–44 y	45–49 y	50–5 4y	55–59 y	>60 y	Grand Total
Bougainville	**124**	**113**	**102**	**75**	**55**	**66**	**52**	**55**	**42**	**23**	**12**	**27**	**746**
Rorovana	**63 (15.2)**	**49 (11.8)**	**48 (11.6)**	**49 (11.8)**	**29 (7.0)**	**37 (8.9)**	**37 (8.9)**	**37 (8.9)**	**19 (4.6)**	**18 (4.3)**	**10 (2.4)**	**19 (4.6)**	**415**
Female	31	16	25	32	15	23	19	22	9	12	3	9	216 (52.1)
Male	32	33	23	17	14	14	18	15	10	6	7	10	199 (47.9)
Sipai	**61 (18.4)**	**64 (19.3)**	**54 (16.3)**	**26 (7.9)**	**26 (7.9)**	**29 (8.8)**	**15 (4.5)**	**18 (5.4)**	**23 (6.9)**	**5 (1.5)**	**2 (0.6)**	**8 (2.4)**	**331**
Female	23	28	20	13	12	13	8	11	9	2	1	3	143 (43.2)
Male	38	36	34	13	14	16	7	7	14	3	1	5	188 (56.8)
East New Britain	**104**	**141**	**137**	**80**	**54**	**72**	**51**	**66**	**30**	**25**	**5**	**20**	**785**
Kokopo	**42 (10.0)**	**77 (18.4)**	**80 (19.1)**	**43 (10.3)**	**19 (4.5)**	**29 (6.9)**	**25 (6.0)**	**50 (11.9)**	**20 (4.8)**	**16 (3.8)**	**5 (1.2)**	**13 (3.1)**	**419**
Female	16	36	41	21	12	16	13	33	9	8	2	4	211 (50.4)
Male	26	41	39	22	7	13	12	17	11	8	3	9	208 (49.6)
Pomio	**62 (16.9)**	**64 (17.5)**	**57 (15.6)**	**37 (10.1)**	**35 (9.6)**	**43 (11.7)**	**26 (7.1)**	**16 (4.4)**	**10 (2.7)**	**9 (2.5)**	**0 (0.0)**	**7 (1.9)**	**366**
Female	32	24	23	21	14	22	14	6	3	4	0	3	166 (45.4)
Male	30	40	34	16	21	21	12	10	7	5	0	4	200 (54.6)
New Ireland	**211**	**118**	**101**	**65**	**61**	**56**	**47**	**57**	**39**	**26**	**12**	**18**	**811**
Lovangai	**95 (23.2)**	**62 (15.2)**	**49 (12.0)**	**42 (10.3)**	**26 (6.4)**	**27 (6.6)**	**34 (8.3)**	**28 (6.8)**	**19 (4.6)**	**10 (2.4)**	**7 (1.7)**	**10 (2.4)**	**409**
Female	35	30	29	19	10	19	18	10	10	4	4	6	194 (47.4)
Male	60	32	20	23	16	8	16	18	9	6	3	4	215 (52.6)
Tanga	**116 (28.9)**	**56 (13.9)**	**52 (12.9)**	**23 (5.7)**	**35 (8.7)**	**29 (7.2)**	**13 (3.2)**	**29 (7.2)**	**20 (5.0)**	**16 (4.0)**	**5 (1.2)**	**8 (2.0)**	**402**
Female	54	23	36	15	19	18	10	20	12	9	3	5	224 (55.8)
Male	62	33	16	8	16	11	3	9	8	7	2	3	178 (46.2)
Oro	**156**	**187**	**141**	**78**	**82**	**71**	**62**	**31**	**28**	**30**	**15**	**19**	**900**
Oro Bay	**65 (15.6)**	**86 (20.7)**	**61 (14.7)**	**35 (8.4)**	**45 (10.8)**	**23 (5.5)**	**34 (8.2)**	**19 (4.6)**	**18 (4.3)**	**11 (2.6)**	**6 (1.4)**	**13 (3.1)**	**416**
Female	26	40	20	16	28	14	18	9	10	4	2	3	190 (45.7)
Male	39	46	41	19	17	9	16	10	8	7	4	10	226 (54.3)
Saiho	**91 (18.8)**	**101 (20.9)**	**80 (16.5)**	**43 (8.9)**	**37 (7.6)**	**48 (9.9)**	**28 (5.8)**	**12 (2.5)**	**10 (2.1)**	**19 (3.9)**	**9 (1.9)**	**6 (1.2)**	**484**
Female	35	43	43	28	22	28	19	4	7	13	5	3	250 (51.7)
Male	56	58	37	15	15	20	9	8	3	6	4	3	234 (48.3)
West New Britain	**119**	**150**	**113**	**82**	**75**	**86**	**52**	**40**	**22**	**22**	**9**	**24**	**794**
Gloucester	**47 (11.5)**	**71 (17.4)**	**53 (13.0)**	**47 (11.5)**	**45 (11.0)**	**49 (12.0)**	**31 (7.6)**	**20 (4.9)**	**12 (2.9)**	**11 (2.7)**	**4 (1.0)**	**18 (4.4)**	**408**
Female	23	25	24	27	22	22	17	11	7	6	2	8	194 (47.6)
Male	24	46	29	20	23	27	14	9	5	5	2	10	214 (52.4)
Vituhu	**72 (18.7)**	**79 (20.5)**	**60 (15.5)**	**35 (9.1)**	**30 (7.8)**	**37 (9.6)**	**21 (5.4)**	**20 (5.2)**	**10 (2.6)**	**11 (2.8)**	**5 (1.3)**	**6 (1.6)**	**386**
Female	36	44	27	23	12	21	10	12	5	4	3	5	202 (52.3)
Male	36	35	33	12	18	16	11	8	5	7	2	1	184 (47.7)
Grand Total	**714**	**709**	**594**	**380**	**327**	**351**	**264**	**249**	**161**	**126**	**53**	**108**	**4036**

Table 3–3 Prevalence of antigenaemia and microfilaraemia at sentinel sites (%, 95% CI [n=total number tested])

Location	TropBio	Microfilaraemia
Bougainville		
Rorovana Total	**0.0 (415)**	
Sipai		
Keakara	58.7, CI:50.5–66.9 (138)	8.9, CI:4.1–13.7 (135)
Sipai	28.9, CI:15.7–42.1 (45)	13.6, CI:4.2–23 (44)
Sisiapai	81.1, CI:74.8–87.4 (148)	33.3, CI:25.6–41 (141)
Sipai Total	**64.7, CI:59.5–69.9 (331)**	**20.3, CI:15.9–24.7 (320)**
Bougainville Total	**28.7, CI:25.5–31.9 (746)**	**20.3, CI:15.9–24.7 (320)**
East New Britain		
Kokopo		
Kerawera Island	62.0, CI:56.8–67.2 (337)	21.3, CI:12.3–30.3 (80)
Utuan Island	65.9, CI:55.6–76.2 (82)	25.7, CI:11.8–39.6 (35)
Kokopo Total	**62.8, CI:58.2–67.4 (419)**	**22.6, CI:15.1–30.1 (115)**
Pomio		
Gar	31.5, CI:24–39 (146)	5.1, CI:1.6–8.6 (136)
Lat	57.6, CI:50.2–65 (170)	17.9, CI:10.8–25 (112)
Mango Station	42.0, CI:28.3–55.7 (50)	14.3 (14)
Pomio Total	**45.1, CI:40.0–50.2 (366)**	**11.1, CI:7.3–14.9 (262)**
East New Britain Total	**54.5, CI:51.0–58.0 (785)**	**14.6, CI:11–18.2 (377)**
New Ireland		
Lovangai		
Lovangai Mission	62.2, CI:53.5–70.9 (119)	16.3, CI:9.4–23.2 (104)
Metvoe	53.4, CI:44.8–62 (131)	18.7, CI:11.8–25.6 (123)
Ungat	52.9, CI:41.2–64.6 (70)	12.3, CI:4.8–19.8 (65)
Vaikeb	51.7, CI:41.3–62.1 (89)	12.5, CI:5.6–19.4 (88)
Lovangai Total	**55.5, CI:50.7–60.3 (409)**	**15.5, CI:11.9–19.1 (380)**
Tanga		
Amfar	5.8, CI:2.6–9 (189)	1.6, CI:0.1–3.1 (182)
Lif	80.0, CI:66.7–93.3 (35)	20.6, CI:7–34.2 (34)
Malesak-Put	67.7, CI:58.3–77.1 (96)	41.5, CI:31.5–51.5 (94)
Tefa	74.4, CI:65–83.8 (82)	23.5, CI:14.3–32.7 (81)
Tanga Total	**41.0, CI:36.2–45.8 (402)**	**17.4, CI:13.6–21.2 (391)**
New Ireland Total	**48.3, CI:44.9–51.7 (811)**	**16.5, CI:13.9–19.1 (771)**
Oro		
Oro Bay Total	**3.1, CI:1.5–4.7 (416)**	**0.0 (402)**
S Total	**1.0, CI:0.2–1.8 (484)**	**0.0 (462)**
Oro Total	**2.0, CI:1.1–2.9 (900)**	**0.0 (864)**
West New Britain		
Gloucester		
Giurisi	75.6, CI:66.2–85 (82)	9.0, CI:2.2–15.8 (67)
Kokopo	36.4, CI:30.0–42.8 (217)	0.0 (195)
Siamatai	77.1, CI:69.2–85 (109)	3.2, CI:0.3–6.1 (93)
Gloucester Total	**55.1, CI:50.3–59.9 (408)**	**2.5, CI:1.0–4.0 (355)**
Vituhu Total	**20.2, CI:16.2–24.2 (386)**	**1.3, CI:0–2.6 (308)**
West New Britain Total	**38.2, CI:34.8–41.6 (794)**	**2.0, CI:0.9–3.1 (662)**
Grand Total	**33.6, CI:32.1–35.1 (4036)**	**8.7, CI:7.7–9.7 (2995)**

Table 3–4 Prevalence of antigenaemia at Amfar and Oro Bay by self-reported place of residence % (n)

Location	TropBio Antigenaemia
Amfar	
Ambaba (1)	0.0 (0)
Amfar (73)	2.7 (2)
Ampotpot (66)	1.5 (1)
Bil (6)	33.3 (2)
Buel (4)	0.0 (0)
Fonly (1)	0.0 (0)
Manman (3)	0.0 (0)
Sasa (29)	3.4 (1)
Sumniul (6)	83.3 (5)
Oro Bay	
Beama (222)	2.7 (6)
Bematu (1)	0.0 (0)
Bendoroda (4)	0.0 (0)
Borau (3)	33.3 (1)
Dc base (2)	0.0 (0)
Dombada (5)	20.0 (1)
Embi estate (1)	0.0 (0)
Eroro (14)	0.0 (0)
Fisheries (63)	1.6 (1)
Harbours (7)	14.3 (1)
Honeorata (3)	0.0 (0)
Katereda (11)	0.0 (0)
Kopure (11)	0.0 (0)
Kurumbo (1)	0.0 (0)
Log point (10)	0.0 (0)
Marine (39)	0.0 (0)
Orowosari (7)	0.0 (0)
Rebai (3)	100.0 (3)
Saroda (7)	0.0 (0)
St Margarets (1)	0.0 (0)
Waipa (1)	0.0 (0)

Figure 3–4 shows the relationship between the prevalence of titre group seven at each site and the percentage of this titre group compared to all TropBio antigenaemics, that is those in titre groups four, five, six and seven. With increasing antigenaemic prevalence, there is a corresponding increase in proportion of those recording the highest titre ($\beta = 0.56$ $t(19) = 3.61$, $p < .01$). Two notable outliers were Amfar, where overall antigen prevalence was only 4.2% yet 72% (n=8) of the antigenaemics recorded the highest titre of seven, and Keakara, with a prevalence of 58.7% with only 13.5% of those antigenaemic in titre group seven. At the collection site of Amfar, of the eight antigenaemic individuals, four of these were from the same village of Sumniul (n=6), which had 66.7% (n=4) in titre group seven with 80% of

the antigenaemics in titre group seven. The percentage of titre group seven in relation to overall antigenaemia for West New Britain sites appeared similar to other sites that had not undergone MDA

Table 3–5 Prevalence of titre group by location % (n)

Location	1	2	3	4	5	6	7
Bougainville							
Rorovana Total (415)	**91.8 (381)**	**6.75 (28)**	**1.45 (6)**	**0.0 (0)**	**0.0 (0)**	**0.0 (0)**	**0.0 (0)**
Sipai							
Keakara (138)	26.81 (37)	4.35 (6)	10.1 (14)	7.97 (11)	22.46 (31)	20.29 (28)	7.97 (11)
Sipai (45)	71.1 (32)	0.0 (0)	0.0.0 (0)	4.4 (2)	8.9 (4)	6.7 (3)	8.9 (4)
Sisiapai (148)	9.5 (14)	4.1 (6)	5.4 (8)	4.1 (6)	10.1 (15)	14.9 (22)	52 (77)
Sipai Total (331)	**25.1 (83)**	**3.6 (12)**	**6.6 (22)**	**5.7 (19)**	**15.1 (50)**	**16 (53)**	**27.8 (92)**
Bougainville Total (746)	**62.2 (464)**	**5.4 (40)**	**3.8 (28)**	**2.5 (19)**	**6.7 (50)**	**7.1 (53)**	**12.3 (92)**
East New Britain							
Kokopo							
Kerawera Island (337)	26.1 (88)	7.7 (26)	4.2 (14)	6.8 (23)	15.4 (52)	13.4 (45)	26.4 (89)
Utuan Island (82)	19.5 (16)	12.2 (10)	2.4 (2)	7.3 (6)	11 (9)	7.3 (6)	40.2 (33)
Kokopo Total (419)	**24.8 (104)**	**8.6 (36)**	**3.8 (16)**	**6.9 (29)**	**14.6 (61)**	**12.2 (51)**	**29.1 (122)**
Pomio							
Gar (146)	34.9 (51)	21.9 (32)	11.6 (17)	8.9 (13)	8.9 (13)	4.1 (6)	9.6 (14)
Lat (170)	16.5 (28)	15.9 (27)	10.0 (17)	8.8 (15)	9.4 (16)	5.3 (9)	34.1 (58)
Mango Station (50)	40.0 (20)	10.0 (5)	8.0.0 (4)	4 (2)	12 (6)	4 (2)	22 (11)
Pomio Total (366)	**27 (99)**	**17.5 (64)**	**10.4 (38)**	**8.2 (30)**	**9.6 (35)**	**4.6 (17)**	**22.7 (83)**
East New Britain Total (785)	**25.9 (203)**	**12.7 (100)**	**6.9 (54)**	**7.5 (59)**	**12.2 (96)**	**8.7 (68)**	**26.1 (205)**
New Ireland							
Lovangai							
Lovangai Mission (119)	16.8 (20)	13.4 (16)	7.6 (9)	2.5 (3)	7.6 (9)	14.3 (17)	37.8 (45)
Metvoe (131)	6.9 (9)	22.9 (30)	16.8 (22)	5.3 (7)	4.6 (6)	7.6 (10)	35.9 (47)
Ungat (70)	38.6 (27)	4.3 (3)	4.3 (3)	8.6 (6)	1.4 (1)	4.3 (3)	38.6 (27)
Vaikeb (89)	4.5 (4)	36 (32)	7.9 (7)	7.9 (7)	4.5 (4)	5.6 (5)	33.7 (30)
Lovangai Total (409)	**14.7 (60)**	**19.8 (81)**	**10.0 (41)**	**5.6 (23)**	**4.9 (20)**	**8.6 (35)**	**36.4 (149)**
Tanga							
Amfar (189)	85.2 (161)	6.3 (12)	2.6 (5)	1.1 (2)	0.5 (1)	0.0 (0)	4.2 (8)
Lif (35)	17.1 (6)	2.9 (1)	0.0 (0)	2.9 (1)	14.3 (5)	5.7 (2)	57.1 (20)
Malesak-Put (96)	22.9 (22)	4.2 (4)	5.2 (5)	4.2 (4)	6.3 (6)	5.2 (5)	52.1 (50)
Tefa (82)	13.4 (11)	8.5 (7)	3.7 (3)	1.2 (1)	7.3 (6)	11 (9)	54.9 (45)
Tanga Total (402)	**49.8 (200)**	**6 (24)**	**3.2 (13)**	**2 (8)**	**4.5 (18)**	**4 (16)**	**30.6 (123)**
New Ireland Total (811)	**32.1 (260)**	**12.9 (105)**	**6.7 (54)**	**3.8 (31)**	**4.7 (38)**	**6.3 (51)**	**33.5 (272)**
Oro							
Oro Bay Total (416)	**85.6 (356)**	**10.6 (44)**	**0.7 (3)**	**1.4 (6)**	**1 (4)**	**0.2 (1)**	**0.5 (2)**
Saiho Total (484)	**95.5 (462)**	**2.9 (14)**	**0.6 (3)**	**0.4 (2)**	**0.6 (3)**	**0.0 (0)**	**0.0 (0)**
Oro Total (900)	**90.9 (818)**	**6.4 (58)**	**0.7 (6)**	**0.9 (8)**	**0.8 (7)**	**0.1 (1)**	**0.2 (2)**
West New Britain							
Gloucester							
Giurisi (82)	15.9 (13)	2.4 (2)	6.1 (5)	11 (9)	11 (9)	11 (9)	42.7 (35)
Kokopo (217)	45.2 (98)	12 (26)	6.5 (14)	7.4 (16)	12 (26)	4.6 (10)	12.4 (27)
Siamatai 109)	11 (12)	7.3 (8)	4.6 (5)	3.7 (4)	15.6 (17)	6.4 (7)	51.4 (56)
Gloucester Total (408)	**30.1 (123)**	**8.8 (36)**	**5.9 (24)**	**7.1 (29)**	**12.7 (52)**	**6.4 (26)**	**28.9 (118)**
Vituhu Total (386)	**69.4 (268)**	**6 (23)**	**4.4 (17)**	**7.3 (28)**	**4.1 (16)**	**2.3 (9)**	**6.5 (25)**
West New Britain Total (794)	**49.2 (391)**	**7.4 (59)**	**5.2 (41)**	**7.2 (57)**	**8.6 (68)**	**4.4 (35)**	**18 (143)**
Grand Total (4036)	**52.9 (2136)**	**9.0 (362)**	**4.5 (183)**	**4.3 (174)**	**6.4 (259)**	**5.2 (208)**	**17.7 (714)**

Figure 3–2 Titre group percentage by location

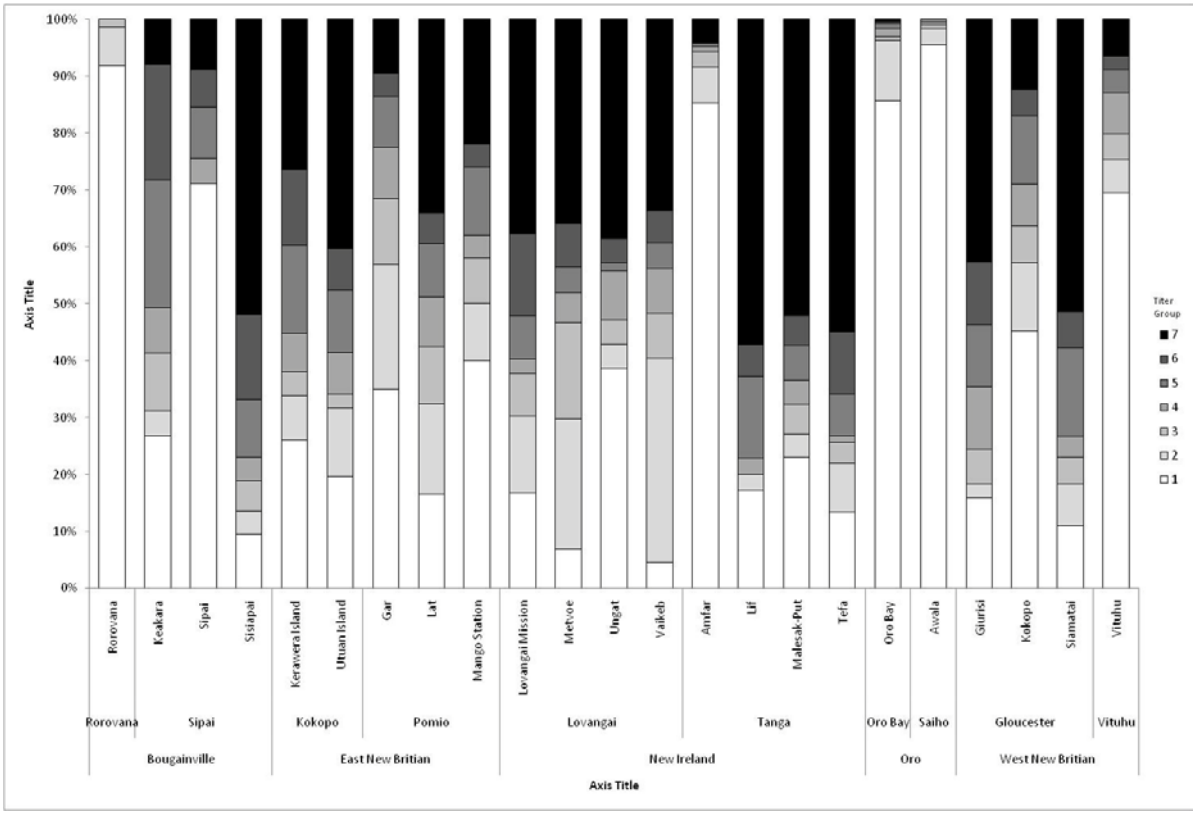


Figure 3–3 Microfilariae blood density by location

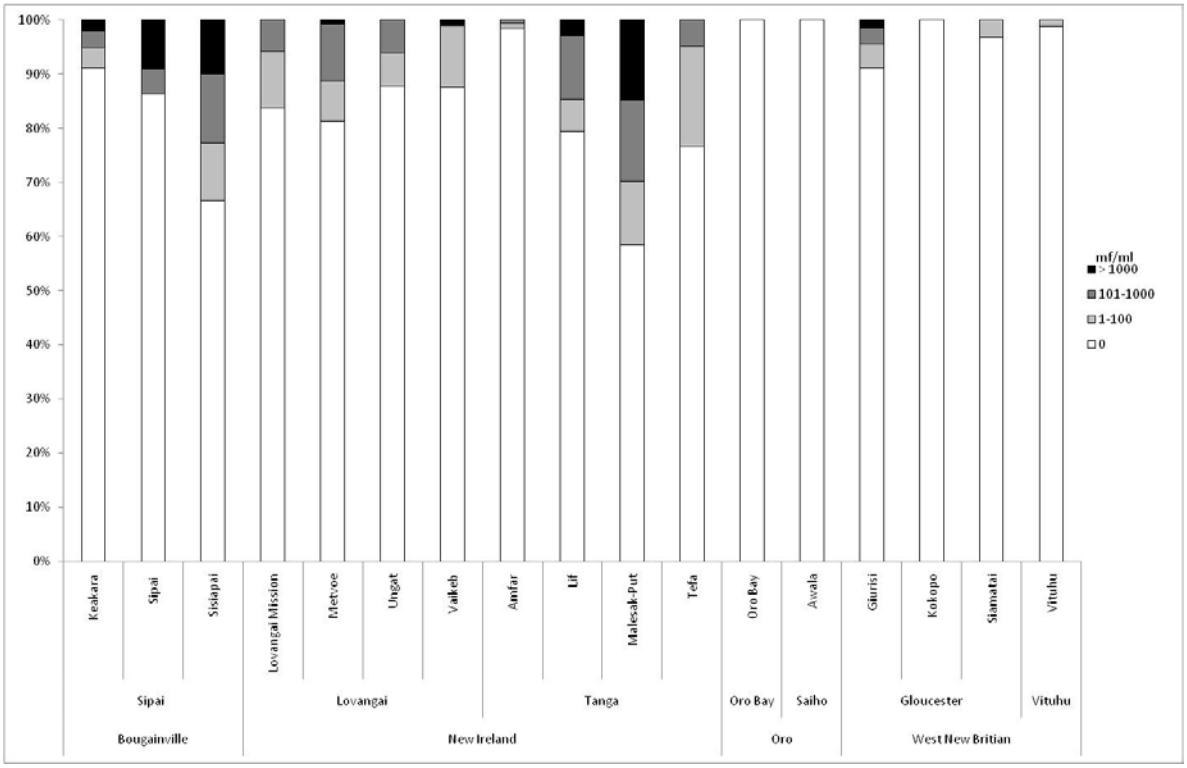
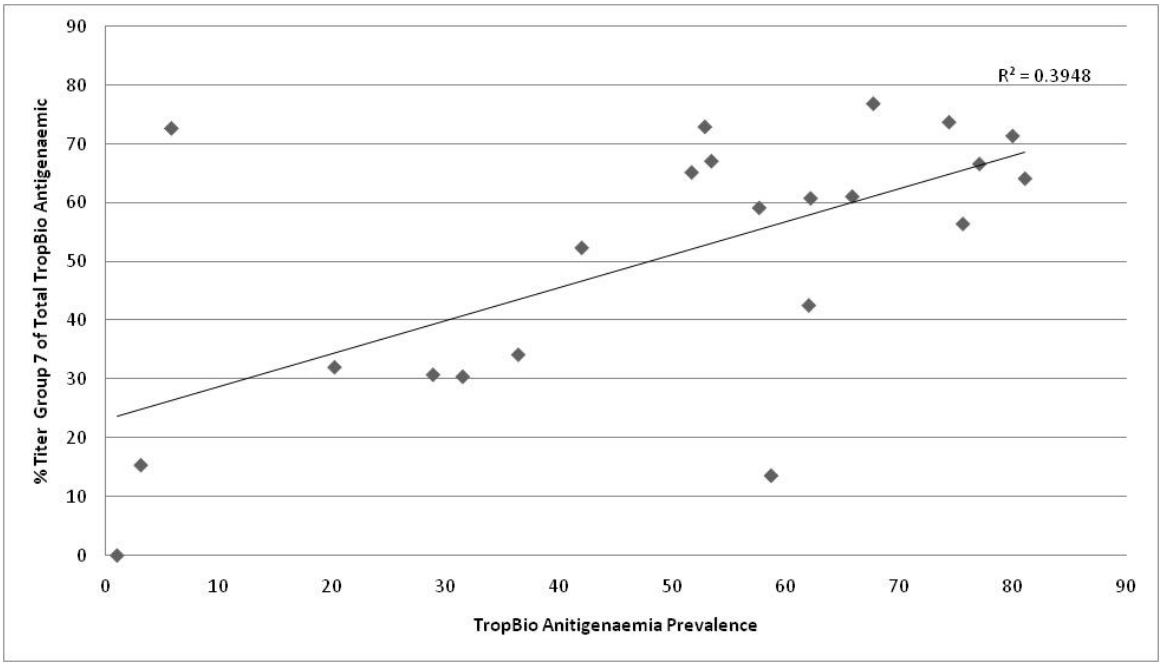


Table 3–6 Microfilariae density by gender

	1–99 mf/ml	100–999 mf/ml	>999 mf/ml
Male (152)	49.3 % (75)	37.5% (57)	13.2% (20)
Female (108)	46.2% (50)	35.1% (38)	18.5% (20)

Figure 3–4 Relationship between the antigen prevalence and the percentage titre group seven of all TropBio antigenaemics at each collection site



.

Table 3–7 Percentage of microfilaraemics by blood microfilariae density % (n)

Location	1–99 mf/ml	100–999 mf/ml	>999 mf/ml
Bougainville			
Sipai			
Keakara (12)	41.7 (5)	33.3 (4)	25.0.0 (3)
Sipai (6)	0.0 (0)	33.3 (2)	66.7 (4)
Sisiapai (47)	31.9 (15)	38.3 (18)	29.8 (14)
Sipai Total (65)	**30.8 (20)**	**36.9 (24)**	**32.3 (21)**
East New Britain			
Kokopo			
Kerawera Island (17)	76.5 (13)	23.5 (4)	0.0 (0)
Utuan Island (9)	55.6 (5)	44.4 (4)	0.0 (0)
Kokopo Total (26)	**69.2 (18)**	**30.8 (8)**	**0.0 (0)**
Pomio			
Gar (7)	85.7 (6)	14.3 (1)	0.0 (0)
Lat (20)	25 (5)	70.0 (14)	5 (1)
Mango Station (2)	100.0 (2)	0.0 (0)	0.0 (0)
Pomio Total (29)	**44.8 (13)**	**51.7 (15)**	**3.4 (1)**
East New Britain Total (55)	**56.4 (31)**	**41.8 (23)**	**1.8 (1)**
New Ireland			
Lovangai			
Lovangai Mission (17)	64.7 (11)	35.3 (6)	0.0 (0)
Metvoe (23)	39.1 (9)	56.5 (13)	4.3 (1)
Ungat (8)	50.0 (4)	50.0 (4)	0.0 (0)
Vaikeb (11)	90.9 (10)	0.0 (0)	9.1 (1)
Lovangai Total (59)	**57.6 (34)**	**39 (23)**	**3.4 (2)**
Tanga			
Amfar (3)	66.7 (2)	33.3 (1)	0.0 (0)
Lif (7)	28.6 (2)	57.1 (4)	14.3 (1)
Malesak-Put (39)	28.2 (11)	35.9 (14)	35.9 (14)
Tefa (19)	78.9 (15)	21.1 (4)	0.0 (0)
Tanga Total (68)	**44.1 (30)**	**33.8 (23)**	**22.1 (15)**
New Ireland Total (127)	**50.4 (64)**	**36.2 (46)**	**13.4 (17)**
Oro			
Oro Bay Total (0)	**0.0 (0)**	**0.0 (0)**	**0.0 (0)**
Saiho Total (0)	**0.0 (0)**	**0.0 (0)**	**0.0 (0)**
Oro Total (0)	**0.0 (0)**	**0.0 (0)**	**0.0 (0)**
West New Britain			
Gloucester			
Giurisi (6)	50.0 (3)	33.3 (2)	16.7 (1)
Kokopo (0)	0.0 (0)	0.0 (0)	0.0 (0)
Siamatai (3)	100.0 (3)	0.0 (0)	0.0 (0)
Gloucester Total (9)	**66.7 (6)**	**22.2 (2)**	**11.1 (1)**
Vituhu Total (4)	**100.0 (4)**	**0.0 (0)**	**0.0 (0)**
West New Britain Total (13)	**76.9 (10)**	**15.4 (2)**	**7.7 (1)**
Grand Total (260)	**48.1 (125)**	**36.5 (95)**	**15.4 (40)**

Table 3–8 Prevalence of TropBio antigenaemia by age group % (n=total number of individual in each age group)

Location	5–9y	10–14y	15–19y	20–24y	25–29y	30–34y	25–39y	40–44y	45–49y	≥50y
Bougainville										
Rorovana Total	**0 (0)**	**0 (0)**	**0 (0)**	**0 (0)**	**0 (0)**	**0 (0)**	**0 (0)**	**0 (0)**	**0 (0)**	**0 (0)**
Sipai										
Keakara	59.4 (19)	36.4 (8)	60.9 (14)	70 (7)	63.2 (12)	70 (7)	50 (1)	40 (2)	70 (7)	80 (4)
Sipai	0 (0)	62.5 (5)	29.4 (5)	25 (1)	33.3 (1)	0 (0)	0 (0)	100 (1)	0 (0)	0 (0)
Sisiapai	61.5 (16)	82.4 (28)	78.6 (11)	83.3 (10)	50 (2)	88.9 (16)	100 (10)	100 (12)	70 (7)	100 (8)
Sipai Total	**57.4 (35)**	**64.1 (41)**	**55.6 (30)**	**69.2 (18)**	**57.7 (15)**	**79.3 (23)**	**73.3 (11)**	**83.3 (15)**	**60.9 (14)**	**80 (12)**
Bougainville Total	**28.2 (35)**	**36.3 (41)**	**29.4 (30)**	**24 (18)**	**27.3 (15)**	**34.8 (23)**	**21.2 (11)**	**27.3 (15)**	**33.3 (14)**	**19.4 (12)**
East New Britain										
Kokopo										
Kerawera Island	23.8 (10)	57.1 (36)	63 (34)	67.6 (23)	61.5 (8)	69.2 (18)	72.7 (16)	85.4 (35)	68.8 (11)	69.2 (18)
Utuan Island	#DIV/0!	64.3 (9)	73.1 (19)	66.7 (6)	83.3 (5)	100 (3)	66.7 (2)	33.3 (3)	75 (3)	50 (4)
Kokopo Total	**23.8 (10)**	**58.4 (45)**	**66.3 (53)**	**67.4 (29)**	**68.4 (13)**	**72.4 (21)**	**72 (18)**	**76 (38)**	**70 (14)**	**64.7 (22)**
Pomio										
Gar	15.2 (5)	27.3 (6)	53.3 (8)	21.1 (4)	38.9 (7)	40 (6)	37.5 (3)	33.3 (2)	0 (0)	62.5 (5)
Lat	48.1 (13)	43.3 (13)	63.6 (21)	60 (6)	69.2 (9)	63.6 (14)	56.3 (9)	85.7 (6)	42.9 (3)	80 (4)
Mango Station	0 (0)	41.7 (5)	44.4 (4)	12.5 (1)	25 (1)	83.3 (5)	50 (1)	33.3 (1)	0 (0)	100 (3)
Pomio Total	**29 (18)**	**37.5 (24)**	**57.9 (33)**	**29.7 (11)**	**48.6 (17)**	**58.1 (25)**	**50 (13)**	**56.3 (9)**	**30 (3)**	**75 (12)**
ENB Total	**26.9 (28)**	**48.9 (69)**	**62.8 (86)**	**50 (40)**	**55.6 (30)**	**63.9 (46)**	**60.8 (31)**	**71.2 (47)**	**56.7 (17)**	**68 (34)**
New Ireland										
Lovangai										
Lovangai Mission	100 (3)	33.3 (10)	70.6 (12)	68.4 (13)	83.3 (5)	62.5 (5)	75 (9)	70 (7)	100 (4)	60 (6)
Metvoe	32.4 (12)	43.8 (7)	50 (7)	60 (6)	72.7 (8)	77.8 (7)	50 (6)	100 (6)	76.9 (10)	33.3 (1)
Ungat	50 (7)	46.2 (6)	50 (3)	87.5 (7)	57.1 (4)	33.3 (1)	33.3 (2)	60 (3)	#DIV/0!	50 (4)
Vaikeb	51.2 (21)	66.7 (2)	41.7 (5)	40 (2)	100 (2)	42.9 (3)	100 (4)	28.6 (2)	0 (0)	83.3 (5)
Lovangai Total	**45.3 (43)**	**40.3 (25)**	**55.1 (27)**	**66.7 (28)**	**73.1 (19)**	**59.3 (16)**	**61.8 (21)**	**64.3 (18)**	**73.7 (14)**	**59.3 (16)**
Tanga										
Amfar	1.8 (1)	8 (2)	3.2 (1)	11.1 (1)	5.6 (1)	0 (0)	0 (0)	25 (3)	0 (0)	14.3 (2)
Lif	57.1 (4)	75 (3)	66.7 (2)	100 (1)	100 (6)	100 (2)	#DIV/0!	100 (2)	60 (3)	100 (5)
Malesak-Put	58.1 (18)	90.9 (10)	25 (2)	71.4 (5)	40 (2)	85.7 (12)	100 (3)	83.3 (5)	66.7 (4)	80 (4)
Tefa	63.6 (14)	75 (12)	90 (9)	83.3 (5)	83.3 (5)	83.3 (5)	100 (2)	66.7 (6)	#DIV/0!	60 (3)
Tanga Total	**31.9 (37)**	**48.2 (27)**	**26.9 (14)**	**52.2 (12)**	**40 (14)**	**65.5 (19)**	**38.5 (5)**	**55.2 (16)**	**35 (7)**	**48.3 (14)**
New Ireland Total	**37.9 (80)**	**44.1 (52)**	**40.6 (41)**	**61.5 (40)**	**54.1 (33)**	**62.5 (35)**	**55.3 (26)**	**59.6 (34)**	**53.8 (21)**	**53.6 (30)**
Oro										
Oro Bay Total	**0 (0)**	**3.5 (3)**	**4.9 (3)**	**0 (0)**	**4.4 (2)**	**0 (0)**	**11.8 (4)**	**0 (0)**	**0 (0)**	**3.3 (1)**
Saiho Total	**0 (0)**	**0 (0)**	**2.5 (2)**	**0 (0)**	**0 (0)**	**0 (0)**	**3.6 (1)**	**0 (0)**	**10 (1)**	**2.9 (1)**
Oro Total	**0 (0)**	**1.6 (3)**	**3.5 (5)**	**0 (0)**	**2.4 (2)**	**0 (0)**	**8.1 (5)**	**0 (0)**	**3.6 (1)**	**3.1 (2)**
West New Britain										
Gloucester										
Giurisi	0 (0)	50 (4)	66.7 (10)	91.7 (11)	72.7 (8)	90 (9)	80 (8)	75 (6)	50 (1)	83.3 (5)
Kokopo	24 (6)	28.6 (12)	33.3 (10)	48 (12)	40 (8)	33.3 (8)	53.3 (8)	22.2 (2)	80 (4)	40.9 (9)
Siamatai	63.6 (14)	66.7 (14)	87.5 (7)	80 (8)	64.3 (9)	93.3 (14)	100 (6)	100 (3)	100 (5)	80 (4)
Gloucester Total	**42.6 (20)**	**42.3 (30)**	**50.9 (27)**	**66 (31)**	**55.6 (25)**	**63.3 (31)**	**71 (22)**	**55 (11)**	**83.3 (10)**	**54.5 (18)**
Vituhu Total	**11.1 (8)**	**21.5 (17)**	**33.3 (20)**	**37.1 (13)**	**13.3 (4)**	**8.1 (3)**	**9.5 (2)**	**20 (4)**	**40 (4)**	**13.6 (3)**
WNB Total	**23.5 (28)**	**31.3 (47)**	**41.6 (47)**	**53.7 (44)**	**38.7 (29)**	**39.5 (34)**	**46.2 (24)**	**37.5 (15)**	**63.6 (14)**	**38.2 (21)**
Grand Total	**23.9 (171)**	**29.9 (212)**	**35.2 (209)**	**37.4 (142)**	**33.3 (109)**	**39.3 (138)**	**36.7 (97)**	**44.6 (111)**	**41.6 (67)**	**34.5 (99)**

Figure 3–5 Total prevalence (excluding Rorovana) of Tropbio antigenaemia by age group

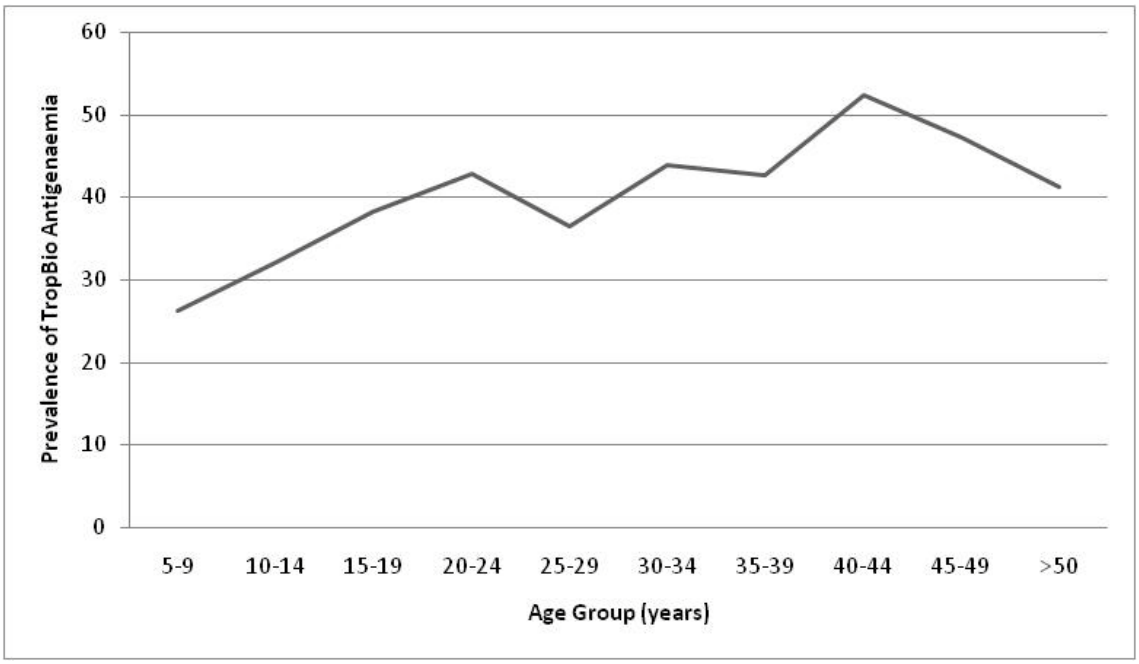


Table 3–9 details the prevalence of TropBio Antigenaemia at each collection site. Figure 3–5 shows the overall prevalence of antigenaemia by age group, excluding Rorovana that had no antigenaemic individuals. Prevalence of antigenaemia rose sharply by age group where from age 20–24 it levelled off with peak prevalence in the 40–44 year old age group. This general pattern was seen in all sentinel sites except for Vituhu, which had a reduction in antigenaemia prevalence between the ages of 25–44.

There were a large number of blood slides (n=626, 17.3%) that were unable to be examined for microfilariae. The main cause was a fungus infestation that covered the glass side likely from contamination of the stain that was used in the district laboratories. Other causes were loss of blood from the slide during staining; particularly using slides prepared using anticoagulated blood, broken slides and slides with unreadable identification. Slides that were examined were less likely ($p<0.001$) to be antigenaemic (35.2%) compared to

unreadable slides (48.2%). Most of the blood slides that were destroyed by fungal infection were from East New Britain with 52% of the slides unreadable from this province. Excluding the East New Britain blood slides from the total sample revealed no difference (p=0.08) between overall prevalence of antigenaemia of those who had blood slides examined (92%) and those who did not (8%).

Figure 3–6 Relationship between TropBio antigenaemia prevalence and microfilaraemia prevalence

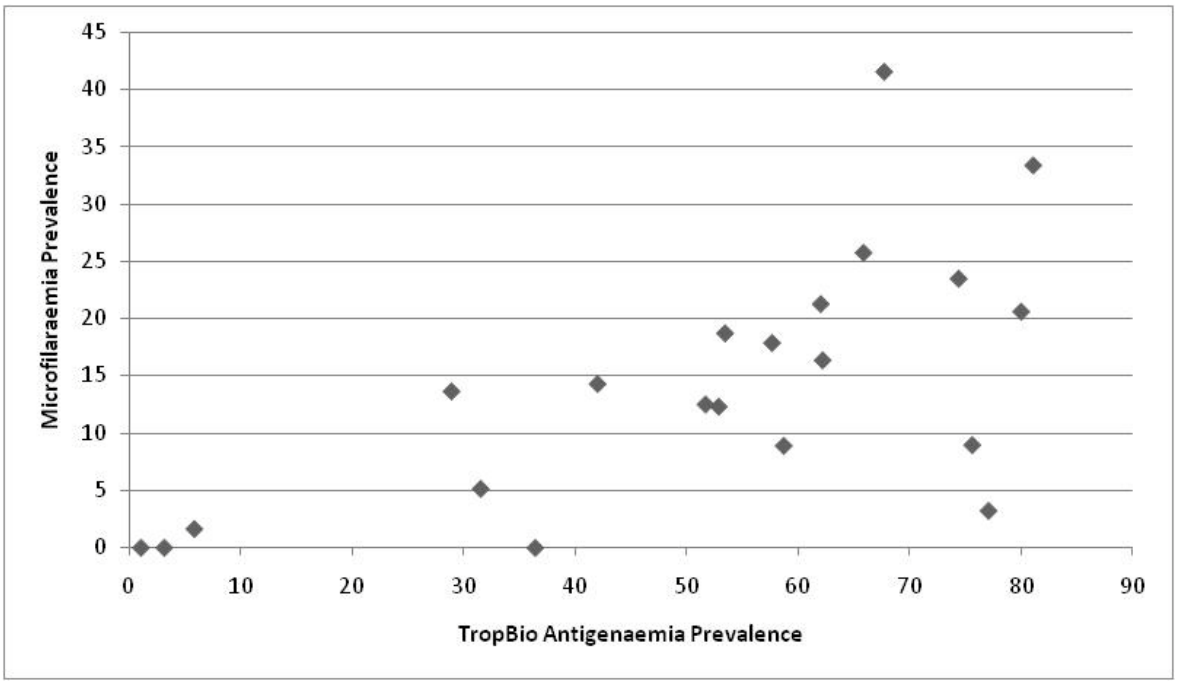


Figure 3–7 Ratio of TropBio antigenaemia prevalence/microfilaraemia prevalence+ 0.1

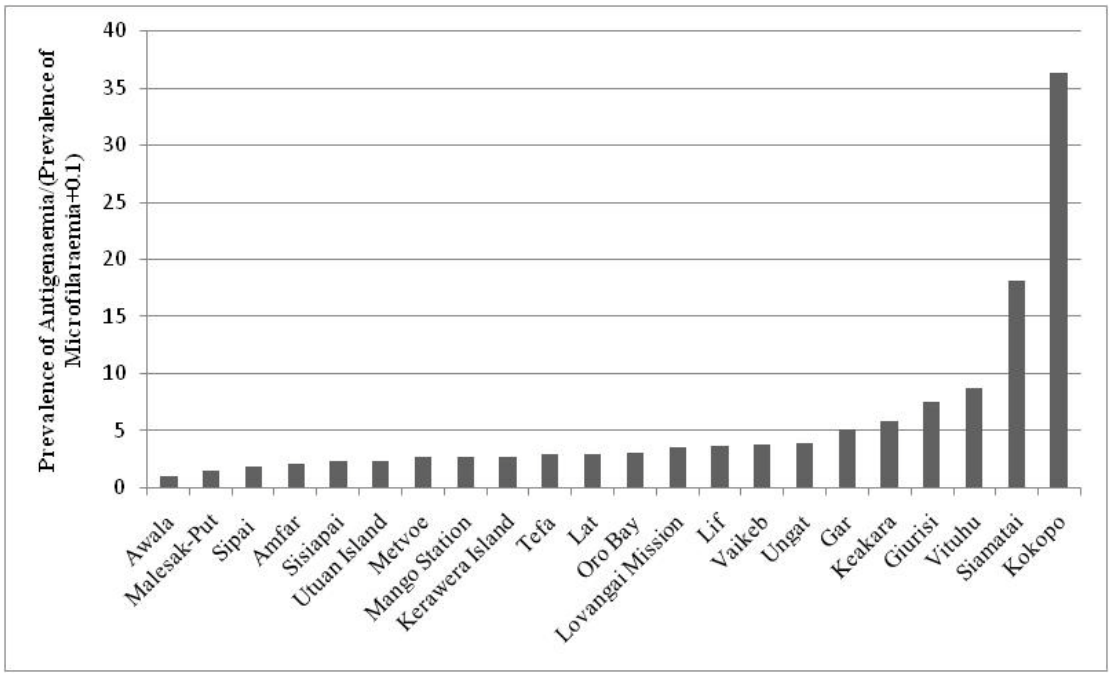


Figure 3–6 shows the relationship between TropBio antigenaemia and microfilaraemia prevalence. It is notable that the microfilaraemia prevalence for those sites that had undergone MDA prior to sampling were comparatively lower than the other sites for a given antigenaemia prevalence. This is shown by the graph showing the ratio of microfilaraemia plus 0.1, to include sites with zero prevalence, to antigenaemia prevalence. The four sites in West New Britain that had undergone MDA were compared to the other sites (n=18) using Mann-Whitney U test. The median ratio for the West New Britain sites and the remainder sites was 3.09 and 2.00 respectively. The ratio was significantly higher for the West New Britain sites ($p<0.01$) and likely due to the MDA. In Kokopo, which had undergone MDA, there was a TropBio antigenaemia prevalence of 36.4% and no cases of microfilaraemia detected.

Figure 3–8 shows the combined prevalence of microfilaraemia increasing with age for all provinces excluding Rorovana. This also seen if East New Britain and West New Britain are excluded due to the large numbers of slides lost and the resulting MDA. When all provinces

excluding Rorovana are considered, the peak age group was in the 35–39 age range or 40–44 when East and West New Britain are excluded. This same peak occurred in this age group for the prevalence of antigenaemia.

Figure 3–8 Prevalence of microfilaraemia by age range

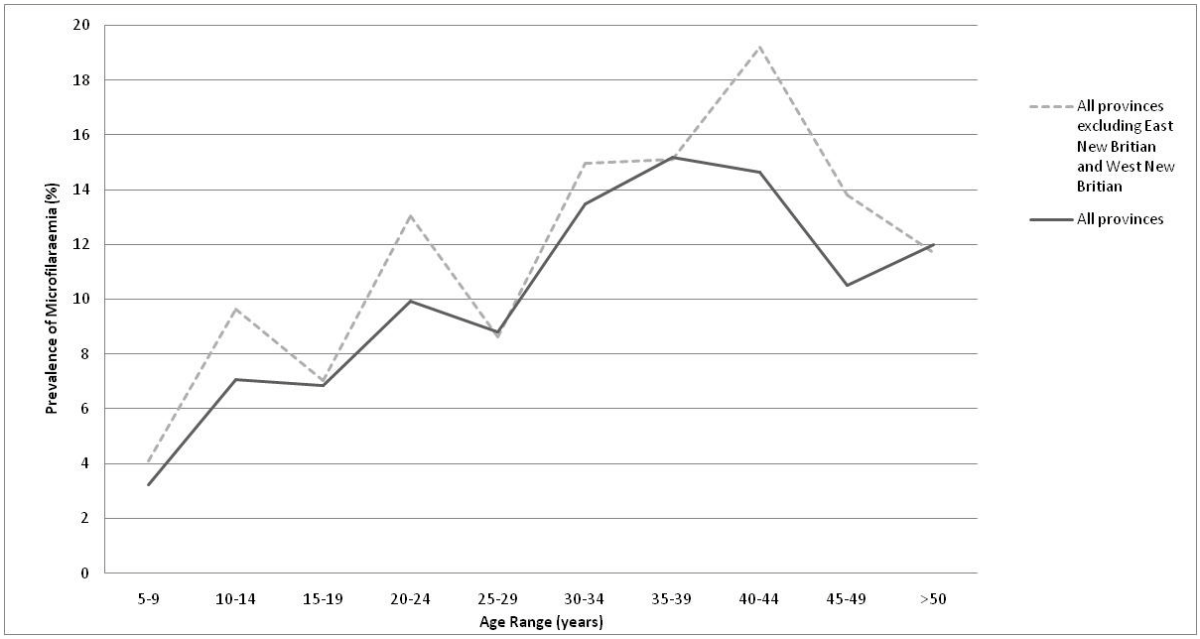


Table 3–7 shows the density of microfilariae by gender. There was no difference in density between male and females ($\chi 2 = 1.39$, df=2, p=0.50.). Table 3–10 shows the gender balance in prevalence for antigenaemia and microfilaraemia. For antigenaemia, the collection sites at Mango Station in Pomio and Vituhu in West New Britain showed a significant difference in prevalence between male and females with a greater proportion of females antigenaemic at both sites. Overall, excluding Rorovana, there was no difference in antigenaemia prevalence (p=0.29) between the genders but males had a higher prevalence of microfilaraemia compared to females (p<0.01).

Table 3–9 Prevalence of antigenaemia and microfilaraemia by gender at each collection site % (n=total number of participants)

	TropBio Antigenaemia				Microfilaraemia	
Location	**Female**	**Male**	**χ^2**	**P**	**Female**	**Male**
Bougainville						
Rorovana Total	0.0 (216)	0.0 (199)				
Sipai						
Keakara	64.4 (45)	55.9 (93)	0.91	0.34	6.98 (43)	9.78 (92)
Sipai	22.7 (22)	34.8 (23)	0.80	0.37	9.1 (22)	18.2 (22)
Sisiapai	86.8 (76)	75 (72)	3.38	0.07	40.3 (72)	26.1 (69)
Sipai Total	69.9 (143)	60.6 (188)			24.8 (137)	16.9 (183)
Bougainville Total	27.9 (359)	29.5 (387)				
East New Britain						
Kokopo						
Kerawera Island	61.1 (175)	63 (162)	0.12	0.73	13.5 (37)	27.9 (43)
Utuan Island	55.6 (36)	73.9 (46)	3.03	0.08	5.9 (17)	44.4 (18)
Kokopo Total	60.2 (211)	65.4 (208)			11.1 (54)	32.8 (61)
Pomio						
Gar	30 (70)	32.9 (76)	0.14	0.71	6 (67)	4.3 (69)
Lat	54.1 (74)	60.4 (96)	0.69	0.41	4.4 (45)	26.9 (67)
Mango Station	59.1 (22)	28.6 (28)	4.71	0.03	16.7 (6)	12.5 (8)
Pomio Total	44.6 (166)	45.5 (200)			5.9 (118)	15.3 (144)
ENB Total	53.3 (377)	55.6 (408)			7.6 (172)	20.5 (205)
New Ireland						
Lovangai						
Lovangai Mission	61.4 (57)	62.9 (62)	0.03	0.87	13.7 (51)	18.9 (53)
Metvoe	55.4 (56)	52 (75)	0.15	0.70	10 (50)	24.7 (73)
Ungat	43.8 (32)	60.5 (38)	1.96	0.16	6.5 (31)	17.6 (34)
Vaikeb	49 (49)	55 (40)	0.32	0.57	16.3 (49)	7.7 (39)
Lovangai Total	53.6 (194)	57.2 (215)			12.2 (181)	18.6 (199)
Tanga						
Amfar	4.2 (119)	8.6 (70)	1.54	0.22	1.7 (116)	1.5 (66)
Lif	85 (20)	73.3 (15)			31.6 (19)	6.7 (15)
Malesak-Put	59.2 (49)	76.6 (47)	3.33	0.07	38.8 (49)	44.4 (45)
Tefa	72.2 (36)	76.1 (46)	0.16	0.69	22.9 (35)	23.9 (46)
Tanga Total	34.4 (224)	49.4 (178)			16 (219)	19.2 (172)
New Ireland Total	43.3 (418)	53.7 (393)			14.3 (400)	18.9 (371)
Oro						
Oro Bay Total	1.6 (190)	4.4 (226)	2.76	0.10	0 (186)	0 (216)
Saiho Total	1.2 (250)	0.9 (234)			0 (242)	0 (220)
Oro Total	1.4 (440)	2.6 (460)			0 (428)	0 (436)
West New Britain						
Gloucester						
Giurisi	71.8 (39)	79.1 (43)	0.59	0.44	3.1 (32)	14.3 (35)
Kokopo	37.9 (103)	35.1 (114)	0.18	0.67	0.0 (93)	0.0 (102)
Siamatai	84.6 (52)	70.2 (57)	3.21	0.07	2.1 (47)	4.3 (46)
Gloucester Total	57.2 (194)	53.3 (214)			1.2 (172)	3.8 (183)
Vituhu Total	25.2 (202)	14.7 (184)	6.68	0.01	1.2 (171)	1.5 (137)
WNB Total	40.9 (396)	35.4 (398)			1.2 (343)	2.8 (319)
Grand Total	32.7 (1990)	34.5 (2046)			7.3 (1480)	10.0 (1515)

3.4 Discussion

There were 22% less females tested for antigenaemia in those aged 5–15 compared to males. The 2000 PNG census showed 10.7% less females in this age range compared to males (National Statistical Office of Papua New Guinea, 2003). Boys are highly favoured in PNG (Gibson and Rozelle, 2004) but this observed gender imbalance is very high. It is possible that parents are more likely to bring their sons for testing. This could this mean girls are less likely to be treated during MDA than boys and be a reservoir source.

Even as late as 1984, Kazura et al. (1984) could write the understated observation that Bancroftian filariasis had been reported in several areas of PNG. The survey conducted here shows widely varying prevalences in microfilaraemia and antigenaemia. Surveys conducted between 1991 and 1997 in PNG also showed a wide range of antigenaemia with no antigenaemia present in Mount Hagan in the Western Highland Province to 88% antigenaemia in Nomad in the Western province. In the Southcoast of New Ireland prevalence of microfilaraemia was 21% and TropBio antigenaemia 32% and on Lihir island, 20% microfilaraemia and 55% antigenaemia. On Witu island in East New Britain, microfilaraemia was 10% and antigenaemia 38% (Melrose et al., 2000). In the 1950s, the prevalence rate of microfilaraemia was 19.5% among 427 indentured native labourers in Rabaul who originated from many different districts (Backhouse and Heydon, 1950).

The concentration of LF in small communities is evident when those surveyed were asked the location of the village with all three people from Rebai antigenaemic at the Oro collection site and five of six people from Sumniul antigenaemic at Amfar. Large differences in antigenaemia and microfilaraemia between nearby villages correlate with transmission potential (Kazura et al., 1997; Tisch et al., 2001). This similar concentration of LF has been

reported in a survey on Lihir Island where nine villages had microfilaraemia prevalence rates ranging from zero to 43%. Here, it was suggested that villages near swampy areas had three to seven times higher prevalence compared to villages located in dry regions (Hii et al., 2000). There can be a difference in microfilaraemia prevalence even in small islands, where villages on the beach and prone to wind may experience different transmission rates compared to villages more inland and surrounded by dense vegetation (Backhouse and Heydon, 1950). The collection sites of Lif, Malesak-Put and Tefa, nearby islands to Amfar, had antigenaemia prevalence's of 80%, 68% and 74%, compared to 5.8% at Amfar. The villages of Amfar and Ampotpot are located beside each other with the medical clinic between them. Possibly, the presence of a medical clinic has contributed to a low prevalence on this island.

The concentration of 'hot spots' of LF makes it important that full coverage is achieved by the MDA. If locations are missed, there is a potential for a residual focus of infection within countries and migration of infected individuals between islands where the main reservoir may reside in non-endemic areas where MDA has not been conducted (Huppatz et al., 2008). In India, Haiti and Samoa migration from other areas and nearby villages has been suggested as a cause for resurgence ongoing transmission and disappointing reduction in prevalence due to MDA (Kimura et al., 1985; Streit and Lafontant, 2008). Migration from one area to another in PNG has also shown to be significant. In rural parts of the Urat and Urim census districts of East Sepik Province, 39% of migrants were microfilaraemic suggesting small areas missed from control programs have the potential to disperse filariasis (Alexander et al., 2001). Transmission from migrants from a nearby village was considered a reason for the high prevalence of Bm14 antibody in a village that had received four annual MDA rounds in PNG (Tisch et al., 2008).

The recent MDA in West New Britain had a measurable effect on microfilaraemia prevalence with the median ratio of antigenaemia prevalence/microfilaraemia prevalence +0.1 greater compared to the sites that had yet to undergo MDA likely due to the reduced microfilaraemia prevalence caused by the MDA. As shown in Table 2–4, after treatment with DEC, the mean antigen level can be less than half one month after treatment yet the West New Britain sites had similar titre group profiles and the proportion of titre group seven compared to all titre groups of these sites appeared similar to other sites. This could be because the treatment reduced the antigen titre similarly across all individuals, an effect on antigen was yet to be evident or the antigen concentration although reduced was still high enough to be grouped into titre seven.

Overall, males had a higher prevalence of microfilaraemia compared to females with density being the same. This is in agreement with other published studies in PNG (Knight et al., 1979; Desowitz et al., 1993; Prybylski et al., 1994; Kazura et al., 1997) though other studies have found no difference (Kazura et al., 1984; Kazura et al., 1997; Hii et al., 2000). Prybyski et al. (1994) suggest men sleep in temporary bush huts and so have greater exposure. Microfilaraemia prevalence rates increased with age. This is also in accordance with other published studies from PNG (Kazura et al., 1984; Prybylski et al., 1994; Kazura et al., 1997; Hii et al., 2000), though Turner et al. (1993) found no trend in three villages in the Western province.

Among the collection sites, there were only two sites showing a difference in prevalence between genders with males showing a higher prevalence at the Mango Station and on Vituhu. There were no gender differences in antigenaemia prevalence reported from the Sepik or Madang (Bockarie et al., 2000; Tisch et al., 2001). The high infection pressure may overwhelm any difference in susceptibility between the genders (Tisch et al., 2001) with the

difference in the two sites due to other behavioural, social or environmental factors. Overall, the prevalence of antigenaemia rose with age peaking in agreement with other surveys performed in PNG (Turner et al., 1993; Bockarie et al., 2000; Tisch et al., 2001).

With increasing prevalence of antigenamics, titre group seven proportion of antigenaemics increased. This indicates that with increasing prevalence, there are relatively higher titre individuals. As expected, increased transmission intensity increases the number of those infected as well as increasing the worm burden on those infected.

The successful baseline surveys conducted in PNG shows that venous blood collected by venipuncture is acceptable to communities. Measuring antigen titre allows the change over time to be monitored providing more information than whether an individual is positive or negative. Using the TropBio assay may extend a program for some years, which will incur extra costs if antigenaemia is used as the basis for deciding when to stop MDA. Antigen assay's may be less useful than anticipated due to the long time in clearance of antigen, and assessing microfilariae concentration may still be the best way of determining treatment success or failure (Eberhard et al., 1997). It has been suggested that monitoring antibody levels may be better suited to monitoring progress due to the slow clearing time from the blood of antigen (Tisch et al., 2008). Simonsen et al. (2005) also state that the minor short-term effect on antigen levels makes it unsuitable for monitoring the immediate effect of treatment. As the aim of elimination programs is to stop transmission, which is thought to require the prevalence of microfilaraemia to be below 1%, then prevalence of microfilaraemia is the important parameter. This needs to be monitored and due to the persistence of antigen, antigen tests cannot eliminate the need for examination for microfilariae.

It is encouraging to have encountered locations with little or no antigenaemia. Rorovana, a community that supplied labour for the Paguna mine, had no cases of antigenaemia recorded. Three people were observed to have leg lymphoedema although they had suffered this for over a decade. It is possible that health care provided to workers and the community by the mining company eliminated lymphatic filariasis and it has not yet returned. It is essential that the elements determined in Haiti for the continuation of MDA; non-compliance, inflated coverage estimates, non-representative sentinel sites and the insensitivity of blood smears for microfilaraemia (Grady et al., 2007); are not replicated in PNG. Using the TropBio antigen test protects against the insensitivity of blood smears. Using locations in each province in remote areas, far removed from central health and coordination services, provides more confidence that the trend encountered over the life of the program is reflective of what is happening nationwide with the MDA operation. Continual monitoring of the LF program is essential.

Chapter Four: Baseline Monitoring of Soil-transmitted Helminths Prevalence in Timor-Leste

4.1 Introduction

Timor-Leste has an elimination of lymphatic filariasis program that includes the use of albendazole. This medication is also used in the treatment of STH infection. Baseline parasitological surveys using faecal samples were conducted at three locations in Timor-Leste to determine the effect of mass anthelmintic administration on STH.

In 2001, the World Health Assembly issued Resolution 54.19, which specified that at least 75% of all school-aged children who are at risk of morbidity from schistosomiasis and STH should be regularly reached and treated by the year 2010 (WHO, 2001). Lymphatic Filariasis Elimination Program's use of albendazole and/or ivermectin allows integration of this goal into national programs. Widespread use of effective drugs has been credited with the decrease of STH infections nationally (Bowie et al., 2004), although their use annually is unlikely to be sufficient to control STH in women and children (Cline et al., 2000).

The global estimates of STH prevalence, using techniques based on extrapolation from survey data, are shown in Table 4–1 with a increase in prevalence in 1994 attributed to population growth (Chan et al., 1994) and a decrease between 1994 and 2002 being attributed to national control activities and social and economic development particularly in Asia and Latin America (de Silva et al., 2003). Sanitation, improvements in water supply and education are likely factors that will aid in the reduction of STH prevalence. However, until this occurs, anthelmintic treatment will be the key intervention. Most control programs attempt to reduce morbidity as a short-term goal using anthelmintics with the likelihood of reduced transmission (Albonico et al., 1999; Crompton and Nesheim, 2002; Horton, 2003).

Although single dose anthelmintic treatments are suitable for hookworm and *A. lumbricoides*, they have poor efficacy against *T. trichiura* (Keiser and Utzinger, 2008).

Table 4–1 Estimates of global prevalence of STH infections, absolute numbers (millions) and prevalence (%)

	1947 Stoll (1947)	1978 WHO (1992)	1994 Chan et al. (1994)	2002 de Silva et al. (2003)
Hookworm	456.8 (22%)	700–900	1297 (31%)	740 (17%)
A. lumbricoides	644.4 (30%)	800–1000	1471 (36%)	1221 (26%)
T. trichiura	355.1 (16%)	500	1048 (25%)	795 (15%)

4.1.1 Contribution of STH to disease

The public health impact of STH is often underestimated because, despite the high worldwide prevalence, there is little evident disease (Albonico et al., 1999). Chan et al. (1994) estimated the prevalence of morbidity of 3.0–5.2% (122–214 million cases), 2.1–3.2% (87–133 million cases) and 1.5–2.4% (61–96 million cases), for *A. lumbricoides, T. trichiura* and hookworm respectively for the global population. This contrasts with a WHO estimate of morbidity in 1978 of 1.5 million, 1 million and 100,000 cases (WHO, 1992). WHO estimates 135 thousand deaths per year due to these three parasites (WHO, 2002).

Hookworms, *A. lumbricoides* and *T. trichiura* have public health significance due to their contribution to malnutrition, iron deficiency anaemia and *Trichuris* Dysentery Syndrome leading to increased maternal deaths, poor growth, impaired cognitive function and decreased educational achievement (Crompton, 2000; O'Lorcain and Holland, 2000; Stephenson et al., 2000). Lower worm burdens are thought to have detrimental effects on school performance and physical fitness while higher burdens cause overt disease (WHO, 2002). Lower worm burdens in children increase time spent in free and structured play, which may indicate increased potential for productivity later in their lives (Stephenson et al., 1993a; Adams et al.,

1994). *A. lumbricoides* can also cause intestinal obstruction in children (de Silva et al., 1997) with changes in villi and crypts in jejunal biopsies, which could cause malnutrition effects (Tripathy et al., 1972). Moderate to high intensity *Trichuris* infection or hookworm infection is a risk factor for moderate to severe wasting or underweight in preschool-aged children (Casapia et al., 2007).

Hookworms contribute to iron deficiency anaemia, causing a daily loss of blood of between 0.03 and 0.15 ml per worm so that even a low worm burden is enough to drain iron stores in women and adolescent girls (Crompton, 2000). In a review of 14 randomised trials on the effect of anthelmintics on haemoglobin, the pool weighted mean difference of the change in haemoglobin was 1.71 g/l. This was projected to reduce anaemia in a population by five to 10% (Gulani et al., 2007). This compares to a mean increase of 7.4 g/l using iron supplements with between 38% and 62% of baseline anaemia being responsive to treatment (Gera et al., 2007). However, in some populations, worm burden in anaemic and normal hosts show no significant differences (Pritchard et al., 1990) nor is baseline helminth prevalence a predictor of haemoglobin response to anthelmintics (Gulani et al., 2007).

The presence of STH infection appears to affect the disease process in other infections. There is a strong association between TB and intestinal helminth infection with the odds of being a TB patient increasing with the number of helminth species (Elias et al., 2006b). This may affect the efficacy of TB vaccinations due to immune modulation (Elias et al., 2006a). There is no consistent effect of co-infection of malaria and intestinal helminths on malaria incidence or disease severity (Hartgers and Yazdanbakhsh, 2006). Deworming does have beneficial effects on CD-4 counts and plasma HIV-1 RNA levels in STH and HIV coinfected patients (Walson et al., 2009).

According to one WHO publication ‘the effects of the deworming are dramatic’ (WHO, 2005, page 1). Deworming helps achieve the Millennium Development Goals by allowing children to earn their way out of poverty by improving intellectual development and cognition, reducing school absenteeism, particularly improving girls’ enrolment and reducing dropout rates, reducing child mortality by reducing anaemia in both childhood and pregnancy and relieving the burden of disease of AIDS and malaria (WHO, 2005). The evidence cited for this dramatic effect, provided by Awasthi et al. (2000), is less overwhelming. This study showed that after two years of six monthly albendazole treatment compared to a placebo, stunting increased from 59.8% to 61.7% in those taking albendazole while stunting rose from 54.8% to 66.2% in those taking the placebo. Although this difference was significant, there was no difference in the proportion change for those categorised as underweight or wasted, or a difference between the two groups in weight, height, weight gain, height gain, haemoglobin level or cognitive function. The findings in this study do not support evidence of dramatic effects due to deworming. Concern has been expressed with the randomisation process in this study (Kapil, 2000).

In a Cochrane review, the evidence was unclear whether worm burden had any effect on cognitive development in children (Dickson et al., 2000). There is possibly some effect from heavy parasite loads (Kvalsvig et al., 1991) while light infections have even been thought to be beneficial (Watkins et al., 1996). This negative effect on cognitive development could be mediated either due to iron deficiency or protein energy malnutrition with depressed appetite as a controlling mechanism (Stephenson et al., 1993b; Drake et al., 2000). There is little evidence to support the use of routine anthelmintic treatment to improve growth and cognitive performance in children in developing countries (Dickson et al., 2000). The Cochrane review conclusion was criticised for the absence of analysis by the intensity of worm burden (Michael et al., 2000), where it is expected that those with the highest burden

would have the greatest health benefit from drug treatment. However, a more recent Cochrane review (Taylor-Robinson et al., 2007) came to a similar conclusion that deworming did not have a positive effect on school performance.

This review (Taylor-Robinson et al., 2007) found that deworming had a small but inconsistent effect on weight gain and did not affect cognitive function. The meta-analysis showed an overall weight gain of 0.34 kg after one dose, whereas multiple doses had no effect on weight or height irrespective of length of time of follow-up. As the authors note, this result is confusing. Single or multiple doses also had no effect on haemoglobin concentration and there was no effect on school performance or cognitive function. When the trials were stratified, reflecting worm intensity and prevalence, a single dose had a significant beneficial effect on weight gain in children.

Miguel and Kremer (2004) consider that many studies underestimate the impact of deworming, as anthelmintic treatment has benefit to both the treated and control group. Within school randomised studies do not take into account external benefits and they argue that individuals who do not receive treatment also reduce their worm burden. Due to treatment, there is less contamination of the soil and consequently reduced reinfection for both treated and control individuals so that both groups receive benefit. They report several studies (Bundy et al., 1990; Simeon et al., 1995) showing this fall in the prevalence or intensity of STH in the placebo group. Bundy et al. (1990) reported age targeted anthelmintic treatment on the island of Montserrat in the West Indies with a total population of 11500. Approximately 2500 children between two and 15 years received four doses of albendazole four monthly. As well as a decline in the intensity and prevalence of *T. trichiura* and *A. lumbricoides* in the treated population, prevalence and intensity also fell in the 16–25 year old age group in which less than 4% had received treatment.

Table 4–2 summarises the studies using albendazole that were included in the Cochrane review (Taylor-Robinson et al., 2007), (as this is the anthelmintic used in Timor-Leste), which examined the effect of weight change. Only one study (Stephenson et al., 1989) showed a significant decrease in prevalence and intensity of *A. lumbricoides* in the placebo group. To observe a reduction in prevalence and intensity of STH in the wider untreated population, it would probably require a large percentage of the population to be treated for untreated individuals to reduce their helminth burden. Eggs of *Ascaris* can remain in the soil for several months so reinfection can still occur despite treatment (Wong and Bundy, 1990). Worm burdens may fall if the loss due to old age of the worm is greater than the reduced reinfection rate as scenscent worms are not replaced (Miguel and Kremer, 2004).

The studies (summarised in Table 4–2) that showed a significant weight gain for children treated with albendazole all occurred at the same school in Kenya. All other studies showed a non-significant weight difference between the placebo and treated groups. The question should be asked what is special about the studies conducted at this school and whether the results are applicable to other communities. Stephenson et al. (1989) note improvements in growth, attributed to one dose of albendazole, was achieved despite 75–90% of the treated group still having STH. The studies in Table 4–2 not associated with this study site did not show this improvement in growth. Why is this not the case for the other studies that showed non-significant weight gain changes?

It is clear in some study areas that there is a rapid reinfection of *A. lumbricoides.* This is shown clearly in Kruger et al. (1996) where prevalence decreased significantly only to return to baseline levels five months after the last treatment. In addition, six monthly treatment with albendazole over two years, as described by Awasthi (2000), did not reduce prevalence, but reduced the gain in prevalence compared to the placebo group. The remaining parasites could

still have an impact burden on the host. Does the STH burden need to be reduced to near zero for an effect to be evident or is the actual burden of disease caused by STH minimal? Worm species may also affect hosts in different ways. The impact of *Ascaris* might not be as great as hookworm and *Trichuris* (Watkins and Pollitt, 1996). What is the effect of multiple species of STH or STH with other parasites including protozoa on the host?

The other feature that Table 4–2 highlights is the often poor reduction of *T. trichiura* prevalence compared to the other STH. While hookworm and *A. lumbricoides* decreased by 50% in five of the nine trials, *T. trichiura* prevalence was reduced by greater than 50% in only one of eight trials.

Stephenson et al. (1993a) identify in one study that weight gain was greatest in children with greatest reduction in intensity of hookworm infection. Rather than prevalence being an important measure, the intensity of infection probably has important health consequences. Table 4–2 again shows the three trials in the same Kenyan school show significant reductions in intensities. The Cochrane review suggests that a single dose anthelmintic treatment for high intensity/prevalence areas may have some positive effect on weight gain (Taylor-Robinson et al., 2007).

Table 4–2 Comparison between albendazole treated and placebo STH prevalence rates and changes in weight gain

Study characteristics	Treated	Placebo	Changes in Weight	Reference
All available children in standard one and two of Mvindeni Primary School, Kwale District, Coast Province, Kenya Single 400 mg dose albendazole Parasitology : Modified Kato technique	N=78 Significant reduction in prevalence and intensity of *A. lumbricoides, T. trichiura* and hookworm six months after treatment. Hookworm prevalence at baseline 95%, at end 77% 89% egg reduction, geometric mean 1183 to 136 epg *A. lumbricoides* prevalence at baseline 44%, at end 13% 98% egg reduction, geometric mean 86 to 2 epg *T. trichiura* prevalence at baseline 98%, at end 91% 63% egg reduction, geometric mean 2857 to 1061epg	N=72 Significant reduction in prevalence and intensity of *A. lumbricoides.* No significant change in *T. trichiura* and hookworm. Hookworm prevalence at baseline 79%, at end 89% 90% egg increase, geometric mean 384 to 729 epg *A. lumbricoides* prevalence at baseline 54%, at end 42% 25% egg reduction, geometric mean 284 to 72 epg *T. trichiura* prevalence at baseline 97%, at end 94% 13% egg reduction, geometric mean 2540 to 2220 epg	Significant increase in weight gain comparing albendazole group to placebo. Six months after baseline Mean weight gain albendazole group = 2.1 kg Mean weight gain placebo group = 0.8 kg	Stephenson et al. (1989)
Children in grades I to V from Mvindeni Primary School, Kwale District, Coast Province, Kenya Treated group received 600 mg albendazole at baseline or 600 mg at baseline and 600 mg 3.6 months later Parasitology : Modified Kato technique	Significant reduction in prevalence and intensity rates for Hookworm, *A. lumbricoides* at 3.6 and 8.2 months after treatment for both treated groups. *T. trichiura* prevalence significantly reduced 8.2 months for both treated groups. Prevalence not significantly reduced 3.6 months after treatment for *T. trichiura* for single dose group. Single 600 mg group N=96 Hookworm prevalence at baseline 85%, at end 45% 99% egg reduction, geometric mean 519 to 15 epg *A. lumbricoides* prevalence at baseline 35%, at end 16% 88% egg reduction, geometric mean 33 to 4 epg *T. trichiura* prevalence at baseline 90%, at end 81% 62% egg reduction, geometric mean 973 to 374 epg 600 mg X 2 group N=95 Hookworm prevalence at baseline 86%, at end 23% 99% egg reduction, geometric mean 590 to 3 epg *A. lumbricoides* prevalence at baseline 25%, at end 5% 78% egg reduction, geometric mean 11 to 0.6 epg *T. trichiura* prevalence at baseline 81%, at end 66% 82% egg reduction, geometric mean 424 to 95 epg	N=93 No significant change in prevalence and intensity rates for *T. trichiura* and *A. lumbricoides* 3.6 and 8.2 months after baseline. Significant increase in hookworm intensity 8.2 months after baseline. Hookworm prevalence at baseline 88%, at end 95% 107 % egg increase, geometric mean 602 to 1248 epg *A. lumbricoides* prevalence at baseline 32%, at end 33% 35% egg increase, geometric mean 20 to 27 epg *T. trichiura* prevalence at baseline 92%, at end 95% 19% egg increase, geometric mean 1142 to 1357epg	Significant increase in weight gain for both single and twice treated groups compared to placebo 8.2 months after baseline. Mean weight gain single albendazole group = 3.3k g Mean weight gain double albendazole group = 3.1 kg Mean weight gain placebo group = 2.20 kg	Stephenson et al. (1993b)

Study characteristics	Treated	Placebo	Changes in Weight	Reference
Nursery and standard one classes of Mvindeni primary school, Kwale District, Coast province, Kenya 3 consecutive daily doses of 400 mg albendazole. Parasitology : Modified Kato technique	N=28 Significant reduction in prevalence and intensity rates for hookworm, *T. trichiura* and *A. lumbricoides* nine weeks after treatment. Hookworm prevalence at baseline 93%, at end 0% 100% egg reduction, geometric mean 1229 to 0 epg *A. lumbricoides* prevalence at baseline 32%, at end 4%, 94 % egg reduction, geometric mean 17 to 1 epg *T. trichiura* prevalence at baseline 79%, at end 50% 93% egg reduction, geometric mean 342 to 24 epg	N=28 No significant change in prevalence and intensity rates for hookworm, *T. trichiura* and *A. lumbricoides* nine weeks after treatment Hookworm prevalence at baseline 93%, at end 85% 40% egg reduction, geometric mean 1701 to 1020 epg *A. lumbricoides* prevalence at baseline 26%, at end 19% 54% egg reduction, geometric mean 13 to 6 epg *T. trichiura* prevalence at baseline 89%, at end 85% 32% egg reduction, geometric mean 705 to 481 epg	Significant difference in weight gain nine weeks after treatment Mean weight gain albendazole group 1.0 kg Mean weight gain placebo group 0.3 kg	Adams et al. (1994)
Children attending grade two to five among 14 primary schools in Jamaica 800 mg albendazole given over two consecutive days at baseline, 12 and 24 weeks Parasitology : Kato technique	N=206 Significant reduced prevalence compared to placebo group 32 weeks after first treatment. *A. lumbricoides* prevalence at baseline 42%, at end 7% *T. trichiura* prevalence at baseline 100%, at end 50% 100% > 1200 epg at baseline 5% > 1200 epg at end	N=201 *A. lumbricoides* prevalence at baseline 50%, at end 42% *T. trichiura* prevalence at baseline 100%, at end 92% 100% > 1200 epg at baseline, 57% > 1200 epg at end	No significant difference in body mass index	Simeon et al. (1995)
Children six to eight years of age attending five primary schools in Worchester region South Africa (n=247) 400 mg albendazole given at baseline and four months later. Overall study design included iron supplementation Parasitology : Filtered and filtrate examined	*A. lumbricoides* prevalence reduced significantly at six months then increased significantly five months after the intervention compared to the placebo group. *T. trichiura* decreased but not compared significantly to the placebo. With iron supplementation *A. lumbricoides* prevalence at baseline 12%, at end 11% *T. trichiura* prevalence at baseline 33%, at end 8% Without iron supplementation *A. lumbricoides* prevalence at baseline 28%, at end 28% *T. trichiura* prevalence at baseline 44%, at end 39%	No significant changes With iron supplementation *A. lumbricoides* prevalence at baseline 19%, at end 7% *T. trichiura* prevalence at baseline 25%, at end 16% Without iron supplementation *A. lumbricoides* prevalence at baseline 29%, at end 33% *T. trichiura* prevalence at baseline 61%, at end 27%	No significant differences in weight gain comparing albendazole group only to placebo group only 11 months after intervention. Mean weight gain albendazole group only= 2.32 kg Mean weight gain placebo group = 2.70 kg	Kruger et al. (1996)

Study characteristics	Treated	Placebo	Changes in Weight	Reference
Children in grades one to four attending primary school in Santa de Maria, Guatemala less than 12 years of age. 400 mg at baseline and 12 weeks Parasitology : Modified Kato technique (two smears)	Significant reduction in prevalence and intensity of *A. lumbricoides* 24 weeks after baseline. Significant difference in prevalence but not intensity of *T. trichiura* after 24 weeks compared to placebo. *A. lumbricoides* prevalence at baseline 91%, at end 51% 90% egg reduction, geometric mean 21677 to 2198 epg *T. trichiura* prevalence at baseline 85%, at end 73% 10% egg reduction, geometric mean 832 to 746 epg	No significant changes in prevalence and intensity of *A. lumbricoides* and *T. trichiura* 24 weeks after baseline *A. lumbricoides* prevalence at baseline 92%, at end 87% 22% egg increase, geometric mean 21528 to 26363 epg *T. trichiura* prevalence at baseline 78%, at end 85% 13% egg reduction, geometric mean 659 to 576 epg	No significant differences in weight gain comparing albendazole group to placebo at 12 or 24 weeks. 24 weeks after baseline Mean weight gain albendazole group = 1.82 kg Mean weight gain placebo group = 1.69 kg	Watkins and Pollitt (1996)
Children aged between 2–5 years of a West Javanese village. Overall study design included iron supplementation 400 mg albendazole Parasitology : Modified Kato technique	Iron and Albendazole N=93. Significantly reduced prevalence of *A. lumbricoides* and *T. trichiura* compared to iron only group ten weeks after baseline Hookworm prevalence at baseline 0%, at end 0% *A. lumbricoides* prevalence at baseline 55%, at end 2% *T. trichiura* prevalence at baseline 29%, at end 16%	Iron only N=93 Hookworm prevalence at baseline 0%, at end 1% *A. lumbricoides* prevalence at baseline 55%, at end 47% *T. trichiura* prevalence at baseline 22%, at end 28% Placebo N=93 Hookworm prevalence at baseline 0%, at end 4% *A. lumbricoides* prevalence at baseline 55%, at end 37% *T. trichiura* prevalence at baseline 33%, at end 32%	No significant difference in weight gain between groups 10 weeks after baseline Mean weight gain Iron and albendazole 0.51 kg Mean weight gain Iron group 0.45 kg Mean weight gain placebo group 0.34 kg	Palupi et al. (1997)
Children recruited through 32 health centres in urban slums of Lucknow, North India. 600 mg albendazole six monthly for two years Parasitology: Direct smear examination	N=610 Significant reduced prevalence compared to placebo group after two years *A. lumbricoides* prevalence at baseline 13%, at end 41%	N=451 *A. lumbricoides* prevalence at baseline 11%, at end 55%	No significant weight gain difference after two years Mean weight gain albendazole group 2.63 kg Mean weight gain placebo group 2.68 kg	Awasthi et al. (2000)

Study characteristics	**Treated**	**Placebo**	**Changes in Weight**	**Reference**
Children aged three to five in Agblangandan, southern Benin (n=177). Overall study design included iron supplementation 200 mg albendazole for three consecutive days and again three daily doses one month later. Parasitology : Kato technique	Significant reduction in *A. lumbricoides* prevalence after one month, but not after three months. Non-significant changes at one and three month prevalence rates for *T. trichiura* and hookworm. Significantly lower prevalence at one and three months for *A. lumbricoides* and hookworm compared to placebo. No significant difference in egg counts between baseline and three months. Significant lower difference in egg counts for *A. lumbricoides* and hookworm compared to placebo Hookworm prevalence at baseline 11%, at end 7% 69% egg reduction, geometric mean 369 to 116 epg *A. lumbricoides* prevalence at baseline 31%, at end 26% 74% egg reduction, geometric mean 4292 to 1098 epg *T. trichiura* prevalence at baseline 38%, at end 44% 49% egg reduction, geometric mean 1159 to 587 epg	Significant increases in hookworm prevalence rates. Non-significant changes for *A. lumbricoides* and *T. trichiura* prevalence. Significantly increased egg counts at three months compared to baseline for *A. lumbricoides* and hookworm. Hookworm prevalence at baseline 14%, at end 31% 28% egg increase geometric mean 186 to 239 epg *A. lumbricoides* prevalence at baseline 47%, at end 65% 437% egg increase, geometric mean 2940 to 15816 epg *T. trichiura* prevalence at baseline 59%, at end 65% 35% egg increase, geometric mean 468 to 634 epg	No significant difference in weight gain albendazole only *versus* placebo at three or 10 months post-baseline At three months Mean weight gain albendazole group only= 0.4 kg Mean weight gain placebo group = 0.5 kg	Dossa et al. (2001)

- Percentages obtained from graphical data

4.1.2 Age and gender profiles of STH infections

Hookworm infection shows a differing age prevalence profile compared to *A. lumbricoides* and *T. trichiura* with prevalence continuing to increase with age. Although children are thought to be more indiscriminate about where they defecate, higher hookworm prevalence's in adults is suggested because in some areas adults defecate in areas more suitable for growth of larvae (Chan et al., 1997). Alternatively, higher infection rates in adults have been attributed to adults going to areas outside the village, such as plantations, not normally accessed by children (Bradley and Chandiwana, 1990; Pritchard et al., 1990).

Gender differences have been reported for hookworm infection with a higher prevalence in males as compared to females being reported by Pritchard et al. (1990) and Behnke et al. (2000) but with more women infected than men reported by Gandhi et al., (2001). Other studies have found no difference in prevalence between the sexes (Pritchard et al., 1990; Chongsuvivatwong et al., 1996). Higher hookworm prevalence is often found in boys compared to girls (Brooker et al., 1999; Brooker et al., 2002a; Tomono et al., 2003; Raso et al., 2005; Raso et al., 2006). Differences are probably due to behaviour and exposure rather than immunological factors (Behnke et al., 2000); for instance boys being less likely to wear shoes (Tomono et al., 2003).

The intensity of *A. lumbricoides* and *T. trichiura* tends to reach a maximum in children and then declines or plateaus (Bundy et al., 1987; Bundy, 1988; Gandhi et al., 2001). There are reports of no significant correlation with sex and age for both *A. lumbricoides* (Ellis et al., 2007; Steinmann et al., 2007) and *T. trichiura* infection (Steinmann et al., 2007). In children, Brooker et al. (1999) found no difference in prevalence between the sexes, but in Madagascar, girls had a higher *A. lumbricoides*

worm burden compared to boys. This was suggested to be related to girls remaining in the village during the day, near the source of infection, while boys left the village to perform their duties (Kightlinger et al., 1998).

4.1.3 Environmental factors in STH transmission

Although STH have similar thermal thresholds, the maximum temperature of viable development, *A. lumbricoides* and *T. trichiura* prevalence are low where land surface temperatures are greater than 38 ºC. Hookworm prevalence remains high up to mean land temperatures of 47 ºC possibly due to its ability to move to an optimum soil temperature and its more rapid development. A survey throughout Chad failed to detect *A. lumbricoides* and *T. trichiura* in schoolchildren, possibly due to high soil temperatures (Brooker et al., 2002a; Brooker et al., 2006b). The spatial pattern of hookworm has been associated with elevation, rainfall and land cover with a higher prevalence in western Cote d'Ivoire schools located in tropical rainforest above 400 m possibly due to the thick tree cover preventing the soil becoming too waterlogged for L3 development or preventing faeces being wash away (Raso et al., 2006). In Yunnan province, China, living above 2150 m is a significant risk factor for *A. lumbricoides*. In this study, there was a low prevalence of hookworm suggested to be due to the cool climate (Steinmann et al., 2007). Land surface temperature, vegetation and rainfall are important variables in determining helminth distribution in Cameroon (Brooker et al., 2002b). *A. lumbricoides* infection has shown positive association with vegetation density with a negative association of reinfection with sun exposure of the soil surface (Saathoff et al., 2005).

Models can be developed and probability maps generated to determine areas where treatment can be targeted (Brooker et al., 2002b; Brooker et al., 2006b). GIS/RS would

be a cost effective way for designing and developing monitoring systems by only targeting areas for mass treatment predicted to have a high STH prevalence. The usefulness of such an approach requires the ability to generate accurate models. In western Cote d'Ivoire, targeted intervention based on epidemiological risk rather than spatial factors would be more useful due to the small correlation of hookworm infection with spatial factors in the models examined (Raso et al., 2006).

4.1.4 Risk factors

Most of the hookworm population is harboured by only a small percentage of the human population (Schad and Anderson, 1985) with clustering of heavily infected individuals of STH occurring within households (Forrester et al., 1988; Killewo et al., 1991; Behnke et al., 2000). Heavily infected individuals prior to treatment tend to reacquire heavy infections post-treatment (Schad and Anderson, 1985; Hlaing et al., 1987; Forrester et al., 1990) with poly-helminth infections also aggregating in families (Ellis et al., 2007). Predisposition to reinfection can also be generalised to the household (Forrester et al., 1990). Infection intensity in a household shows predisposition in an individual suggesting a genetic basis. Ecological, social or behavioural factors, such as defecation around the house, may also be important (Schad and Anderson, 1985; Bundy et al., 1987; Forrester et al., 1988; Drake et al., 2000).

The host genetic component of the variation in *A. lumbricoides* worm burden in the Jiral population in Eastern Nepal has been estimated to be between 30% and 50% with 3% to 15% due to shared environmental effects in the Jiral population in Eastern Nepal (Williams-Blangero et al., 1999). Host genetic factors have been estimated to cause 37% of variation of hookworm burden in a Zimbabwean sample (Williams-Blangero et al., 1997).

Individual behaviour, such as contact children have with dirt, may account for differences in intensity (Bundy and Cooper, 1988). Behaviours such as hand washing before meals have been shown to be protective against *A. lumbricoides* (Steinmann et al., 2007), but not necessarily the simple availability of soap in a household or the hygiene status of a child (Kightlinger et al., 1998). Shoe wearing has been shown to have a protective effect in hookworm infection (Chongsuvivatwong et al., 1996; Tomono et al., 2003) but also not shown to be protective although this was possibly due to footwear not being worn outdoors at all times (Behnke et al., 2000).

One interesting cultural behaviour implicated in infection is geophagia, the consumption of soil, mostly practised by women and children, ironically sometimes to relieve gastric irritation due to intestinal parasites (Abrahams and Parsons, 1996). Apart from soil eaten from the ground (Wong et al., 1988), geophagia occurs with dirt from the walls of hut and often from dirt of termite hills and is associated with a higher prevalence and intensity of infection of *A. lumbricoides* and *T. trichiura* (Geissler et al., 1998; Saathoff et al., 2002; Luoba et al., 2005). Termite mounds have infective eggs of *A. lumbricoides* and *T. trichiura* possibly due to the defecation around termite mounds and the transport of infected soil by termites, domestic or wild animals (Geissler et al., 1998). In Zanzibar, despite the practice of geophagia, no association of geophagia with hookworm or *T. trichiura* infection was found. Those who practised it tended to eat 'clean' samples prepared by sun drying, which would be likely to reduce transmission (Young et al., 2007).

It has been known since the 1920s that *Ascaris* infection is more common in some families compared to others in the same locality and the unit of dissemination is the family (Cort et al., 1930). Transmission occurs in both public and household domains. Household transmission can be attributed to people's behaviour (Cairncross et al.,

1996). The household is a major risk factor for *A. lumbricoides* infection, which could reflect socio-economic status of a household and sanitation and hygiene conditions (Ellis et al., 2007) although availability of sanitation facilities between houses as an effect has been disputed as availability does not predict their use (Bundy, 1988). Latrine availability has not been associated with the presence of *Ascaris* (Forrester et al., 1990; Killewo et al., 1991; Kightlinger et al., 1998; Naish et al., 2004; Nguyen et al., 2006) and hookworm infection (Bradley et al., 1993; Chongsuvivatwong et al., 1996). In Vietnam, despite 98% latrine coverage, high STH rates are still experienced possibly due to the use of human faeces for fertiliser (Yajima et al., 2008).

The degree of household clustering has been shown to be more pronounced in communities with covered drains and sewers; therefore, domestic factors such as the size of the floor area become more important (Moraes and Cairncross, 2004; Moraes et al., 2004). Water contact has also been identified as a risk factor, suggesting contamination of water as a source of infection for *A. lumbricoides* and *T. trichiura* (Ellis et al., 2007) with significantly more uninfected children living in housing with plumbing compared to those who used communal sources (Holland et al., 1988).

As reviewed by Brooker et al. (2006b), there are differences in *A. lumbricoides* and *T. trichiura* prevalence rates between similar age and socio-economic groups in urban and rural communities though in no systematic manner. Hookworm is considered to be more often found in rural communities where the environment favours its free living stage (Crompton and Savioli, 1993). A low socio-economic status is associated with STH infection (Holland et al., 1988; Killewo et al., 1991; Carneiro et al., 2002; Raso et al., 2005; Raso et al., 2006). In Cote d'Ivoire, children from poorer households have less access to health services compared to richer households, which may be a factor in their

increased hookworm prevalence (Raso et al., 2005). Agricultural wealth can be a poor predictor of *Ascaris* infection (Kightlinger et al., 1998).

Children living in poorer housing consisting of bamboo or wood with dirt floors are more likely to be infected with *A. lumbricoides, T. trichiura* or hookworm and intestinal protozoa compared to those living in concrete blocks (Holland et al., 1988). Living in a cement house for children in rural Cote d'Ivoire was also found to be protective against STH (Raso et al., 2005). This could be due to children in poorer households being more exposed to contaminated soil. There is a significant relationship between dirt floors and familial infection with heavily infected families with *A. lumbricoides* more likely to have a dirt floor (Forrester et al., 1990). The type of floor in latrines was not associated with *A. lumbricoides* infection in Tanzania (Killewo et al., 1991) nor was there an association between concrete housing and *A. lumbricoides* infection in Southeast Madagascar (Kightlinger et al., 1998). The size of the family or household has both been found as a significant risk for reinfection (Hlaing et al., 1987; Carneiro et al., 2002) as well as not being a significant factor (Forrester et al., 1990; Kightlinger et al., 1998). The interaction between large numbers of young children in a household, dirt floors and their inability to be clean and the propensity for children to engage in geophagia could be a factor in acquiring infection within households.

Improving housing conditions by cementing floors is an important and inexpensive measure to reduce transmission (Raso et al., 2005). Kightlinger et al. (1998) make the important point that where burdens and intensities of *A. lumhricoides* are high, there are few practical measures that an individual household can take to protect its children. However, a community worm control program can reduce prevalence.

4.1.5 STH interventions

Even though the cost of drug provision is negligible, it is still unlikely that the health budgets of developing countries can afford STH programs on their own and they will need to integrate with other programs (Crompton and Nesheim, 2002). Helminth control should easily be integrated into other health care programs (Savioli et al., 1992) as the side effects of anthelmintic drugs are usually mild and transient allowing non-health staff to administer them (Savioli et al., 2004). The major problem facing helminth control is the low priority given to the problem compared to other health interventions (Horton, 2003)

The required intervention is based on prevalence and intensity, as shown in Table 4–3 and Table 4–4 (WHO, 2002). Any intervention also requires sanitation and health education.

Table 4–3 Classes of intensity for STH (WHO, 2002)

Helminth	Light intensity	Moderate intensity	High intensity
A. lumbricoides	1–4999	5000–49999	>49999
Hookworm	1–1999	2000–3999	>3999
T. trichiura	1–999	1000–9999	>9999

Table 4–4 Classes of intensity and treatment strategy for STH (WHO, 2002)

Category	Result of School Study	Treatment Strategies
High prevalence or high intensity	≥ 70% prevalence of any STH infection ≥ 10% moderate to heavy STH infections	Targeted treatment of school age children two to three times per year Systematic treatment of preschool children and women of childbearing age in mother and child health programs
Moderate prevalence but low intensity	≥ 50% prevalence but less than 70% of any STH infection < 10 % moderate to heavy infections	Targeted treatment of school age children once per year Systematic treatment of preschool children and women of childbearing age in mother and child health programs
Low prevalence and low intensity	< 50% prevalence of any STH infection < 10% moderate to heavy infections	Selective treatment of preschool children, schoolchildren and women of childbearing age

Table 4–5 Community treatment strategies (WHO, 2006)

Category	Prevalence of any STH infection among school-aged children	Treatment Strategies
High-risk community	≥ 50% prevalence of any STH infection	Treat all enrolled and unenrolled school age children twice per year and if resources available three times per year Treat preschool children, women of childbearing age, pregnant women in second and third trimesters and lactating women Treat adults at high-risk in certain occupations
Low-risk community	≥ 20% prevalence but less than 50% of any STH infection	Treat all enrolled and unenrolled school age children once per year Treat preschool children, women of childbearing age, pregnant women in second and third trimesters and lactating women Treat adults at high-risk in certain occupations

The levels of intensity of infection were decided by a 1987 WHO Expert Committee (WHO, 1987). Intensities for hookworm infection were developed examining faecal loss of haemoglobin in hookworm infection by Stoltzfus et al. (1996) and suggested as category examples only (WHO, 1998). An updated version (WHO,

2006) ignores intensity and relies only on prevalence as shown in Table 4–5. Where prevalence is less than 20%, treatment of known infected individuals is offered. The aim of these interventions is to reduce morbidity, including diarrhoea, abdominal pain, general malaise and weakness, which may affect working and learning capacities, impaired physical growth and anaemia caused by hookworm.

The rationale behind the differentiation between high-risk and low-risk communities and different treatment strategies is not given in the manual. In personal communication with WHO, it was indicated that the rational was high prevalence settings will also have high intensity. Where hookworm has high prevalence in adults, modelling suggests that targeting children alone may be insufficient (Chan et al., 1997). Surveying only children can give a distorted community profile of the actual STH infections present. For instance, in Nukufetau, only 2.2% of those aged less than 20 years were hookworm positive compared to 18.6% of those over 20 years of age (Speare et al., 2006). Information on the community profile could be helpful in determining the likely reinfection rate and therefore influence the frequency of anthelmintic administration. Since STH infections aggregate in families, it would be valuable to treat the whole family when a child is identified with STH.

The guidelines no longer require intensity to be calculated. Areas with low prevalence, but containing individuals with high intensity, need only receive one dose of anthelmintic. The assumption appears that areas with higher prevalence have higher intensity and therefore require more doses. Although this is likely, no evidence was found in the literature why schoolchildren in lower prevalence sites should receive only one dose. The Cochrane review showed one dose to have some beneficial effect on weight gain while a combined analysis of studies using multiple doses showed no effect (Taylor-Robinson et al., 2007). Another Cochrane review determined that a single dose

anthelmintic for pregnant women during the second trimester had no effect on anaemia in the third trimester, low birth weight, perinatal mortality and preterm birth (Haider et al., 2009). There are at least two reasons why evidence for a beneficial effect for anthelmintic treatment is slight. The average STH infection causes no real health problem in individuals and reduction in STH burden has no measurable effect. In this case, mass administration of anthelmintics has no benefit. Alternatively, current treatment regimes and reinfection means the remaining STH burden in the host is still causing a negative effect and the benefits will only be realised once STH are eliminated from the host. In this case, current guidelines are inadequate and far more needs to be done in terms of treatment for the whole community to prevent reinfection, treatment of individuals until STH are no longer detectable, upgrading sanitation within a community and health education. The current WHO guidelines appear to be a middle way, possibly benefitting the few people with a very heavy burden but with low probability of benefiting the majority. More research is needed to determine exactly what is being achieved with current STH programs.

4.1.6 Monitoring and evaluation by parasitological methods

The choice of strategy for mass treatment is critically dependent on sampling effort (Booth et al., 2003). Gyorkos (2003, page 278) states that 'the importance of conducting a baseline study cannot be over-emphasised. Without baseline estimates of parasite prevalence, intensity and morbidity, it is not possible to ascertain the impact of a control program.' Baseline surveys and monitoring involves faecal egg counts, as there is a strong correlation between hookworm egg burden and worm burden (Pritchard et al., 1990). Prevalence is not a sensitive indicator for the impact of mass chemotherapy, as prevalence may not fall significantly after mass treatment (Hlaing et al., 1987; Hall et al., 1992). WHO states that no significant reduction in overall prevalence may be seen,

but the reduction in heavily infected cases is what is relevant. The rationale to this is that most of the morbidity is accounted for by the 10–15% who are most heavily infected (WHO, 2002).

Kato and Miura (1954) described in Japanese the thick smear for the examination of faecal samples. The first English translation of the method and an evaluation was done by Komiya and Kobayashi (1966). The technique examines faecal material pressed between a glass slide and cellophane soaked in glycerine-malachite solution. The thin smear uses about 10 mg of faeces compared to about 60–75 mg for the Kato technique. Therefore, the sensitivity is improved although Komiya and Kobyashi (1966) found no difference in sensitivity in detecting *Ascaris* infection between the two techniques since they found that the thin smear was able to detect eggs from one female worm. Compared to the direct smear or Stoll method, it has shown an improved sensitivity (Sehgal et al., 1977; Vinayak et al., 1978).

Modifications of the technique soon followed. Martin and Beaver (1968) introduced removing coarse particles by straining the faeces before making the smear. Eggs of hookworm and *T. trichiura* were uniformly spread throughout the smear and it reliably detected 100 hookworm eggs per gram or infection with only one or two female worms. Katz (1972) improved the thick smear method by introducing a disposable card with a 6 mm hole in the centre in which a statistically uniform 43.5 mg of strained faeces could be added. However, the weight of faeces added to the slide can vary greatly. In a template designed for 23.0 mg of faeces, 95% of samples were between 12.0 and 34.0 mg (Engels et al., 1997). The Kato-Katz is based on the assumption that the density of faeces is 1.0 as templates use a defined volume not weight (Kongs et al., 2001).

Other modifications have included substituting the cellulose acetate with a thick glass cover slip (Teesdale and Amin, 1976b), though without a clearing stage this can make it more difficult to examine helminth eggs and can result in reduced sensitivity (Engels et al., 1996). Odongo-Aginya et al. (1995) replaced the malachite green in 50% glycerol with 7.5% nigrosine and 5% eosin in 10% formaldehyde and found similar results to the standard Kato-Katz, but with no significant loss of hookworm eggs, which still could be visualised months later. Teesdale and Amin (1976a) used 25 mg faeces emulsified into 50 µl of glycerine in water on a thick cover slip and found similar *S. mansoni* egg counts with the Kato technique. Peters et al. (1980) reduced the size of the template to 20 mg to introduce a quick version of the test to count *S. mansoni* eggs as clearing only took 15 minutes.

Generally, the Kato-Katz technique compares favourably in sensitivity with other techniques. It shows better sensitivity than direct smear examination (Engels et al., 1996) and has recorded significantly higher egg count for helminths compared to other methods (Sehgal et al., 1977; Goodman et al., 2007; Utzinger et al., 2008) although Santos et al. (2005) found the direct smear more reliable for *T. trichiura* infection. The Kato-Katz technique has higher sensitivity for hookworm infections compared to the formol ether method (Dacombe et al., 2007). However, compared to the sedimentation technique, the Kato-Katz detects less unfertilised *Ascaris* ova (Goodman et al., 2007).

Although comparing favourably in sensitivity to other technique its use in epidemiological settings has been queried (Kongs et al., 2001; Berhe et al., 2004; Utzinger et al., 2008). When compared to multiple (25) specimens, a single examination using the Kato-Katz technique for hookworm reveals a sensitivity of between 18 and 43% (Utzinger et al., 2002). This was confirmed by Booth et al. (2003) who determined a sensitivity for the technique of only 8% for hookworm-only infections and 18% for

dual hookworm *S. mansoni* infections. They also showed a sixfold increase in prevalence from the use of the first specimen to the twenty fifth specimen (five samples from five separate specimens) with modelling showing that the true prevalence was still likely to be 22% higher than that determined by 25 specimens. Similarly, Berhe et al. (2004) showed 25%, 47% and 65% of *A.* lumbricoides, hookworm and *T. trichiura* infections respectively were missed using a single Kato-Katz compared to five Kato-Katz slides from a single specimen. When compared to both the formol-ether concentration method and Kato-Katz combined, Raso et al. (2006) found only a 78% sensitivity for the Kato-Katz and 59% for the formol-ether method for hookworm while Utzinger et al. (2008) reported 68% for duplicate Kato-Katz from the same stool sample and 38% for a single examination using the formol-ether method for hookworm infection. Duplicate Kato-Katzs was also shown to have a higher sensitivity than single examinations of both the formol-ethyl acetate and a modified version for *Ascaris*, *Trichurus* and hookworm. Sensitivities ranged from 77% to 97%, compared to combined results as a true measure of prevalence using the Kato-Katz, formol ethyl acetate, modified formol-ethyl acetate, modified Wisconsin and simple gravity sedimentation methods (Goodman et al., 2007). In a low intensity setting using the Kato-Katz technique, Knopp et al. (2008a) calculated that eight separate specimens were needed to have a less than 1% false negative rate and that diagnostic methods with high sensitivity and low technical demands are needed.

The Kato-Katz method is simple, low cost, easy to learn, requires no special materials except the slides and cellulose cover slips, involves no hazardous chemicals, rapid and is suitable for large-scale epidemiological studies. Since it uses fresh faeces and contamination is common the Kato-Katz has a potential for high risk of infection of the laboratory worker and it is not suitable for cysts, larvae, small fluke or thin-shelled

nematode eggs such as hookworm (Kongs et al., 2001; Santos et al., 2005). Hookworm eggs disappear rapidly with the optimum time for reading being between 30 minutes and one hour (Martin and Beaver, 1968) although even at one hour, sensitivity can be reduced (Zamen and Cheong, 1967).

Problems can be experienced in hot arid areas when the Kato-Katz slides can quickly dry making them unsuitable to be read (Talla et al., 1992). When using the Kato-Katz technique for counting *S. mansoni* eggs, there is large variations between counts of specimens taken over different days, but this is less than the variation between observers (Garcia and Shimizu, 1981; Kongs et al., 2001). Liquid faecal specimens can be difficult to apply to the Kato-Katz method (Talla et al., 1992) with the sample escaping the boundaries of the coverslip. The consistency of the stool appears to influence the count with higher counts of *S. mansoni* eggs observed with dryer stools (Teesdale et al., 1985) although there was no significant difference in odds ratios of infection with the three helminths of diarrhetic stool compared to normal stool using a combination of methods (Goodman et al., 2007). Even the quick Kato-Katz method is considered tedious (Engels et al., 1996) and the technique is not routinely used in developing countries where the formol-ether concentration method is the method of choice (Kongs et al., 2001).

The formol-ether concentration method (Ritchie, 1948) concentrates cysts and eggs about 20 or 30 fold without distortion (Ridley and Hawgood, 1956). Diethyl ether is potentially hazardous to laboratory staff being both explosive and potentially toxic and shows concentration of some faecal material (Truant et al., 1981; Methanitikorn et al., 2003) and substitutes have been investigated. The formalin–ethyl acetate method (Young et al., 1979) shows similar or better recovery for helminth eggs, cysts and larvae with no distortion of morphology (Young et al., 1979; Erdman, 1981; Garcia and

Shimizu, 1981; Truant et al., 1981) although the plug of debris can be sometimes difficult to remove and bubbles of undissolved ethyl-acetate may obscure the microscopic field (Erdman, 1981; Methanitikorn et al., 2003). Other substitutes for the diethyl ether component that appear suitable for concentrating helminths include tween (Methanitikorn et al., 2003) and acetone (Parija et al., 2003). The sensitivity is also increased by using multiple specimens (Marti and Koella, 1993). Although formalin reduces infectivity, delays in processing and the subsequence decrease in sensitivity may not confer any advantage (Dacombe et al., 2007).

The sensitivity of the method to detect true positive cases is determined by the intensity of infection and hence the density of eggs in the sample, the volume or mass of faeces examined and the intrinsic loss rate of the method for example the disintegration of hookworm eggs in the Kato-Katz (Feldmeier and Poggensee, 1993). As intensity reduces following treatment this will mean reduced sensitivity of the test and overestimation of the cure rate by the drug (Utzinger et al., 2002). Of the studies detailed in Table 4–2, only one study indicated two smears taken from one sample were examined while the others appear to have used only one sample and one smear. The true difference in the prevalence between the treated and control groups is likely to be smaller than that which was reported.

4.1.7 STH in Timor-Leste

The aim of this research was to determine the baseline prevalence of STH in Timor-Leste monitor the effects of the anthelmintic used in the filariasis elimination program.

A. lumbricoides, *T. trichuria* and hookworm are present in Timor-Leste (Fraga de Azevedo et al., 1956). As part of the filariasis elimination program, albendazole is given annually, which will also have an effect on these STH. Children between one and 16

years of age are given albendazole twice yearly and pyrantel pamoate twice yearly to those under the age of one.

4.1.8 Conditions influenced by STH in Timor-Leste

Data extracted from the 2003 Health and Demographic survey in Timor-Leste (Ministry of Health and National Statistics Office et al., 2004) are shown in Table 4–6 and Table 4–7 and show the high prevalence of anaemia and poor growth in children. There are multiple causes of anaemia (Recht, 2009) and poor growth in children (Walker et al., 2007), but STH may be a cause.

Table 4–6 Prevalence of conditions in Timor-Leste in children under five years of age (Ministry of Health and National Statistics Office et al., 2004)

	Urban		Rural East		Rural Central		Rural West	
	< -2 Z	< -3 Z	< -2 Z	< -3 Z	< -2 Z	< -3 Z	< -2 Z	< -3 Z
Underweight (low weight for age)	41.9	12.5	43.7	12.1	44.8	15.6	55.9	20.7
Stunting (small length for age)	42.7	21.0	51.3	30.0	50.5	29.2	53.4	33.2
Wasting (low weight for length)	13.6	2.1	9.0	2.3	11.8	2.6	16.7	5.0

Table 4–7 Prevalence of anaemia among children and women in Timor-Leste (Ministry of Health and National Statistics Office et al., 2004)

	Urban			Rural East			Rural Central			Rural West		
	<110 (children) <120 g/l (women)	< 90 g/l	< 70 g/l	<110 (children) < 120 g/l (women)	< 90 g/l	< 70 g/k	<110 (children) < 120 g/l (women)	< 90 g/l	< 70 g/l\	<110 (children) < 120 g/l (women)	< 90 g/l	< 70 g/l
Children < 5 years	30.1	5.8	0.7	35.9	9.6	1.4	27.4	3.2	0.6	35.4	7.5	0.6
Non-Pregnant Women	33.1	5.6	1.0	35.4	9.3	1.8	28.9	2.5	0.5	25.7	3.3	0.4
Pregnant Women	44.3	8.0	0.0	49.2	12.4	3.4	21.3	1.6	0.0	28.3	4.4	0.9

4.2 Method

4.2.1 Study area

People living in the districts of Lautem, the furthest Eastern District, and Cova-Lima in the Southwest were chosen for past reports of clinical filariasis. The villages of Suai Loro in Cova-Lima and Buihomau and Sika in the Lautem District were chosen on recommendation by the local District Health Administrators. The study population were residents of the village aged five years and older

Upon arrival in the village, WHO health staff discussed with village leaders the requirements of the study and obtained permission for the survey to be undertaken. This was then communicated to the residents of the villages. Several residents of each village were employed and trained to assist in conducting the survey.

4.2.2 Questionnaire

The study was conducted in February 2006 and consisted of two parts: a questionnaire and a faecal parasitological survey. Several residents in each village with good literacy skills were employed to assist in the survey. They were instructed to go to every household in the village, fill out the questionnaire for each household and deliver small faecal containers for each member of the household. Each house was identified by a unique number and this was written on each householder's questionnaire so members of a household could be identified. Additionally, each person was assigned a unique identifier that was attached to the questionnaire.

The questionnaire, in the East Timorese language of Tetum, asked questions relating to those residing in the household and to the household itself. This

questionnaire was designed to be very short and simple and interviews were conducted by trained personnel within the household.

Questions (Appendix 3) asked of the individuals were name, gender, age, the relationship of the individual to the head of the family, length of time residing in the village, whether anthelmintics had been taken and if so the name of the medication and the date taken and the individual's ability to write. Questions for the household included: Did the household own a buffalo, pig, goat or chicken? What materials were used in the construction of the house? Did the household use a public toilet or use the field? Did they have access to piped water or use water from a well?

4.2.3 Parasitology field procedures

The initial study design included lymphatic filariasis testing by antigen testing and night blood smears as well as examination of faeces for soil-transmitted helminths. The unique identifier was attached to the faecal container including the person's name and age. Participants were instructed to place a piece of faeces into the container and have the containers delivered to a central location within the village. Sodium-acetic acid-formalin (SAF) was added and the sample shaken. Samples that were collected overnight had SAF added to them the next morning. Samples were transported back to James Cook University, Townsville, Australia where they were then examined for STH and later for other parasites. Laboratory facilities in the small medical clinics with their monocular light microscopes were ill suited for performing the Kato-Katz technique on large numbers of faecal samples.

4.2.4 Parasitology laboratory procedures

A portion of the faecal sample was emulsified into 10 ml of normal saline in a weighed tube and then filtered through two layers of gauze into another tube. This was centrifuged for 900 g for one minute and decanted. Five millilitres of 10% formalin was added and allowed to stand for five minutes. Three millilitres of ethyl acetate was added and the suspension shaken vigorously. This was centrifuged for one and half minutes at 900 g and then decanted. The deposit was then examined for STH.

Initially, only prevalence of STH was to be determined, but after analysing the specimens for STH, it was decided it may be informative to determine the intensity of infection. Samples identified as having hookworm and that had a sufficient quantity of faecal material were concentrated again using the same method. The amount of faeces that was present before the addition of formalin and ethyl-acetate was recorded. After concentration the volume was adjusted to 200 µl with SAF and 20 µl microlitres of suspended deposit was added to a glass slide and the number of eggs was then counted. The number of eggs per gram of filtered faecal material was then calculated using the following formula: egg count x 10/weight of filtered faecal material.

The faecal concentrate was examined for the presence of protozoan cysts. One drop of D'Antoni's iodine solution was added to one drop of concentrate and both were examined by microscopy. A smear was prepared from the concentrate and air-dried. It was then flooded with Kinyoun's carbol fuchsin, gently heated and left for five minutes. The slide was rinsed thoroughly with water. It was then decolourised with 1% sulphuric acid for two minutes then rinsed with water. The slide was then counterstained with methylene blue for one minute, rinsed with water and air-dried. The slide was then examined for *Cryptosporidium* and *Cyclospora* species.

4.2.5 Analysis

All data was entered in Microsoft Excel (2003) and statistical analysis was performed with SPSS version 13 (SPSS Inc. Chicago, IL). Only participants who had completed a questionnaire and had provided a faecal sample were included in the final analysis. Participants were subdivided into five-year age ranges. Infections with hookworm were stratified into light, moderate or heavy infections (see Table 4–3).

4.3 Results

4.3.1 Basic demographic profile

Information was collected on 686 residents from the three villages. The median age was 18 years (range: 1–95 y) with 48.8% male. Figure 4–1 shows that 466 faecal samples were collected and the additional laboratory tests performed.

Figure 4–1 Study characteristics

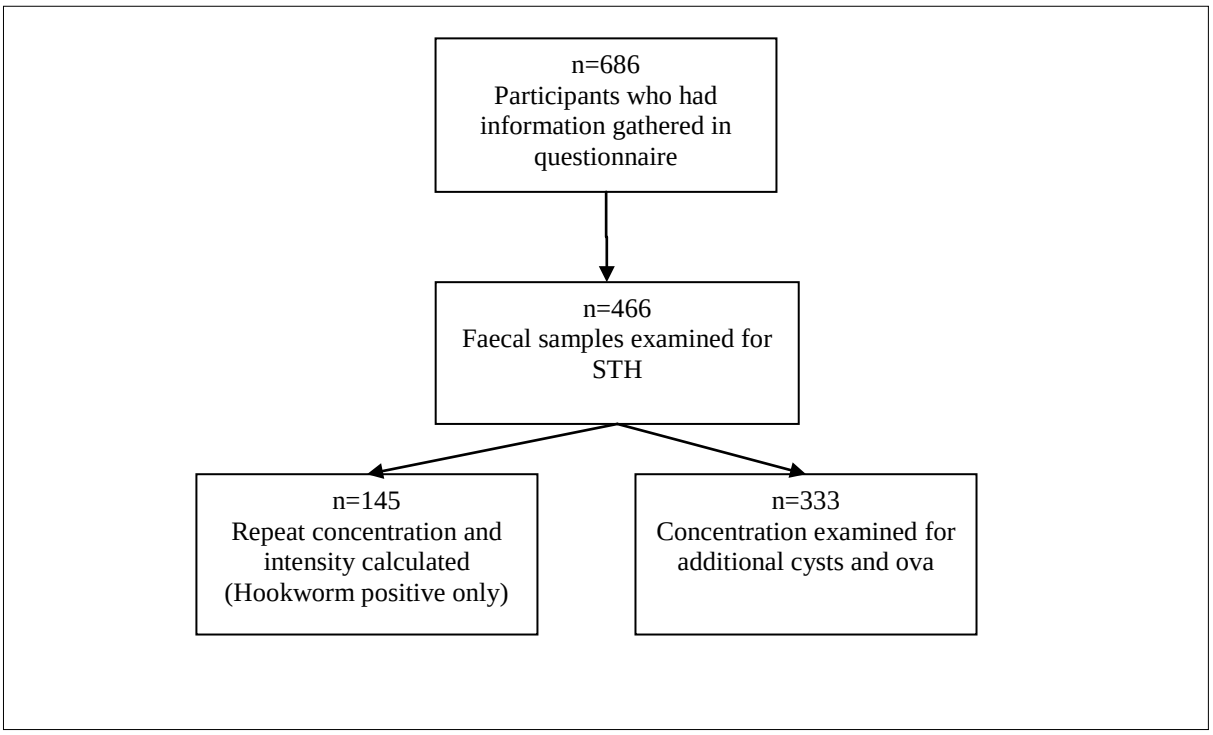
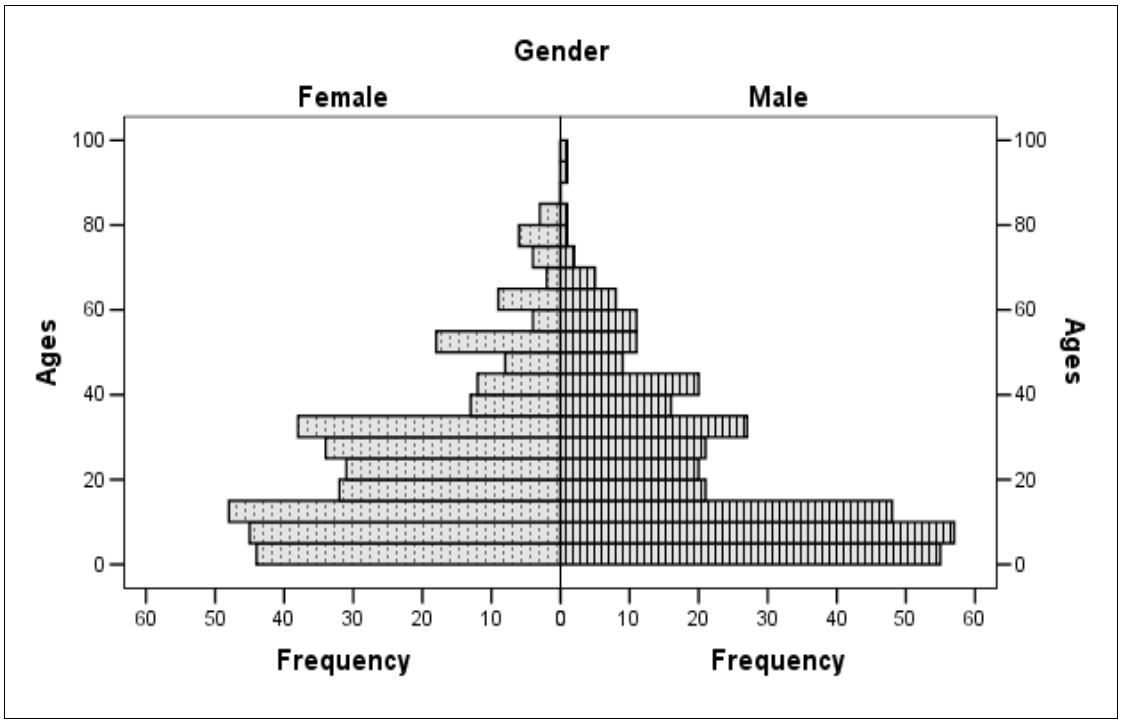


Table 4–8 shows the number of responses from the three villages. There was no difference in the gender structure between villages (χ^2 = 1.10, df=14, p=0.58). The demographic profile as shown in Figure 4–1 shows the age and gender structure of the total population. There is a sudden drop off in frequency of males between the ages of 20–35 years of age.

Table 4–8 Age and gender by village

	Buihomau		**Sika**		**Suai Loro**		
Age range	Male	Female	Male	Female	Male	Female	**Total**
0–4	10	14	21	9	24	21	99
5–9	15	18	16	8	26	19	102
10–14	18	9	15	20	15	19	96
15–19	2	8	8	11	11	13	53
20–24	1	5	3	7	16	19	51
25–29	5	8	4	11	12	15	55
30–34	6	11	7	9	14	18	65
35–39	6	4	2	1	8	8	29
40–44	2	1	5	4	13	7	32
45–49	2	3	3	2	4	3	17
50–54	4	4	3	4	4	10	29
55–59	1	0	2	0	8	4	15
60–64	1	2	4	1	3	6	17
65–69	1	1	1	0	3	1	7
>=70	1	1	1	3	4	9	19
Total	**75**	**89**	**95**	**90**	**165**	**172**	**686**

Figure 4–2 Demographic profile of responders to questionnaire



Based on the survey 640 individuals of the 686 could be allocated to 144 households throughout the three villages. Forty-six individuals could not be attributed to a household in Sika where a household identifying number was not recorded by one team of surveyors. The information collected on these 46 individuals was removed from further analysis. On average, a household contained 4.44 members with a range from 1 to 11 members. Table 4–9 details livestock of the household. Pig ownership was common among the three villages. Household data was collected on the whether a latrine was used. Latrine availability for a household was 19.1% (42) in Buihomau, 90.0% (10) in Sika and 17.7% (62) in Suai Loro. Concrete was used in some part of the construction in 21% of houses in Buihomau, 22% houses in Suai Loro and no houses in Sika.

Table 4–9 Percentage of household livestock ownership (%)

Livestock	Buihomau (number of households=41)	Sika (number of households=31)	Suai Loro (number of households=70)
Buffalo	14.0	27.6	17.7
Pig	97.7	93.1	86.8
Goat	76.7	90.0	2.9
Chicken	95.4	93.1	75.0

There was no association between age range (χ^2 = 14.37, df=14, p=0.42) or gender (p=0.09) and supplying a sample for faecal analysis. There was a significant difference (χ^2 = 16.33, df=2, p<0.01), with 79.5% of Sika residents supplying faecal samples compared to 66.5% and 62.3% for Buihomau and Suai Loro respectively, between the three villages.

Table 4–10 Characteristics of sub-population who had faecal samples examined

	%
Gender	
Male (351)	64.8
Female (335)	70.9
Age Range (years)	
0–4 (99)	65.7
5–9 (102)	76.6
10–14 (96)	69.8
15–19 (53)	52.8
20–24 (51)	60.8
25–29 (55)	72.7
30–34 (65)	70.8
35–39 (29)	65.5
40–44 (32)	62.5
45–49 (17)	82.4
50–54 (29)	69.0
55–59 (15)	73.3
60–64 (17)	64.7
65–69 (7)	57.1
≥ 70 (19)	63.2
Village	
Buihomau (164)	66.5
Sika (185)	79.5
Suai Loro (337)	62.3
Total (686)	**67.9**

4.3.2 Parasite examination for hookworm, *A. lumbricoides* and *T. trichiura*

The overall prevalence of hookworm, *A. lumbricoides* and *T. trichiura* was 32.8%, 1.3% and 2.4% respectively. There were only six cases of *A. lumbricoides* detected, three each in the villages of Buihomau and Sika, three of whom were female and three whom were male. Hookworm prevalence was not significant by gender (p=0.56) although in each village a greater percentage of females were infected than males. Suai Loro, in the west, had a lower prevalence of hookworm but there was no significant difference between the three villages (χ^2 = 4.80, df=2, p=0.09). There were seven cases of *T. trichiura,* five of whom were female and two male. Five cases were in Suai Loro and two cases in Sika. The seven cases were spread among the age ranges with two cases aged between five to nine years, two cases in the 10–14 age ranges and one each in 20–24, 30–34 and 35–39 age ranges.

Table 4–11 Prevalence of hookworm, *A. lumbricoides*, *T. trichiura* and combined STH

	Hookworm (%)				***A. lumbricoides* (%)**				***T. trichiura* (%)**				**Combined STH (%)**			
Village	Total (95%. CI)	Male	Female	p	Total	Male	Female	p	Total	Male	Female	p	Total (95%. CI)	Male	Female	p
Buihomau (109)	37.6 (28.6–46.6)	32.7	42.7	0.43	2.8 (0.0–5.9)	2.0	3.3	1.00	0.0	0.0	0.0		38.5 (29.4–47.7)	34.6	41.7	0.29
Sika (147)	40.1 (32.2–48.0)	39.2	41.2	0.87	2.1 (0.0–4.4)	2.5	1.5	1.00	1.4 (0.0–3.3)	1.3	1.5	1.00	42.9 (34.9–50.9)	41.8	44.1	0.45
Suai Loro (210)	29.5 (23.4–35.6)	28.1	30.6	0.76	0.0	0.0	0.0		2.4 (0.3–4.5)	1.1	3.3	0.40	30.9 (24.7–37.2)	29.2	32.2	0.38
Total (466)	34.8 (30.5–39.1)	33.2	36.1	0.56	1.3 (0.3–2.3)	0.9	1.6	1.00	1.5 (0.4–2.6)	0.9	2.0	0.46	36.5 (32.1–40.9)	35.0	37.8	0.30

As shown in Figure 4–3, the prevalence of hookworm increased steadily with age until after the 20–24 age group. The highest prevalence was reported in the ≥ 70 age group of 83%. Those between 45–49 and 60–64 years also showed a high prevalence comparative to the other age ranges although no one in the 60–65 age group (n=7) had hookworm detected. The difference between age ranges was significant ($\chi^2 = 74.1$, df=14, $p<0.001$). There was also a difference in hookworm prevalence ($p<0.001$) in those under 20 years of age (26.6%) and those 20 years and older (44.4%). Of those under 20 years of age, 32.5% of girls were infected while only 20.9% of boys were. This was significantly different ($p=0.046$) whereby there was a non-significant difference between the genders over the age of 20 ($p=0.12$).

Figure 4–3 Prevalence of hookworm infection by age

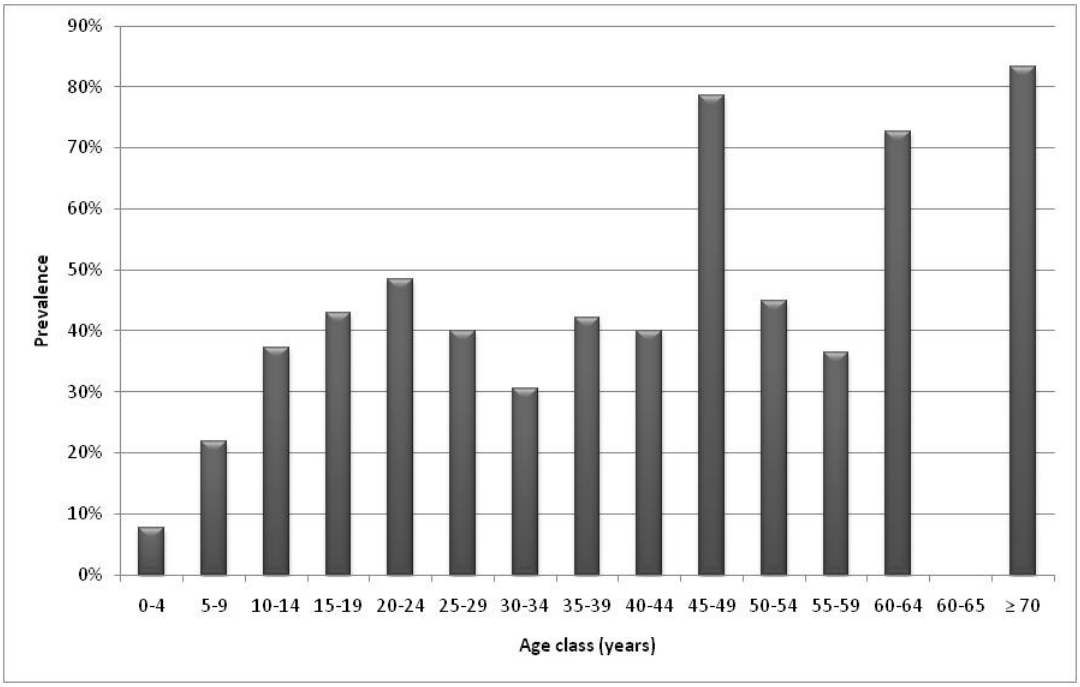


Table 4–12 shows the intensity of hookworm infection. There were only two heavy infections, both found in Sika, and two moderate infections one on Sika and the other Susi Loro.

Table 4–12 Intensity of hookworm infection based on WHO criteria (2002)

Village	Light (%)	Moderate (%)	High (%)
Buihomau (39)	100	0.0	0.0
Sika (58)	94.8	1.7	3.5
Suai Loro (45)	97.8	2.2	0.0
Gender	**Light**	**Moderate**	**High**
Female (80)	100	0.0	0.0
Male (62)	93.6	3.2	3.2
Age range	**Light**	**Moderate**	**High**
< 20 years (60)	98.1	1.9	0.0
≥ 20 years (82)	96.6	2.3	1.1
Total (142)	**97.2**	**1.4**	**1.4**

The overall prevalence for school-aged children of those in the age range from 5–19 years of age was 31. 2% with 93.8%% light infections and 6.2% with a moderate infection. The other moderate infection and two heavy infections occurred in the 60–64 year old age range.

4.3.3 Parasitology examination for other cysts, eggs and larvae

The gender (p=0.59) and age group characteristic of subjects who had specimens (χ^2=16.47, df=14, p=0.29) examined for additional cysts and eggs were not different from those that were examined and all those who were surveyed. There was a difference (χ^2 = 29.8, df=2, p<0.01) between the number of samples examined between the three villages with 62.8% of Buihomau residents participating in the survey faecal concentrates examined compared to 50.3% and 40.7% for Sika and Suai Loro.

Table 4–13 shows the prevalence of other cysts and eggs at the three sites and separated by gender. There was a difference between the three villages for *E. coli.* (χ^2=6.50, df=2, p=0.04), *Entamoeba polecki* (χ^2=8.11, df=2, p=0.02) and *Entamoeba histolytica/dispar* (χ^2=22.68, df=2, p<0.01). Suai Loro had higher prevalence's of 81.8%, 25.5% and 20.4% compared to 67.9%, 17.5% and 2.9% in Buihomau and 78.5%, 10.8% and 5.4% in Sika. There was no significant difference between the

villages with infections of *Blastocystis hominis, Giardia lamblia* and *Endolimax nana.* There was no difference between genders or between those over or under 20 years of age for infection of *E. coli, E. histolytica/dispar, E. polecki, B. hominis, G. lamblia, E. nana* or *Iodamoeba butschlii*.

Of possible serious importance, there were eight samples that contained eggs of *Taenia* species (see Table 4–14). These tended to predominate in the younger age groups with six of the eight cases younger than 20 years of age.

Table 4–13 Prevalences of additional cysts and eggs in Timor-Leste

	Buihomau (103)	Sika (93)	Suai Loro (137)	Male (159)	Female (174)	<20 y (167)	≥20 y (166)	Total % , 95% CI (333)
Entamoeba coli	68.0	78.5	81.8	76.7	76.4	80.8	72.3	76.6, CI:72.0–81.1
Entamoeba polecki	17.5	10.8	25.5	18.2	19.5	15.0	22.9	18.9, CI:14.7–23.1
Entamoeba histolytica/dispar	2.9	5.4	20.4	11.3	10.3	7.8	13.9	10.8, CI:7.5–14.2
Blastocystis hominis	46.6	58.1	54.7	52.2	54.0	55.7	50.6	53.2, CI:47.8–58.5
Giardia lamblia	4.9	8.6	5.8	6.3	6.3	4.2	8.4	6.3, CI:3.7–8.9
Endolimax nana	9.7	10.8	10.2	10.1	10.3	11.4	9.0	10.2, CI:7–13.5
Dientamoeba fragilis	0.0	1.1	0.7	1.3	0.0	0.6	0.6	0.6, CI:0–1.4
Iodamoeba butschlii	2.9	5.4	5.8	5.7	4.0	3.6	6.0	4.8, CI:2.5–7.1
Taenia spp.	1.0	5.4	1.5	1.9	2.9	3.6	1.2	2.4, CI:0.8–4.0
Chilomastix mesnili	1.0	0.0	0.7	0.6	0.6	0.6	0.6	0.6, CI:0–1.4
Fasciolopsis buski	1.0	3.2	0.7	1.9	1.1	0.6	3.0	1.5, CI:0.2–2.8
Hymenolepis diminuta	0.0	4.3	0.7	1.9	1.1	3.0	0.0	1.4, CI:0.0–1.9
Rodentolepis nana	1.9	0.0	0.0	0.0	1.1	1.2	0.0	0.6, CI:0.0–1.4
Cryptosporidium parvum	1.9	0.0	0.0	1.3	0.0	1.2	0.0	0.6, CI:0.0–1.4
Retortamonas intestinalis	1.0	0.0	0.0	0.0	0.6	0.0	0.6	0.3, CI: 0.0–0.9

Figure 4–4 Prevalence of *E. coli* infection by age

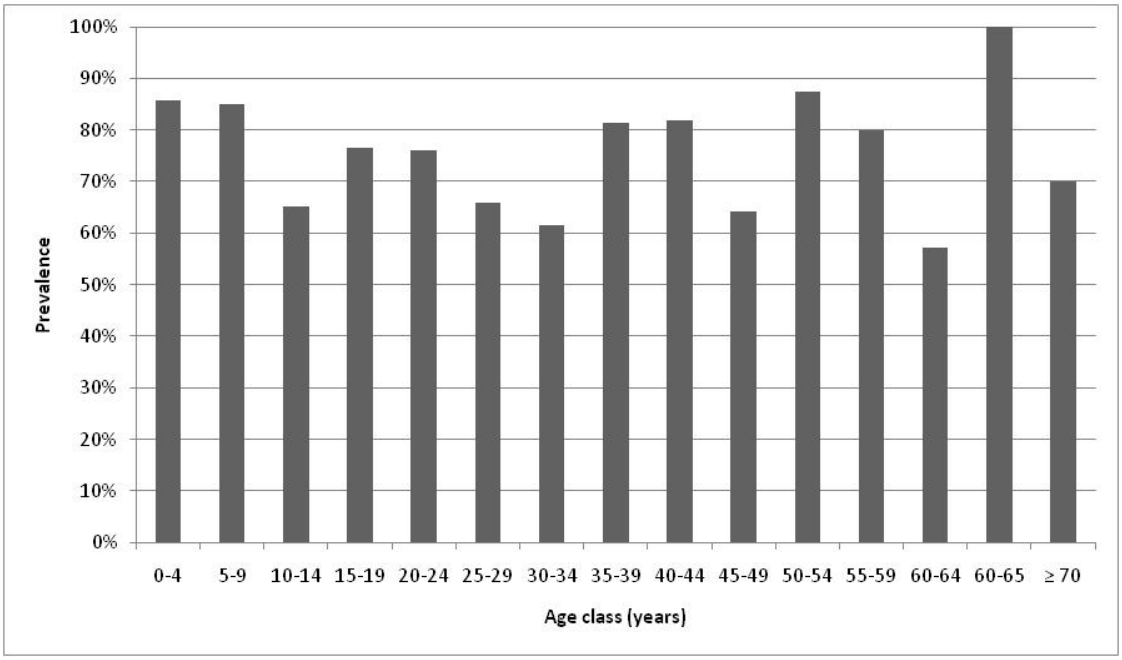


Table 4–14 Demographics of *Taenia* infection

Village	Gender	Age
Buihomau	Female	11
Sika	Male	7
	Male	10
	Male	34
	Female	4
	Female	17
Suai Loro	Female	11
	Female	32

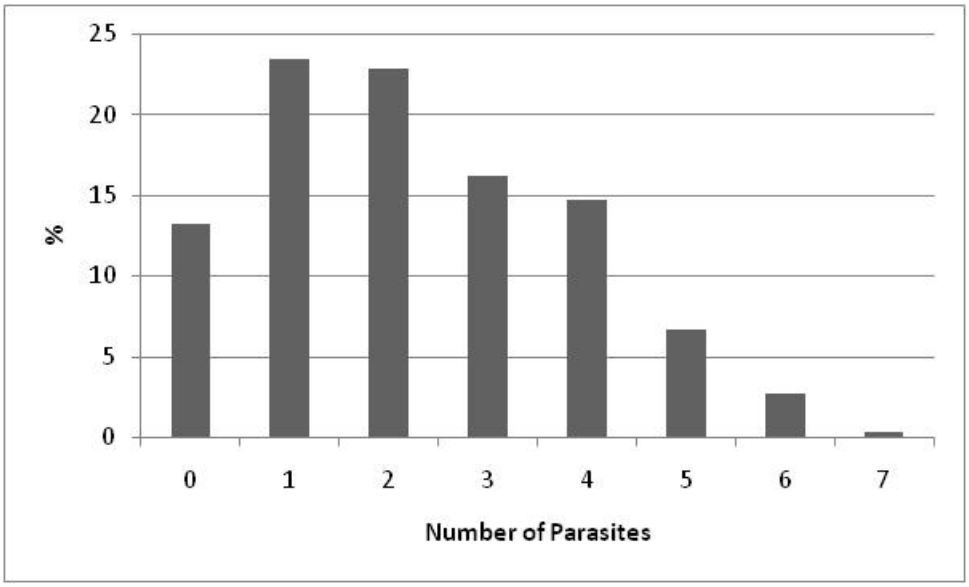
There was one case of *Strongyloides stercoralis* diagnosed by detection of a rhabditoid larvae (see Figure 4–5). This was from a male from the village of Sika.

Figure 4–5 A rhabditoid larvae of *Strongyloides stercoralis* from a faecal sample from the village Sika

The worm above is 290 µm long and 19.5 µmwide.

Figure 4–6 shows the frequency of individuals with varying numbers of parasites including those that are non-pathogenic. Of those that were examined for other parasites besides STH, 24.3% had four or more parasites detected.

Figure 4–6 Percentage of individuals (n=333) with total number of intestinal parasites detected



4.3.4 Household survey

Only 46 households (32%) supplied samples on all members of their household for examination for STH. Of these, five households consisted of only one member.

Figure 4–7 Household size and the number of households not providing faecal samples

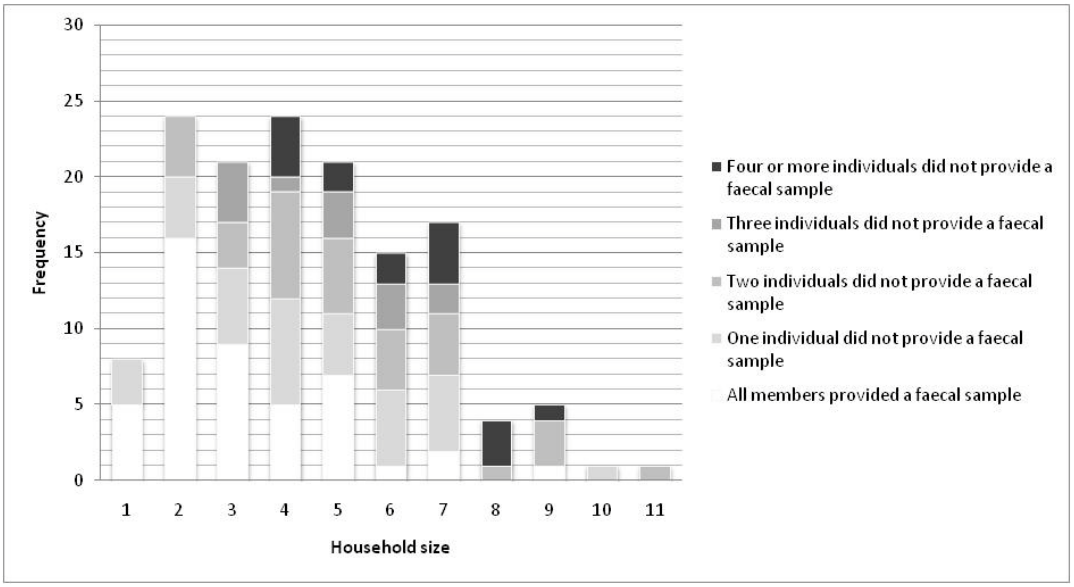
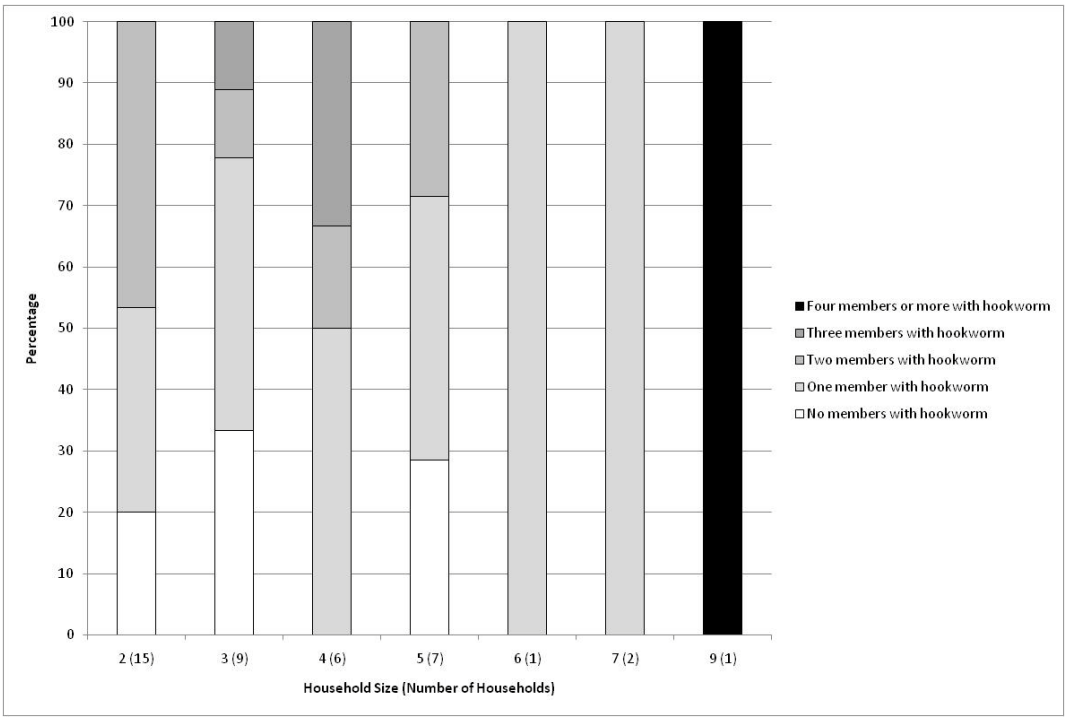


Figure 4–8 Percentage of households, with more than one member and in which all members provided a sample, with varying number of members with hookworm



Of the 41 households with more than one member where all members supplied a specimen, 80.5% (33) had at least one member that was infected by hookworm. Sample sizes were too small to analyse for clustering within household.

4.4 Discussion

The demographic profile shows fewer males in the 15–30 age range in comparison to the younger age groups and the 35–40 age group. The 2003 Health and Demographic survey (Ministry of Health and National Statistics Office et al., 2004) notes the same deficit in the rural west and east and attributes this to the annexation of Timor-Leste by Indonesia in 1975 and the warfare and relocation of populations at this time influencing the birth rate. This would explain the lower number of males in this survey. The mean household size was 4.4, which was lower than the household sizes of 6.4 and 6.1 in the rural east and west reported in the 2003 survey. This is a similar average of 4.3 to Indonesia (Ministry of Health and National Statistics Office et al., 2004). The lower

household size may reflect that only those currently present in the village at the time were enumerated and those who resided but were currently away from the village were not included.

Hookworm was the predominant STH with an overall prevalence of 32%. *T. trichiura* was found in 2.4% and surprisingly only six infections with *A. lumbricoides* were identified. There are few published studies on STH prevalence rates in Timor-Leste. It was reported in 1956 prevalence rates for children between the age of seven and 14 were between 5% and 94% for *A. lumbricoides* and between 2.5% and 70% for hookworm with no association with altitude (Fraga de Azevedo et al., 1956). In the Venilale District, for all age groups, 49% were infected with *A. lumbricoides,* 67% had hookworm and 1% had *T. trichiura*. Ninety per cent of the children by the age of three were infected with *Ascaris* with no difference between genders under the age of 14 but in adults, significantly more males were infected. All hookworm infections were light except one that was considered a moderate infection (Jones, 1976). A more recent survey of children (Melrose, 2002) found *A. lumbricoides* rates of 22.0%, 13.6% and 75.6% in Batugade in the west, Rembor in central Timor-Leste and in Dili, the capital city of Timor, respectively. No hookworm was identified. Melrose speculates this could be due to the wearing of footwear and that hookworm is more often found in adults. Jones (1976) considered the low amount of shade, the long dry season and well-drained limestone soil would be particularly unsuitable for eggs and larvae at Venilale. However, as 67% had hookworm these factors do not appear to have had much influence in acquiring infection. The actual hookworm prevalence is likely to be far higher due to the delay in processing the specimens. Delays in processing or adding preservatives by more than three hours can reduce sensitivity by 50% for hookworm

(Dacombe et al., 2007). Falsely low hookworm prevalence may be obtained due to the fragility of hookworm even with the addition of SAF (Utzinger et al., 2008).

It was surprising, due to higher *A. lumbricoides* in previous reports (Fraga de Azevedo et al., 1956; Jones, 1976; Melrose, 2002), that only a prevalence rate of 1.3% was found for *A. lumbricoides. Ascaris* eggs are very robust and may even maintain viability in 10% formalin (Sandars, 1951). The presence of protozoa and hookworm eggs would indicate that the preservation technique was suitable.

Due to STH having considerable spartial heterogeneity (Knopp et al., 2008b; Stothard et al., 2008) it is difficult to determine whether the prevalence found in this study show a decrease compared to previous reports. Attached to the walls of a guesthouse in Cova Lima were newspaper articles detailing the donation of large amounts of albendazole from doctors in Australia to be given out to the area. If the *A. lumbricoides* prevalence is lower than in the past, this could be due to Non-Governmental Agencies being involved in uncoordinated activities distributing anthelmintics resulting in a reduced incidence. It has been suggested that the gradual fall in prevalence in Sri Lanka over a decade in the absence of a coordinated program is due to wide use of inexpensive over the counter anthelmintics (De Silva et al., 2003). Conversely, as the studies in Table 4–2 show, reducing *Ascaris* prevalence through medication alone is difficult and the reasons for the absence of *A. lumbricoides* from these communities should be confirmed and reasons identified. This could be done by comparing other communities with high *A. lumbricoides* prevalence and identifying the differing environmental, behavioural and sociological factors between them in order to identify the risk factors.

Hookworm prevalence in this study increased with age up to adulthood as has been shown in some other countries (Pritchard et al., 1990; Gandhi et al., 2001). Prevalence remaining high in adults shows that that the immune response to hookworm is not protective (Behnke, 1987) with mature hookworms surviving by protecting the integrity of the host mucosa (Croese and Speare, 2006). Girls had a higher prevalence of hookworm infection compared to boys and this could be due to as yet unidentified behavioural differences between the genders.

Most hookworm infections were light (97.2%). Due to the time delay in processing the specimens, it could be expected that moderate and high intensity infections are underestimated. The combined STH prevalence of among children aged five to nineteen at the villages of Buihomau, Sika and Suai Loro were 10.8%, 40.3% and 42.9% respectively (WHO, 2006). The current Timor-Leste treatment strategy is albendazole twice yearly for children between one and 16 years of age and pyrantel pamoate twice yearly to those under the age of one. Under the current WHO guidelines (2006), the second albendazole dose is not required. However, as seen in Sri Lanka, prevalence of STH was unchanged during three years of annual MDA (Gunawardena et al., 2008). Reinfection can be rapid even in children receiving three daily anthelmintic treatments to completely clear STH infection (Hesham Al-Mekhlafi et al., 2008). This would indicate more doses are required.

This is the first report of a *Strongyloides* species in Timor-Leste. This was identified as *S. stercoralis* since larvae, not eggs were present in faeces (Speare, 1988). Using Harada-Mori culture Jones (1976) found no *Strongyloides* spp. However, Harada-Mori cultures are not the most sensitive technique for diagnosis of *Strongyloides* spp. infection (Marchi Blatt and Cantos, 2003). Collection of additional specimens from the family of this individual was arranged but internal unrest in Timor-Leste prevented staff

returning to the village. It is interesting that strongyloidiasis was unreported in Solomon Islands until after a multinational peace-keeping force had been present for several years (Pattison and Speare 2008). Although evidence that the parasite was introduced by peace-keepers is lacking, it is one option of which to be aware.

The identification of *Taenia* eggs is further discussed in Chapter Five. However, it is worth noting the high number of households that owned pigs in all three villages. Unlike Jones (1976) who reported the absence of free roaming pigs, pigs were free to wander around the three villages visited. With only 40% of households reporting that they had a latrine available, the conditions are present for the spread of cysticercosis. Three species of *Taenia* infect humans in Asia (Ito et al., 2004) and evidence is presented in Chapter Five that *T. solium* may be present in Timor-Leste.

The high prevalence of the non-pathogenic *E. coli* shows the likely extent of faecal contamination of fresh water. Only 42% in Buihomau and 62% of households said they had a latrine available. Jones (1976) considered that the scarcity of latrines, the poor supply of domestic water and poor quality of houses as factors increasing the spread of the parasites while the scattered houses and cooking food would mitigate against spread. Latrine availability should be considered at a community level since the risk of infection is dependent on the infectivity of the soil. This is dependent on the amount of defecation occurring in on the soil therefore those who have latrines are still at risk of infection (Chongsuvivatwong et al., 1996). Thus, there is likely to be a certain level of investment in sanitation in a community before the effects in lowering prevalence and intensity are noticeable (Asaolu et al., 2002). Behavioural and cultural factors may still influence where defecation occurs for, as Chongsuvivatwong (1996) shows, although latrines may be available to all households, a percentage routinely do not use one. Reasons for non-use may include no flush water or not being close to one when one is required. Children

may not use them because the children are too small (Kiyu and Hardin, 1993). Feachem et al. (1983) report that there was no difference in STH infection in an African village between those who had private flush toilet, communal aqua privies and fouled bucket latrines, suggesting that provision of superior facilities to a few households in a community does not offer protection to those who use them. Without social barriers to doing so or unsuitable latrines, it may be more convenient to defecate in the bush in remote communities. Once social norms of behaviour have become accepted and once the supply industry has adapted to the new situation (in this case latrine and sewerage systems) then it requires no effort for the individual to maintain the behaviour (Rose, 1985). It may be that the whole community requires suitable latrines for a decrease in STH prevalence. In a review of the literature on the role of sanitation on helminth infections, Asaolu and Ofoezie (2003) conclude that sanitation interventions have an important role to play in controlling helminth infections with their success depending on coverage, operational state and community acceptance. The facilities must be affordable, effective, simple to use, compatible with local technology and available in the local market. As shown in Korea, not only was mass regular treatment of anthelmintics required to reduce STH prevalence to near zero among children but improved sanitation, changes in agricultural practice such as using not using human faeces for fertiliser and health education were also required (Hong et al., 2006).

For households with more than one member in which all members provided a sample, 80.5% had at least one member infected by hookworm. Significant levels of household clustering of high intensity hookworm infections have been observed along with spatial clustering in Brazil. In this study, it was suggested that the peri-domiciliary, family practices and behaviours overrode any genetic basis for infection (Brooker et al.,

2006a). Investigation of such factors in the East Timorese context and mitigation of them could assist in the reduction of STH infection.

Only 21% of houses in Buihomau, 22% houses in Suai Loro and no houses in Sika contained concrete. Cement or concrete block housing offers some protection for children against infection with STH (Holland et al., 1988; Raso et al., 2005). Improvements in housing as well as sanitation are likely to help in reducing the burden of disease due to STH.

The study could have been improved by the research team spending more time involved with the survey at the collection sites, but budget constraints prevented this. The sensitivity of the parasitology methods could have been improved by collecting more faecal samples from the residents (Booth et al., 2003). Increasing the number of samples, in agreement with Berhe et al. (2004), was not feasible or practical and could have introduced substantial costs (Booth et al., 2003). More time could have been spent in training villagers recruited to assist in the study and administer the questionnaire. A pilot of the questionnaire could have allowed more additional useful information to be collected. As Dacombe et al. (2007) note, there is real difficulty in conducting helminth studies in rural environments with poor infrastructure. The rural laboratories with their monocular optical microscopes are ill equipped to deal with large numbers of faecal specimens and in this project, it necessitated the need for analysis back in Australia.

Routine parasitological techniques have changed little in half a century. For routine parasitological specimens, single specimens, depending on parasite, offer poor sensitivity. However, the cost and time consuming procedures tend to make collecting additional specimens impractical. Integration of STH elimination into filariasis elimination programs is a logical step. However, the need for additional albendazole

distribution six monthly is questionable when considered against WHO guidelines (2006). It is likely there are areas of high STH prevalence in Timor-Leste that do require six monthly doses. It would be an informative exercise to compare the economics and logistics of surveying all schools for STH and mass treating once or twice per year based on the results compared to the current practice of mass treatment of all children twice per year. The baseline surveys at these three sites will be used to monitor the effects of MDA. Concentration on determining if cysticercosis is present is an urgent matter.

Chapter Five: Immunodiagnosis of Neurocysticercosis in Papua New Guinea and Timor-Leste

5.1 Introduction

Neurocysticercosis has not been described in PNG or Timor-Leste. Serum specimens from previous parasitological surveys were analysed for serologic evidence of NCC.

Taenia solium is acquired by humans when undercooked pork containing a cysticercus is consumed. The protoscolex is not digested and attaches to the intestinal wall growing a chain of proglottids. When eggs from the tapeworm are ingested or possibly enter the duodenum from reverse peristalsis, the oncosphere hatches and penetrates the intestine, then travels in the bloodstream and lodges in tissue, particularly the central nervous system, skeletal muscle, subcutaneous tissue and eye (Carpio et al., 1998; Markell et al., 1999).

NCC is one of the most common parasitic infections of the nervous system and globally the single most common cause of epilepsy. It is responsible for approximately 10% of neurologic admissions to hospitals in endemic countries. It is also considered an emerging infection in developed countries with immigration of people from high prevalence to low prevalence areas (Garcia and Del Brutto, 2000; Garcia et al., 2002a; Wallin and Kurtzke, 2004).

5.1.1 Clinical signs and symptoms

The clinical picture of NCC can vary depending on the location of the parasite. If the parasite is found in the parenchyma or subarachnoidal space of the sulci, there is a

benign presentation. When it is found in the subarachnoidal space at the base of the brain or in the ventricles, NCC has a greater probability of being severe. This is related to increased intracranial pressure with the cysticerci obstructing the flow of cerebral spinal fluid or the inflammatory reaction of the ependyma (Takayanagui and Odashima, 2006; Fleury et al., 2007). In a review of the literature in the USA, Wallin and Kurtzke (2004) determined that seizures were the most frequently reported onset symptom followed by symptomatic hydrocephalus and headaches. Other symptoms included meningitis or encephalitis, focal neurological deficits and altered mental states. Most patients (91%) presented with parenchymal cysticercosis, with ventricular cysts in 6%, subarachnoid cysts in 2% and 0.2% with spinal cysts. Epilepsy and headache were also the most common symptoms of NCC in Mexico (Sotelo et al., 1985).

Sotelo et al. (1985) devised a classification of NCC based on disease activity. In active cases, arachnoiditis is a common finding as well as obstructive hydrocephalus secondary to inflammatory occlusion of Luschka and Magendie foramina and vasculitis. Parenchymal cysts are also found. In inactive forms, parenchymal calcified granulomas are the most commonly found.

Treatment is based on the number of cysts, the location and their viability. Albendazole and praziquantel are effective antiparasitic drugs with growing cysts actively managed by antiparasitical drugs or surgery. Patients with raised intracranial pressure secondary to NCC are managed for the hypertension while those with seizures are given antiepileptic drugs (Garcia et al., 2002a).

5.1.2 Diagnosis of neurocysticercosis

Criteria for diagnosis are based on four categories; absolute, major, minor and epidemiologic, stratified according to their diagnostic strength. Definitive diagnosis

consists of one absolute criterion or two major, one minor and one epidemiological criterion. Absolute criteria are either by histological demonstration of the parasite by biopsy, cystic lesions showing the scolex in CT or MRI or direct visualisation of subretinal parasites by funduscopic examination. Major criteria, consisting of highly suggestive lesions on imaging studies, a positive serum immunoblot for anti-cysticercal antibodies, resolution of intracranial lesions following antiparasitic drugs or spontaneous resolution of a single lesion. Minor criteria consist of lesions compatible with NCC, frequent non-specific manifestations such as clinical signs, cysticercosis outside the central nervous system and demonstration of anti-cysticercal antibodies or cysticerci antigens by ELISA in the CSF. Epidemiological criteria consist of being associated with a household contact or an individual coming from an endemic area (Del Brutto et al., 2001).

Due to NCC being a common cause of epilepsy in developing countries (Senanayake and Roman, 1993) patients should be tested for cysticercosis. However, most endemic areas occur in developing countries where image analysis is not available due to the high cost. When images are available, single or similar looking lesions may often be attributed to *Mycobacterium tuberculosis* or other inflammatory causes that may be difficult to distinguish from *T. solium* cysts. Non-inflamed extraparenchymal cysticerci are usually not visible on CT and may be only subtly present in the MRI. The collection of biopsy tissue for demonstration of the parasite is also rarely justified (Carpio et al., 1998; Del Brutto et al., 2001; Margono et al., 2003; Mandal et al., 2006; Takayanagui and Odashima, 2006); therefore, a definitive diagnosis based on absolute criteria cannot be made in many endemic regions.

Serology is an important tool in providing information towards the diagnosis of NCC. Early diagnosis of NCC may allow treatment before development of symptoms,

monitoring of treatment and evaluating vesicular cysts, which may be difficult to image (Dorny et al., 2003; Lopez et al., 2004). Serology can be used to identify areas that require preventative and control measures, such as presumptive treating of suspected cases and their families and surveillance after control measures are applied (Dorny et al., 2003; Montresor and Palmer, 2006). As CSF collection is an invasive procedure and impractical for surveys, the majority of sampling in endemic countries is by blood collection (Scheel et al., 2005; Mandal et al., 2006).

5.1.3 Antibody response in NCC

Antibody levels are related to the anatomical position of the cyst with greater levels in individuals with cysts in the base of the brain, in the major cisterns or in the ventricles. Those who have cysts in the brain cortex generally have lower levels (Zini et al., 1990). Fleury et al. (2007) have also noted antibody levels to crude antigen are increased in patients with NNC patients with vesicular cysticerci in the subarachnoid space or the ventricles. Antibody production is more intense in cases with intraventricular lesions, signs of CSF inflammation and multiple clinical syndromes (Zini et al., 1990; Odashima et al., 2002).

In CSF, there is more IgG to specific antigens compared to the other antibody classes (Odashima et al., 2002). Higher IgG levels are seen in CSF to reactive antigens in the vesicular stage, lower in the degenerative stage and lowest in the calcified stage (Lopez et al., 2004) with corresponding decreases of sensitivity in the ELISA assay (Proano-Narvaez et al., 2002). Zini et al. (1990) found no correlation between antibody levels in serum and CSF with the type of cyst or the number of cysts in individuals with NCC with complex neurological symptoms compared to those who were considered benign.

Garicia et al. (2001) have noted a discrepancy between the high seroprevalence of cysticercosis in communities and the relatively low numbers of symptomatic cases. They note three tiers of individuals who are serologically positive; those with symptomatic NCC, those with cysticercosis outside the CNS or cysticercosis of the CNS without any symptoms and those exposed but in whom no cysts can be found. In longitudinal surveys, they found that about 40% of those who were antibody positive were antibody negative one year later indicating exposure but a non-viable infection and implying an exposure rate higher than previously realised.

Solitary cysts are more common radiological finding than multiple lesions in India and Latin America (Garg and Nag, 1998; Prabhakaran et al., 2004; Montano et al., 2005; Kumar et al., 2006). The presence of a single cyst rather than multiple cysts may be due to low incidence and prevalence of taeniasis with low level of egg ingestion (Prabhakaran et al., 2004). The number of brain lesions shows no correlation with antibody titre with single lesions sometimes showing higher antibody levels than multiple cysts (Odashima et al., 2002; Mandal et al., 2006). However, serologic detection of antibodies for single cysts is quite low compared to multiple cysts (Proano-Narvaez et al., 2002; Prabhakaran et al., 2004; Bueno et al., 2005). A case report by Ito et al. (1999) used glycoproteins purified by isolectric focusing to detect a single cyst with antibody disappearing a year after treatment.

5.1.4 Antibody assays using whole crude antigen

Arambulo et al. (1978) were the first to describe an ELISA method for antibody detection for human cysticercosis using delipidised saline extracts of *T. solium* and its cysticercus. Using whole or component crude antigen from scolex, membrane or vesicular fluid has been widely used with large variations in reported sensitivity that can

be often lower than 70% (Ramos-Kuri et al., 1992; Bueno et al., 2000; Proano-Narvaez et al., 2002; Biswas et al., 2004; Arruda et al., 2005) or greater than 90% (Dekumyoy et al., 2000; Suzuki et al., 2007).

As pigs naturally infected with *T. solium* cysticerci can be difficult to maintain an alternative source of antigen is from the larval form of *Taenia crassiceps,* which reproduces asexually by intraperitoneal passage in mice (Larralde et al., 1990) as there are common antigenic determinants between the two species (Espindola et al., 2005). Using crude antigen of *T. crassiceps* the sensitivity and specificity is comparable in the ELISA using antigen from *T. solium* (Bueno et al., 2000; Arruda et al., 2005).

These assays suffer from poor specificity due to cross-reactions with patients with toxoplasmosis, Hepatitis B, cytomegalovirus, infectious mononucleosis, schistosomiasis, hydatidosis, hookworm, gnathostomiasis, toxocariasis, onchocerciasis, opisthorchiasis (Dekumyoy et al., 2000; Ishida et al., 2003; Arruda et al., 2005). Cross-reaction in patients with intestinal taeniasis due to *T. solium* or *Taenia saginata* is also a problem (Ev et al., 1999).

Using crude antigen has been described as unreliable and producing disappointing results (Ramos-Kuri et al., 1992). Ng and Ko (1994) determined that the efficacy of the ELISA using crude antigen depends on the source of the antigen from membrane, scolex or vesicular fluid, the ELISA format and the course of infection. Observed variations in the performance of immunoassay may also be due to criteria for selecting patients, the heterogeneity of the patients, time of sampling relative to infection, the number, stage and localisation of the parasite, the immune status of the patient, how the results are presented and the cut-off used (Arruda et al., 2005). The low sensitivity of the ELISA and its use in low prevalence areas result in very low positive predictive

values indicating its use in locating transmission rather than identification of individual cases (Proano-Narvaez et al., 2002).

Although enzyme-linked immunoelectrotransfer blot is considered the gold standard, crude antigen cyst fluid based ELISA is still used widely in the developing world (Ferrer et al., 2005).

5.1.5 Glycoproteins for diagnosing cysticercosis

Gottstein et al. (1986) originally showed that detection of polypeptides from soluble *T. solium* metacestode proteins, prepared by homogenising and sonicating sediment of thawed cysts, by antibody in human serum using enzyme-linked immunoelectrotransfer blot could be a suitable diagnostic test for cysticercosis. Two polypeptides (26 kDa and 8 kDa) were found that reacted in sera from individuals with cysticercosis, but not other parasitic infections including those infected by the closely related cestode, *Echinococcus.*

Using lentil-lectin affinity purified glycoproteins from cysts in EITB is the test of choice that identifies seven major bands at 13, 14, 18, 21, 24, 39–42 and 50 kDa and is very sensitive (98%) and specific (100%) for diagnosis of human cysticercosis (Tsang et al., 1989) in patients with more than one cyst (Tsang et al., 1989; Wilson et al., 1991; Schantz et al., 1994; Del Brutto et al., 2001). These glycoproteins consist of a closely related family of 8 kDa proteins with one to three N-linked glycosylation sites. At least 32 nucleic acid sequences encoding 18 unique 8 kDa proteins have been identified as encoding members of the 8 kDa family that are extracellular secreted proteins that are found in cyst fluid. These 8 kDa proteins in the LLGP EITB are found at 14, 18, 21 kDa and in the 24 kDa band with those in 39–42 kDa bands possibly being sulphide

multimers (Greene et al., 1999; Sako et al., 2000; Obregon-Henao et al., 2001; Hancock et al., 2003).

There is a difference in banding patterns of glycoproteins recognised by patients with cysticercosis between the two genotypes of *T. solium* with the difference in size of the glycoproteins due to different glycosylation. Principally, a 22-kDa protein from the American/African genotype is detected as an 18-kDa protein using the Asian type. There is no difference in diagnostic sensitivities with either of the two genotype glycoproteins (Sato et al., 2006).

The lentil-lectin purification procedure is complicated, up to ten times as expensive, has a lengthy purification process and does not give a quantitative result. There is also poor reproducibility between laboratories possibly due to nature and purity of antigen extracts. The produced glycoproteins give a high background reading in an ELISA due to the proteins binding to polystyrene at a higher affinity than the specific antigens (Ito et al., 1998; Proano-Narvaez et al., 2002; Fleury et al., 2003; Hancock et al., 2003; Espindola et al., 2005; Scheel et al., 2005).

Other purification techniques for *T. solium* or *T. crassiceps* cysticerci that have been used such as ion exchange, electro-elution and immunoaffinity chromatography have also shown good sensitivity and specificity for use in an ELISA (da Silva et al., 2000; Peralta et al., 2002; Lopez et al., 2004; Espindola et al., 2005).

Ko and Ng (1998a) showed that isoelectric-focusing could be used as a purification method to isolate glycoproteins from the cystic wall or cystic fluid with good specificity for immunodiagnosis of cysticercosis in pigs. They subsequently showed that the method could be used with good specificity for diagnosing human cysticercosis (Ko and Ng, 1998b). In the EITB, NCC serum samples recognise three major bands, 10–26 kDa,

using glycoprotein prepared this way and this has shown 100% sensitivity (Ito et al., 1998). There is little background reading in patients with cystic echinococcosis, alveolar echinocococcosis or healthy controls in the ELISA format. The ability to use these glycoproteins in an ELISA format allows for mass screening in endemic areas and monitoring progress of NCC (Ito et al., 1998).

Macromolecules in the cyst fluid of *T. solium* metacestode also have the potential to be used as diagnostic antigens. A 120 kDa protein consisting of two major components, themselves comprised of six subunits originating from the 14 or 18 kDa precursors has shown 86% sensitivity with active NCC though only 35% with chronic cases and some cross-reactions with alveolar or cystic echinococcosis (Lee et al., 2005a).

5.1.6 Recombinant/synthesised glycoproteins

There has been a progression in development of antigens for use in the ELISA format. Tsang et al. (1989) initially identified a 14-kDa glycoprotein that reacted with sera from patients with parasitologically confirmed cysticercosis and is a post-translationally modified 8-kDa protein. This antigen purified from lentil lectin-bound glycoprotein 14 showed 100% sensitivity with parasitologically confirmed cysticercosis specimens by EITB using sera and 100% specificity with healthy controls or individuals with other helminth infections (Greene et al., 1999). This antigen has subsequently been chemically synthesised as a 66 amino acid without its signal sequences and in the ELISA format shows 90% sensitivity with more than one viable or racemose cysts, but only 50% with one cyst. Specificity was 85% using a variety of sera from parasite-infected individuals (Scheel et al., 2005). Other recent recombinant and synthesised proteins used for diagnosis of NCC are shown in Table 5–1.

Since maintaining a supply of cyst fluid for production of native glycoproteins can be difficult and extraction of cysts from infected muscle is laborious and time consuming (Sako et al., 2000; Lee et al., 2005b), recombinant proteins could be used in diagnostic tests based on antibody detection.

Sensitivity of individual glycoproteins or peptides can be less than native proteins used in the Western blot, which may be due to either the less sensitive ELISA procedure or the difference between native and recombinant proteins (Bueno et al., 2005).

Table 5–1 Recent recombinant/synthesised proteins used for diagnosing NCC

Recombinant Antigens	ELISA	Reference
TsRS1 chemically synthesised 8 kDa protein with a long chain biotin	▪ 100% sensitive (n=32) with samples from cases of NCC and that reacted in the EITB • 100% specific (n=49) with individuals with other parasitic infection or no known infection	Hancock et al., 2003
Ts18 var 1 chemically synthesised 8 kDa protein with a long chain biotin	▪ 97% sensitive (n=32) with samples from cases of NCC and that reacted in the EITB ▪ 100% specific (n=49) with individuals with other parasitic infection or no known infection	Hancock et al., 2003
	▪ 90.4% sensitive (n=52) with sera samples and 90.2% (n=41) using CSF from cases of NCC and that reacted in the EITB ▪ 90.3% specific (n=145) with sera and 98.0% (n=50) using CSF from individuals with other parasitic infection or no known infection	Bueno et al., 2005
rTsM10-ELISA. A baculovirus expressed recombinant protein.	• 91.1% sensitivity (n=41) using sera and 97.8% (n=44) using CSF with active NCC • 96.4% specificity (n=139) with individuals with other parasitic infection or no known infection	Lee et al., 2005b
Combined synthesised HP6-3, Ts45W-1 and Ts45W-5 peptides from *T.saginata* oncosphere molecules	• 85% (17/20) using sera for both clinically inactive and active cases • 83.8% (119/142) specificity from individuals with other parasitic infections.	Ferrer et al., 2005
Ts14 a 14 kDa glycoprotein initially identified by Tsang et al. (1989) and described by Green et al. (1999) with identical amino acid homology to a 12 kDa protein described by Obregon-Henar et al. (2001).	• 97% sensitivity (n=30) with serum • 100% (n=41)sensitivity with CSF • 100% specificity (n=59) with sera from healthy control or individuals with schistosomiasis and Chagas' disease • 100% specificity using CSF (n=29	da Silva et al., 2006
Ag1V1/Ag2 chimeric recombinant antigen (Sako et al., 2000). Ag2 is in the TsRs1 clade and Ag1V1 in the Ts18 clade (Hancock et al., 2003)	• 94.3% (n=66) sensitivity • 100% specificity using sera from individuals with other parasitic infections (n=115)	Sako et al., 2006
Ts8B2. Recombinant antigen showing similarity to 8 kDa glycoproteins and shared partial identity with 8 kDa subunit of antigen B of *Echinococcus* spp. and with hydrophobic ligand binding proteins of *H. diminuta* and *M. expansa*	• 96.8% (n=31)sensitivity with active NCC using serum • 67% (n=31) sensitivity with inactive NCC using serum • 38.5% (n=26) sensitivity with active NCC using CSF • 93.1% (n=87) specificity using sera from individuals with other parasitic infections (	Ferrer et al., 2007

The absence of post-translational modification may be the cause of reduced antigenicity, sensitivity or specificity (Obregon-Henao et al., 2001; Hancock et al., 2003; Bueno et al., 2005) although the absence of post-translational modification has been shown not to cause an effect in the ELISA (da Silva et al., 2006).

Binding to the ELISA plate of peptides may cause a difference in sensitivity, though coupling peptides to protein carrier makes no difference using *T. saginata* oncosphere-derived synthetic peptides (Fleury et al., 2003) or peptides bound to bovine serum albumin (Hernandez et al., 2000). Scheel et al. (2005) concluded that direct coating of 8 kDa synthetic peptides to polystyrene plates for detection of NCC is not successful as the antigens are too insensitive or highly cross-reactive. However, those with biotin tails for capture by streptavidin coated polystyrene sticks performed well in the ELISA (Hancock et al., 2003). Sensitivity increased without loss of specificity when glycoproteins were partially unfolded using urea (Prabhakaran et al., 2007). Possibly, multiple peptides are required in the ELISA assay to increase sensitivity, but this may cause a decrease in specificity due to more false positive reactions (Hancock et al., 2003; Lopez et al., 2004; Ferrer et al., 2005).

Sensitivity of ELISA assays can depend on whether individuals are in the active, mixed or chronic stages. Assays can be less sensitive in detecting chronic cases (Chung et al., 1999; Fleury et al., 2003; Lee et al., 2005a; Lee et al., 2005b) possibly due to lower antibody levels in the calcification process (Espindola et al., 2005). Possibly, as the metacestodes develop, die and become calcified different antigens are released. Only viable metacestodes can release E/S antigens although the inflammatory system may cause the release of antigenic structures such as vesicular membrane (Lopez et al., 2004). This could be important for differential diagnosis as active and mixed cases are treated with anthelmintics while chronic cases are given anticonvulsants (Lee et al.,

2005b). Differences in peptide recognition may also be due to genetic, geographical, behavioural differences in the performance of the assay or the balance between short and long-term infections (Ferrer et al., 2005). Non-responsiveness may be due to immune status of the patient rather than the antigenic properties of the peptide (Chung et al., 1999).

5.1.7 Antigen detection

Dorny et al. (2004) consider that antigen detection is more useful than antibody assays for detection of active cysticercosis cases and follow-up after treatment. Detection of antigen indicates live parasites and patients may benefit from antiparasitic therapy (Garcia et al., 2002b). Antigen levels are also shown to drop after albendazole treatment in those who respond to treatment as evidenced by radiologic disappearance of active lesions (Garcia et al., 2000).

There are at least two validated antigen detecting ELISA assays (Dorny et al., 2004) using monoclonal antibodies. Harrison et al. (1989) created a monoclonal antibody, HP10, from mice immunised with *T. saginata* cysticerci that detected an antigen that was only present with viable parasites. This antigen was shown to be present in *T. saginata*-infected cattle, but they also reported it to be present in the CSF of humans infected with *T. solium.* Antigen detection has shown good sensitivity (86%) with a correlation between antigen levels and number of live cysts (Garcia et al., 1998). Sensitivity is high in active cases, but very low in chronic cases (Ferrer et al., 2005) and lower in patients with only one cyst or multiple degenerating lesions (Garcia et al., 1998). Patients with calcified lesions have shown no circulating antigen (Garcia et al., 2002b) and specificity is high when tested against a variety of infected individuals

(Garcia et al., 1998). Detection of HP 10 is as sensitive and specific also using CSF and is a potential indicator for ventricular or subarachnoidal NCC (Fleury et al., 2007).

Brandt et al. (1992) developed a set of monoclonal antibodies using E/S products of 10-week-old cysticerci of *T. saginata.* Living cysticerci could be detected in cattle five weeks post-infection. They also reported the assay could detect cases of human cysticercosis. Sensitivity was 94% compared to NCC diagnosed by CT scan, biopsy of subcutaneous nodules or both with no cross-reactions in individuals with a wide variety of infections including those with hydatid cysts (Erhart et al., 2002). In a comparison between antibody detection using recombinant antigen (Sako et al., 2000) and antigen detection (Brandt et al., 1992) in 504 epileptics in Cameroon, antigen was detected in 1.2% and antibody in 44.6% possibly indicating that epilepsy in NCC is associated with dead or dying cysticerci (Zoli et al., 2003). This antigen detection method is also usually negative in calcified NCC but high values may indicate the presence of subarachnoid NCC (Rodriguez et al., 2009).

5.1.8 Background of study

In September 2004 at the Department of Parasitology, Asahikawa Medical College, Asahikawa, Japan, the Second International Seminar on Technology Transfer for Immunodiagnosis of Cysticercosis/Taeniasis and Echinococcosis was conducted. This seminar was designed to transfer knowledge of the methodology of immunological and molecular diagnosis of these cestode zoonoses. There have been no reports of NCC in Timor-Leste or PNG yet it was suspected it might be present due the close proximity of these countries to Indonesia where cysticercosis is found (Margono et al., 2006). Specimens were available from other parastilogical surveys performed in Timor-Leste, Irian Jaya and PNG and these were taken to Japan to be tested for evidence of NCC.

5.2 Method

5.2.1 Purification of *T. solium* glycoproteins from cyst fluid using preparative isoelectric-focusing

Antigens were supplied ready for use for use in the ELISA and immunoblot as described by Ito et al. (1998). Briefly, cyst fluid and antigen was extracted from *T. solium* metacestodes extracted from skeletal muscles of pigs and rapidly frozen as described by Gottstein et al. (1986). The sediment was added to PBS and homogenised in a blender. It was then frozen and thawed twice more and ultrasonicated for three minutes then centrifuged for 45 minutes at 48000 g. The supernatant was dialysed against a 5mM Tris buffer (pH 7.4), mixed with Tris buffer and ampholyte and subjected to preparative isoelectric-focusing in a Rotofor (Bio-Rad laboratories) using 12W of constant power for four hours. The fraction collected at pH 9.2–pH 9.6 was used for the ELISA and immunoblot.

5.2.2 ELISA for NCC

The supplied glycoprotein solution was diluted in PBS to 1 µg/ml. One hundred microlitres was added to a 96 well ELISA plates (Maxisorp, Nunc, Roskilde, Denmark) and incubated overnight at 4 ºC. The plates were washed with PBS and then blocked with 250 µl of casein buffer and incubated for one hour at 37 ºC. Plates were then washed twice with PBS-Tween 20 and 50 µl of serum, diluted 1:100 with coating buffer, was added to the wells and incubated overnight at 37 ºC. Plates were then washed five times in PBS-Tween 20 and incubated with 50 µl of anti-human IgG-peroxidase (1:2000 dilution in casein buffer) for 1 hour at 37 ºC. Plates were washed five times with PBS-Tween and a final wash with PBS. Substrate, ABTS (Kirkegaard and Perry, Gaitersburg, MD) was added and kept at room temperature for 30 minutes. Absorbances were read at 420 nm with a microplate reader (model 450, Bio-rad

Laboratories). The cut-off value was calculated as three times the average absorbance of a negative control performed in triplicate. Samples that were above the cut-off were repeated twice in duplicate. Specimens that were repeatedly positive were considered sero-positive for NCC by ELISA.

Samples that were repeatedly positive using purified glycoproteins were tested using the recombinant antigen Ag1V1/Ag2 recombinant protein in the ELISA as described by Sako et al. (2000).

5.2.3 SDS-PAGE and immunoblot

Serum samples that were positive by ELISA were confirmed by immunoblotting. Serum was diluted 1:20 and *T. solium* glycoproteins diluted 1:1 in casein buffer. The glycoprotein solution was incubated at 95 ºC for five minutes and separated on a 12.5% acrylamide gel at 30 mA. The proteins were then transferred to a nitrocellulose sheet for five hours at 30 V. The sheet was cut into narrow strips and each was placed in an incubation trough and blocked for one hour at room temperature. The buffer was removed and diluted serum added to the strip and incubated for one hour at room temperature. Strips were washed five times in PBS-Tween and incubated with horseradish peroxidase conjugated anti-human IgG (Cappel). This was incubated at room temperature for 40 minutes and strips then washed five times with PBS-Tween and a last wash with PBS. Strips were then incubated with 4-chloro-1-naphtol for 15 minutes then stoped with water. Locations of visible bands were compared to a marker containing proteins with known molecular weights.

5.2.4 Samples

Venous serum samples were collected previously as part of parasite surveys undertaken in Timor-Leste, PNG and Irian Jaya. Samples were stored at -70 ºC before being transported to Japan for testing where they were then stored at 4 ºC.

Table 5–2 Date of collection of samples used for testing for NCC

Location	Date of Collection	Number of Samples
Timor-Leste	February 2002	279
Rembor, Manatuto, Timor-Leste	February 2002	52
Dili , Timor-Leste	February 2002	14
Basalaki Island, PNG	April 1996	43
Siaman, New Ireland, PNG	May 1997	20
Opau, Kereama, PNG	September 1993	51
Strickland, PNG	August 2004	77
Timuka, Irian Jaya	April 1998	51

5.3 Results

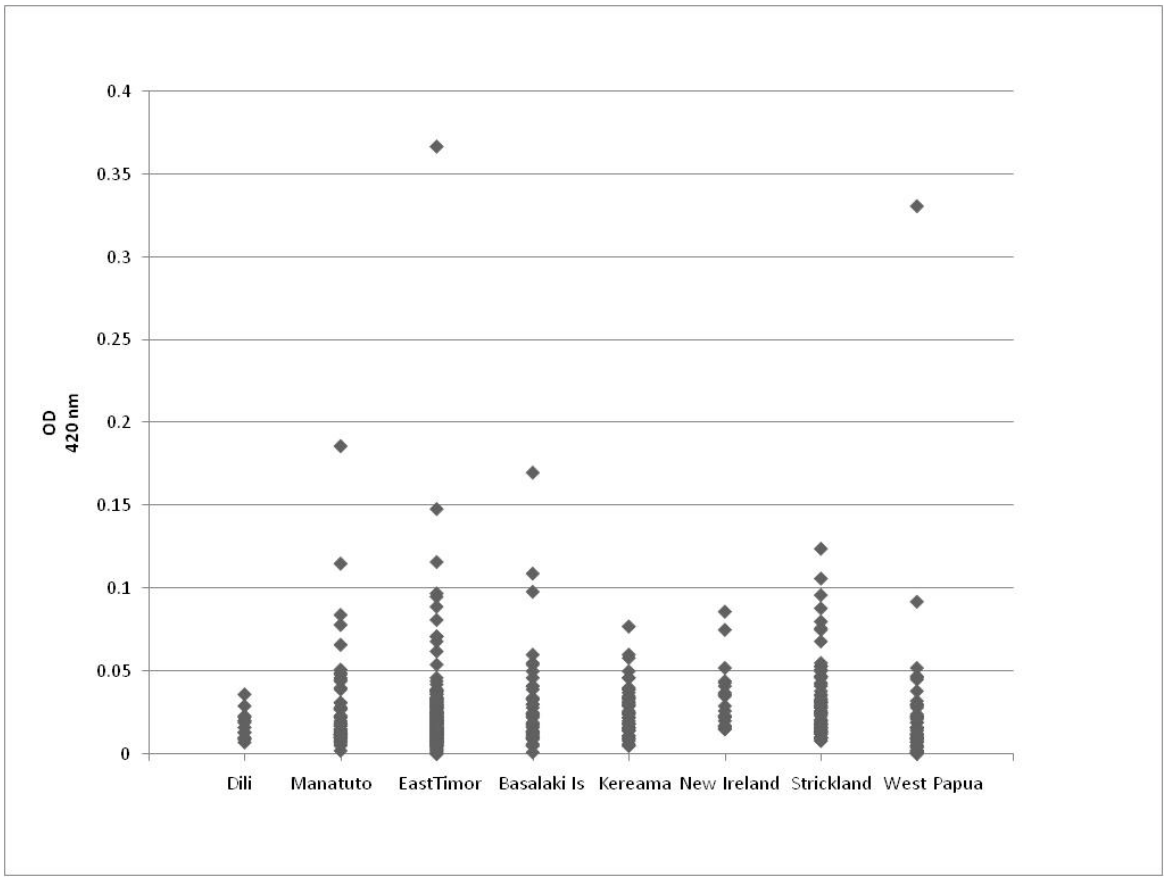
5.3.1 ELISA results

The cut-off absorbances ranged from 0.063 to 0.078. Figure 5–1 shows the absorbance values of the initial screening of the serum specimens from each of the three countries tested. Table 5–3 summaries the percentage positive for specimens that were repeatedly positive for each country and region tested.

Table 5–3 Prevalence of sero-positive individuals repeatedly positive by ELISA for NCC

Location	Number of Samples	Number above cut-off during screening	Number repeatedly above cut-off	Percentage repeatedly above cut-off
Timor-Leste	279	11	4	1.4 %
Rembor, Manatuto, Timor-Leste	52	6	2	2.6 %
Dili , Timor-Leste	14	0	0	0 %
Total Timor-Leste	**345**	**17**	**6**	**1.7%**
Basalaki Island, PNG	43	3	2	4.7 %
Siaman, New Ireland, PNG	20	1	1	5.0 %
Opau, Kereama, PNG	51	1	0	0 %
Strickland, PNG	77	6	2	2.6 %
Total PNG	**191**	**12**	**5**	**2.1%**
Timuka, Irian Jaya	**51**	**2**	**1**	**2.0 %**
Grand Total	**587**	**30**	**12**	**2.0 %**

Figure 5–1 Graph of the optical density by ELISA using purified glycoproteins to detect antibody for NCC from samples originating from Timor-Leste, PNG and West Papua



5.3.2 Immunoblot

Table 5–4 shows the results of the serological testing of specimens that were identified as positive by the ELISA. Of those that were repeatedly positive in the ELISA (n=12), 75% (n=9) were positive by immunoblot. This compares to only one specimen (6.6%) that was positive by immunoblot from those specimens that were above the cut-off during initial screening but did not give a repeatable positive ELISA result (n=15). This difference was significant (χ^2=13.35, df=1, p= <0.01). The specimen that did not give a repeatable ELISA, but was positive by immunoblot using purified glycoproteins, was also negative by ELISA and positive by immunoblot using recombinant antigen. Only one specimen of the three that were repeatedly positive by ELISA and negative by

immunoblot had further testing due to insufficient sample volume. This specimen was also positive in the ELISA and immunoblot using recombinant antigen. There were four specimens positive by all four tests, two from Timor-Leste and two from PNG.

Table 5–4 Results of further serological tests of specimens identified as positive in the ELISA screening test

Location	Sample ID	Repeatable LE Positive ELISA using native glycoprotein	Immunoblot using purified glycoproteins	ELISA using recombinant antigen	Immunoblot using recombinant antigen
Manatuto (Timor-Leste)	M9	Yes	Negative	Positive	Positive
Manatuto (Timor-Leste)	M18	No	Negative		
Manatuto (Timor-Leste)	M28	No	Negative		
Manatuto (Timor-Leste)	M34	No	Negative		
Manatuto (Timor-Leste)	M46	Yes	Positive	Negative	Positive
Manatuto (Timor-Leste)	M51	No	Negative		
Timor-Leste	MRS25	Yes	Positive	Positive	Positive
Timor-Leste	MRS50	No	Insufficient		
Timor-Leste	MRS155	No	Positive	Negative	Positive
Timor-Leste	MRS166	Yes	Negative		
Timor-Leste	MRS218	No	Negative		
Timor-Leste	MRS224	Yes	Positive	Positive	Positive
Timor-Leste	MRS272	Yes	Positive	Negative	Negative
Timor-Leste	MRS274	No	Insufficient		
Timor-Leste	MRS302	No	Negative	Negative	Negative
Timor-Leste	MRS380	No	Negative		
Timor-Leste	MRS434	No	Negative		
New Ireland (PNG)	NI 30	No	Negative		
Strickland (PNG)	S2	No	Negative		
Strickland (PNG)	S21	No	Negative		
Strickland (PNG)	S30	No	Negative		
Strickland (PNG)	S33	Yes	Positive	Positive	Positive
Strickland (PNG)	S34	Yes	Positive	Negative	Positive
Strickland (PNG)	S43	No	Negative	Negative	Negative
Basalaki Island (PNG)	BA4	Yes	Positive	Positive	Positive
Basalaki Island (PNG)	BA9	No	Negative		
Basalaki Island (PNG)	BA11	Yes	Positive	Negative	Positive
Kereama (PNG)	K170	No	Negative		
Irian Jaya	I16	Yes	Positive		
Irian Jaya	I17	Yes	Negative		

5.4 Discussion

The serological results suggest that cysticercosis is present in both Timor-Leste and PNG. Using glycoproteins purified by isoelectric-focusing (Ito et al., 1998) four samples from Timor-Leste and five samples from PNG were sero-positive in both the ELISA and immunoblot. Additionally, two of these specimens from Timor-Leste and two from PNG were positive using recombinant antigen in both the ELISA and immunoblot.

There have been no reports of cysticercosis in Timor-Leste or PNG and only a single case report of infection with *T. solium* (Abu-Salem and Hassan, 2003). Human and porcine cysticercosis is a major problem in Irian Jaya that borders PNG and is one of the worst endemic areas in the world (Subahar et al., 2001; Margono et al., 2006). Although *T. solium* is thought to have entered Irian Jaya relatively recently, prevalence of taeniasis is very high (Simanjuntak et al., 1997). The Jayawijaya District of Irian Jaya borders PNG. In the Gran Dani valley of this district, prevalence of cysticercosis and epilepsy increased from 2% and 14% to 27% and 48% respectively between 1977 and 1993 (Handali et al., 1997). In the Assologaima Sub-District, there was increased reporting of epileptic seizure from 1991 to 1995, with histological examination of a few subcutaneous nodules and mitochondrial DNA analysis from people and one pig confirmed cysticerci of *T. solium*. Serological testing of serum by immunoblot showed about two thirds were serologically confirmed NCC (Wandra et al., 2000). Other serological surveys have shown a high prevalence of serologically positive individuals and pigs for NCC (Subahar et al., 2001; Margono et al., 2003).

Owens (2006) reports that in PNG, there have been no cases of intestinal taeniasis as distinct from cysticercosis. *Taenia* could enter PNG through the traditional movement of people and pigs along the border with Irian Jaya and seems inevitable (Thompson et al., 2003) despite the people travelling across the border (Wandra et al., 2000). Other favourable factors in PNG that could allow transmission include high pig numbers, the rapid transport of pigs around a region, pigs roaming free eating human faeces and the absence of carcass monitoring (Dwyer, 2006; Owen, 2006).

In a serological survey of Irian Jaya refugees living in refugee camps with PNG in 1986, one person was positive by EITB. This individual had no history or signs of cysticercosis. However, he was from an area in Irian Jaya endemic for *T. solium* (Fritzsche et al., 1990). A serological survey in 1999 among villages in southern PNG bordering Irian Jaya showed no cysticercosis among the residents despite the presence of *Trichinella* antibodies among 29% of the residents, which is believed to be associated with their consumption of undercooked pork (Owen et al., 2001). Ito et al. (2002; 2004) refer to unpublished data of a survey in PNG of 541 people using ELISA and immunoblot that detected one strongly positive, three positive and 12 weakly positive individuals. Most of these, including the strongly positive individual, were PNG nationals not Irian Jaya refugees. The strongly positive individual suffered frequent headaches and had a spouse who was a West Papuan refugee.

The current serological results therefore add further evidence that *T. solium* may be present in PNG. Although specimens were serologically positive, they were very weak positive in both the ELISA and immunoblot. Absorbance readings were close to the cut-off and bands in the immunoblot faded very quickly. In the initial report by Ito (1998) using this method, OD readings of most samples with NCC were over an absorbance of 1.0. This compares to the average OD of the positive samples of 0.15.

It is desirable to continue to survey for cysticercosis in these two countries to determine the true situation. Antigen and antibody tests are available (Flisser and Gyorkos, 2007) that could be used to rapidly survey both countries. Additionally serological testing and lingual examination of the pig population could occur to assist in establishing prevalence (Krecek, Michael et al. 2008). If evidence of infection is found confirmatory, testing could be then undertaken. If *T. solium* is then found to be present then intervention measures can be put in place. The information presented here suggests that this should be performed as soon as possible.

Chapter Six: Immunological Responses of *Necator americanus* Infection in Subjects with Crohn's Disease and Healthy Volunteers

6.1 Introduction

Crohn's Disease patients and healthy patients were inoculated with *N. americanus* and the immune response to antigen from various microbiological species were analysed.

6.1.1 Hygiene hypothesis

The hygiene hypothesis (Strachan, 1989), when initially proposed, suggested that the size of families, improved amenities and higher standards of personal cleanliness could lead to decreased cross-infection and increased atopy. This hypothesis was suggested as an explanation to explain differential hay fever risk within families (Strachan, 2000), but has since been expanded to explain allergic and autoimmune diseases as incidences of infectious diseases in developed countries have decreased and incidences of allergic and autoimmune diseases have increased (Bach, 2002). Asthma, inflammatory bowel disease, multiple sclerosis and type 1 diabetes are immune related diseases that have become common in industrialised countries but are less common in underdeveloped countries (Elliott et al., 2007).

The hygiene hypothesis has been suggested as a cause of IBD although the question is not yet settled (Lashner and Loftus, 2006). Economic development that introduced running water and sewerage have been noted between areas with differing incidence rates (Elliott et al., 2005). Hospitalisation due to IBD is more common in northern states of the USA, compared to southern USA states (Sonnenberg et al., 1991) and incidence

rates higher in northern Europe compared to southern Europe (Shivananda et al., 1996). This suggests that environmental factors such as diet, climate, economic wealth or genetic susceptibility may be involved (Shivananda et al., 1996). The rise of incidence of IBD in young Bangladeshis who are born in the United Kingdom (UK) in comparison to those who living in the UK, but born in Bangladesh has been suggested to be attributable to improved hygiene (Tsironi et al., 2004). A proxy measure of hygiene, the availability of a fixed hot water supply in early childhood, is associated with an increased risk of Crohn's Disease in England (Gent et al., 1994; Duggan et al., 1998).

It has been suggested that loss of colonisation by helminths permits the development of immune related diseases (Elliott et al., 2007). There is evidence to suggest that there is a relationship between intestinal helminths and allergic diseases. The risk of wheeze is reduced by hookworm or *Ascaris* infection with an increased occurrence in an urban population being suggested due to a loss of protective effect from intestinal parasites (Scrivener et al., 2001; Dagoye et al., 2003), although a meta analysis determined the risk of asthma was found to be increased with *Ascaris* infection and reduced with hookworm infection (Leonardi-Bee et al., 2006). Atopy, as measured by skin test pricking, has shown an inverse association with intestinal helminth infection (Nyan et al., 2001; Cooper et al., 2003a; Cooper et al., 2003b; Cooper et al., 2004; Davey et al., 2005). Positive associations have also been reported (Scrivener et al., 2001; Palmer et al., 2002). The variations found between studies could be due to the type of helminth, the age at which the helminth was acquired and intensity of infection (Flohr et al., 2009).

Elliott et al. (2000) proposed that a major environmental factor predisposing to IBD is underexposure to intestinal helminths that cause a Th2 type environment within the

intestine. The question then should be asked of parasite elimination programs: if parasites are eliminated or reduced in a population, is there is a corresponding increase in incidence of allergic or autoimmune diseases?

Giving treatment to helminth-infected individuals has shown a conversion to reactivity to skin applied allergens. In a randomised controlled trial using praziquantel and mebendazole, children who were treated and had cleared infection of *A. lumbricoides* or *T. trichiura* had a 3.5 higher risk of converting to a positive skin test for house dust mites compared to children who were treated, but remained infected (van den Biggelaar et al., 2004). Ethiopian immigrants dewormed with albendazole showed a significant increase in the delayed type hypersensitivity response to tuberculin purified protein derivative compared to those treated, but whose helminths remained (Borkow et al., 2000). There was increased prevalence of sensitisation to allergens in the skin prick test of Vietnamese schoolchildren compared to those receiving a placebo in an helminth eradication campaign (Flohr et al., 2007).

6.1.2 Crohn's disease

IBD is characterised by intestinal inflammation and sufferers present with symptoms of rectal bleeding, abdominal pain and diarrhoea. Diagnosis is based on clinical symptoms, radiologic or endoscopic appearance of the bowel and histology. The cause is thought to be multi-factorial and due to environmental factors triggering an abnormal host immune response in genetically susceptible individuals (Farrokhyar et al., 2001).

CD is an IBD and can occur in any part of the gastrointestinal tract with areas of inflammation separated by normal tissue. There can be small superficial ulcerations and

focal chronic inflammation extending to the sub-mucosa sometimes with a non-caseating granulomata (Hendrickson et al., 2002).

The gut immune system must be able to react to pathogens, but at the same time remain unresponsive to food antigens and commensal bacterial. The total number of intestinal bacteria is estimated to be 10^{14} with over 500 species (Ott et al., 2004). A single microbial species has not been identified as the cause of inflammation (Hendrickson et al., 2002) though suspect candidates include *Mycobacterium avium* subsp *paratuberculosis*, adherent invasive *E. coli*, *Helicobacter* spp., viruses and yeasts (Pineton de Chambrun et al., 2008). Notably, IBD is most frequently observed in the terminal ileum and colon, which have the highest bacterial concentrations (Duchmann et al., 1999) while in experimental animal models of inflammation a requirement for development of inflammation is normal commensal gut microflora (Blumberg et al., 1999).

Inflammation is thought to be due to an excessive cell-mediated response to commensal bacteria (Macdonald and Monteleone, 2005). Lamina propria mononuclear cells derived from inflamed tissue proliferate in response to bacterial sonicates derived from autologous intestine. Peripheral blood mononuclear cells or LPMC from uninflamed tissue of CD patients or controls do not proliferate. When bacterial sonicates derived from heterologous intestine are used, PBMC and LPMC from uninflamed and inflamed tissue proliferate showing that the systemic and intestinal immune system is tolerant to autologous intestinal flora but is broken in inflamed intestine (Duchmann et al., 1995). A mutation in the gene *Nod2* on chromosome 16 is associated with an increased risk of developing CD (Hugot et al., 2001; Ogura et al., 2001a). This gene is expressed in neutrophils, macrophages, dendritic cells and Paneth cells in the small intestine (Ogura et al., 2001b; Ogura et al., 2003) and is involved in recognising

muramyl dipeptide derived from peptidoglycan, which is present in the bacterial cell wall (Inohara et al., 2003).

6.1.3 Immunology of Crohn's disease

IBD is characterised by a T cell dysregulation with an imbalance of activated Treg/Th1/Th2 and Th17 cells (Sanchez-Munoz et al., 2008). Granulomatous diseases such as CD display Th1 cytokine profile producing IL-2 and IFN-γ, which activate macrophages releasing proinflammatory cytokines IL-1, IL-6 and TNFα (Melmed and Abreu, 2004). The cytokine profile in CD has shown to be a Th1 environment within the lesion. CD patient's LP CD4+ T cells stimulated via the CD2/CD28 pathways have increased IFN-γ secretion with a corresponding increased number of IFN-γ secreting cells in LP compared to controls. LP CD4+ T cells also have decreased secretion of IL-2, IL-4 and IL-5 while PB CD4+ T cells have increased IFN-γ and decreased IL-2 secretion (Fuss et al., 1996). This is confirmed by Parronchi et al. (1997) who cultured IFN-γ secreting T cell clones from gut biopsy specimens from CD patients, but not IL-4 secreting clones. In the granuloma itself, IFNγ+ lymphocytes detected by immunostaining are also more common while IL-4 and IL-10 staining lymphocytes are rare (Kakazu et al., 1999). Resection samples from CD show increased IL-8, TNFα and IL-6 compared to controls despite patients being on anti-inflammatory medication (McCormack et al., 2001).

6.1.4 Helminth immunology

The Th2 immune response appears responsible for protection or clearance against intestinal helminth infection in animal and human infections. Infection of mice with the gastrointestinal nematode, *Heligmosomoides polygyrus*, results in an inflammatory cell infiltrate resembling a cyst, which shows accumulation of $CD4^+$ T cells in sites adjacent

to the parasite with high elevations of the Th2 cytokines, IL-4 and IL-3 in non-lymphoid tissue surrounding the cyst (Morimoto et al., 2004). IL-10 or IL-4 deficient mice fail to eliminate *Trichuris muris* (Schopf et al., 2002). In humans, the intensity of *A. lumbricoides* infection is inversely associated with Th2 cytokines (Turner et al., 2003) and with lower Th1 cytokine responses in those with high parasite load compared to endemic controls (Geiger et al., 2002). Further evidence for the regulating role of helminths in the mucosa is shown by a patient with faint signs of ulcerative colitis with an *Enterobius vermicularis* infection who was subsequently treated and then developed active UC. Analysis of mRNA showed up-regulation of TGF-β and IL-4 during the *E. vermicularis* infection, slightly increased IFN-γ and detectable IL-10 mRNA. FoxP3+ cells were abundant in the mucosa during the infection but decreased in the active UC (Buning et al., 2008). Infections with helminth parasites appear to modify cytokine responses to other antigenic stimuli. For example, PBMC from *S. mansoni* infected people with asthma produce less Th2 cytokines to dust house mite antigen than PBMC from uninfected asthma patients, which is thought to be attributable to IL-10 (Araujo et al., 2004).

Immune response to *N. americanus* appears not protective, as evidenced by the continued susceptibility to infection by those living in endemic areas (Behnke, 1987). As hookworms are not affected by the immune response, it suggests that they subvert the response towards a phenotype that ensures their survival. Some E/S products from *N. americanus* bind to natural killer cells and stimulate the production of IFN-γ therefore potentially skewing a protective Th2 response seen in other parasitic infections to a Th1 response (Hsieh et al., 2004).

Volunteers inoculated with low numbers of *N. americanus* larvae show variable responses with often meagre rises in total and parasite specific IgG and IgE (Maxwell et

al., 1987; Wright and Bickle, 2005). In contrast, multiple large doses of larvae given to a volunteer show large rises in IgG specific *N. americanus* E/S products though total IgE only increased with multiple doses (Ogilvie et al., 1978). In endemic communities, there is a strong antibody response to larval and adult antigens by all five isotopes (Pritchard et al., 1992). Combined IgG, IgA and IgM to E/S products and cuticular collagen are positively correlated with egg production (Pritchard et al., 1990) while IgE shows a negative correlation between with hookworm weight and fecundity showing it plays some role in immunity (Pritchard et al., 1995).

Response to *N. americanus* antigen gave rise to a mixed cytokine response in individuals from an endemic area with production of Th1 cytokines and Th2 cytokines (Pit et al., 2000; Pit et al., 2001; Geiger et al., 2007). In a comparison with endemic controls, in hookworm-infected patients and cured hookworm patients there was no significant difference in IFN-γ, TNF-α and IL-12 production by PBMC in response to stimuli by hookworm between hookworm-infected and endemic controls but there was significantly higher production of IL-5, IL-13 and IL-10 by endemic controls (Geiger et al., 2004). Despite a mixed cytokine profile, there is possibly a bias towards a Th2 pattern as there is a negative correlation with egg burden and production of IFN-γ and once treated, hookworm-infected individuals produce more IFN-γ. Pre-treatment IL-5 response is an also negatively correlated with reinfection egg burden (Quinnell et al., 2004). The Th2 bias appears more pronounced in adults than in children with IFN-γ/IL-10 ratios from adult PBMC lower in hookworm patients than in endemic controls (Fujiwara et al., 2006).

6.1.5 Using parasites to cure disease

Since intestinal helminths are thought to induce a Th2 cytokine environment and diseases such as CD have elements of a Th1 inflammatory process, investigators have used helminths to treat some conditions. Three of four CD and three UC patients achieved remission after being given a single oral dose of 2500 infective *Trichuris suis* eggs orally every two weeks (Summers et al., 2003). Although remission was only temporary, regular three weekly doses showed a remission rate of 73% in active CD over 24 weeks (Summers et al., 2005a). Over 12 weeks, in a double blind study, patients with ulcerative colitis were given *T. suis* fortnightly and showed significant improvement compared to controls (Summers et al., 2005b). *T. suis* was considered a good option as it could be raised in large numbers in pathogen free pigs, does not multiply in the host and has never found to cause human disease (Elliott et al., 2007). However, it was noted that *T. suis* is invasive and could potentially migrate within the human body ending up within other organs (Van Kruiningen and West, 2005). A sexually mature *T. suis* male worm was found in the cecum of a 16-year-old boy following oral doses of *T. suis* eggs (Kradin et al., 2006). Van Kruiningen and West (2007), commenting on this case, note that that a sexually adult male in the large intestine shows that *T. suis* is invasive as it must penetrate the small intestine mucosa and move to the large intestine as part of its development. The USA Food and Drug Administration currently detains all imported *T. suis* eggs into the USA citing these concerns (US Federal Drug and Administration, 2009).

Hookworm infection may be more preferable than *T. suis.* Since adult hookworms have been reported to survive over 18 years (Beaver, 1988), only one inoculation may be needed compared to multiple doses of *T. suis*. Hookworm infection tends to be asymptomatic (Pritchard and Brown, 2001) and hookworm is unlikely to be spread in

societies where lavoratories are routinely used, as the larvae require incubation in soil for around seven days to become infective (Markell et al., 1999). *N. americanus* is preferable to *Ancylostoma duodenale* due to its longer survival time in the human host, its less severe pulmonary symptoms (Markell et al., 1999) and its lesser effect in causing iron deficiency anaemia (Albonico et al., 1998). *N. americanus* has been used to establish whether hookworm could be maintained in and have an influence on CD patients (Croese et al., 2006a). Hookworm is also being considered as a modifier of the allergic response in atopic individuals (Mortimer et al., 2006).

As is apparent, helminths are considered to both regulate both Th1 type disease such as CD and Th2 type disease such as asthma and UC indicating the Th1/Th2 paradigm over simplifies the complex immunological interactions occurring.

The aim of these experiments was to determine if hookworm infection had a demonstrable effect on the immunological response in CD patients.

6.2 Methods

6.2.1 Antigen preparation

Crude bacterial antigens and a whole yeast suspension were prepared to stimulate immune cells for the lymphocyte proliferation stimulation and whole blood cytokine assay. *Escherichia coli* ATC 25922, *Enterococcus faecalis* ATC 29212 and a wild type *Pseuodomonas fluorescens* were cultured aerobically at 37 ºC overnight on 5% sheep blood agar (Oxoid). A wild type *Bacteroides fragilis* (Queensland Pathology Services, Townsville) was cultured anaerobically overnight at 37 ºC on 5% sheep blood agar and *Saccharomyces cerevisiae* from commercial baker's yeast (Goodman Felder Consumer Foods LTD) cultured aerobically at 37 ºC for 48 hours on Mycological agar (Difco). A human faecal specimen was cultured overnight at 37 ºC aerobically on 5% sheep blood

agar and for 48 hours anaerobically. The human faecal sample would give a large variety of bacteria. Bacteria were harvested into deionised water and a small volume of zirconia/silica 0.1 mm beads (Biospec Products Inc) was added. Their cellular structures were then disrupted in a mini Bead Beater (Biospec Products Inc) for four minutes. The suspensions were centrifuged at 13000 RPM for 10 minutes and filtered through a 0.2 µm filter (Braun).

Protein concentration was assessed using Coomassie® Brilliant Blue G-250 (Bio-Rad). Two millitres of reagent was diluted with eight millilitres of deionised water. A 1000 µg bovine serum albumin standard was prepared by adding 2000 µg to two millilitres of deionised water. Three standards were prepared: 125 µg, 250 µg and 500 µg. Twenty microlitres of crude antigen or standard was added to one millilitre of reagent and incubated at room temperature for ten minutes. The absorbance was read 595 nm and protein concentration calculated from the standard curve. The protein concentration of each crude antigen was adjusted to 200 µg/ml with deionised water.

S. cerevisiae was harvested into sterile PBS with 2.5% formalin and left for 15 minutes. These were washed three times (1000 g, 10 min) in PBS and concentration adjusted to 1 X 10^8/ml.

The sterility of the soluble antigens and *S. cerevisiae* suspension were checked by culturing a small volume onto 5% sheep blood agar (Oxoid) and incubating overnight aerobically at 37 ºC. The soluble antigens and *S. cerevisiae* suspension were stored at -70ºC until used.

N. americanus E/S antigen was made and supplied by Professor David Pritchard, University of Nottingham (Carr and Pritchard, 1987).

6.2.2 Subjects

Ten CD patients, diagnosed by Dr John Croese by standard clinical and pathological criteria, were inoculated on the forearm with L3 larvae of *N. americanus*. Four patients were inoculated with 25 L3 and then reinoculated with another 25 larvae 27 weeks later. Five patients were inoculated with 50 L3 larvae with one patient reinoculated 27 weeks later with 50 larvae. One patient was inoculated with 100 L3 larvae. Eight of these subjects were taking anti-inflammatory medication: prednisone (n = 6), methotrexate (n = 7), azathioprine (n = 1) or a combination of these.

Three subjects without CD were also inoculated with *N. americanus*. Control 1 was inoculated with 50 L3, Control 2 was inoculated with 100 L3 and Control 3 with 31 L3 and 27 weeks later with an additional 50 L3.

Ten subjects with CD acted as CD controls. Of these CD patients without *N. americanus,* seven were taking methotrexate, five were taking prednisone and three were taking azathioprine or a combination of these. One subject was not taking any anti-inflammatory medication. Lymphocyte stimulation and whole blood cytokine assays were set up using blood collected on the same day.

Eleven subjects without known CD or *N. americanus* infection acted as normal controls.

6.2.3 Lymphocyte proliferation assay

Blood was collected into lithium heparin tubes (Becton Dickinson) from 10 patients with CD inoculated with *N. americanus*, 10 patients with CD and 11 controls without CD or hookworm infection. Blood was collected 16–22 weeks after inoculation for four of the CD patients and between 41–53 weeks for the other six patients.

PBMC were separated from heparinised blood using Ficoll-Hypaque (Amersham Biosciences). Twenty millilitres of heparinised blood was diluted in an equal volume of RPMI-1640 (Invitrogen, Australia) and slowly added to overlay 3 ml Ficoll-Hypaque and then centrifuged at 500 g for 20 minutes. The PMBC layer was siphoned off and PMBC washed twice in RPMI-1640 and then suspended in 10 ml of RPMI-1640. Cells were counted in a counting chamber and adjusted to 1 X 10^6/ml in RPMI-1640. One hundred microlitres was added to 96 well plates (Nunc) with 100 µl ds-RPMI 1640 medium supplemented with penicillin (100 U/ml), streptomycin (100 µg/ml), L-glutamine (2 mM), Hepes buffer (25 mM) and 10% foetal calf serum. Triplicate wells were then stimulated with 2 µg/ml of the mitogen PHA (Sigma) and 10 µg/ml of the *E. coli, B. fragilis, E. faecalis, Ps. fluorescens,* mixed faecal aerobic, mixed faecal anaerobic antigens and 5 X10^6 whole *S. cerevisiae*. For each subject triplicate wells were included that had no antigen added. Culture plates were incubated in 5% CO_2 at 37˚C and proliferation was determined at 96 hr, 120 hr and 144 hr by measuring [^{3}H]-thymidine incorporation (Amersham-Pharmacia Biotech; 1.0 µCi/ml) for four hours. Results were expressed as the Stimulation Index (SI), the proportion of counts per minute in stimulated cultures compared to that of unstimulated cultures. The maximum SI out of the three time points in individual proliferation assays was chosen and then compared statistically between the three groups.

6.2.4 Whole blood cytokine assays

Either one millilitre of whole blood or one millilitre of whole heparinised blood diluted 1:4 with RPMI (Joseph et al., 2004) was added to 24 well plates (Nunc). The prepared antigens (10 µg/ml), *S. cerevisiae* (5 X10^6) and PHA (2 µg/ml) were added to individual wells. The plates with whole blood were incubated at 37˚C in 5% CO_2 for 24 hours while the diluted blood was incubated under the same conditions for 48 hours.

The contents of the well were removed and centrifuged at 3000 RPM for seven minutes, the plasma collected and stored frozen at -70˚C until used.

The cytokine, IFN-γ was analysed on the blood incubated for 24 hours. The cytokines Interleukin (IL)-2, IL-5, IL-6, IL-10, IL-13 were analysed using whole blood diluted in RPMI incubated for 48 hours. The concentration of cytokines in the samples was determined with kits supplied by Mabtech. Capture antibody was diluted in PBS and 50 µl added to 96 well plates (Costar) then incubated overnight at 4 ºC. Plates were washed twice with PBS then blocked with 100 µl PBS-0.1% BSA and incubated one hour at room temperature. Plates were then washed five times with PBS-Tween and 50 µl of sample and seven standards added in duplicate to wells and plates incubated two hours at room temperature. Plates were washed a further five times, 50 µl of biotin-secondary antibody added and incubated for one hour at room temperature. Plates were washed five times in PBS-Tween, 50 ul of Strepavidin-Horse radish peroxidase added and incubated for one hour at room temperature. Plates were then washed five times and 50 µl TMP peroxidase substrate (Bio-Rad) added. The reaction was allowed to develop, 50 µl of 0.5 M Sulphuric Acid added and absorbance read at 450 nm (Multiskan Ex). Concentration of cytokine in samples was calculated from the standard curve.

Serum samples were also collected during the study and stored at -20˚C. Interleukin-6, IL-10, IL-13, IFN- γ and TNF-α were analysed on three of the CD subjects with *N. americanus* and not taking methotrexate, one who was on methotrexate and three control subjects without CD who were also infected with *N. americanus*.

6.2.5 IgG ELISA assay to E/S *N. americanus* antigen

Serum samples collected from baseline from CD and control subjects infected with *N. americanus* were collected at intervals during the course of the study and used to measure IgG and in the Western Blot.

Ninety-six well plates (Maxisorp) were coated with 50 µl with 50 µg/ml of E/S of adult *N. americanus,* crude L3 larvae antigen or E/S antigen from L3 larvae then diluted in PBS-5% milk and left at 4˚C overnight. Plates were blocked with PBS with 5% milk and left for two hours. Serum was diluted 1:500 with blocking buffer and 50 µl added in triplicate to wells and left overnight at 4˚C. Plates were washed 5 times with PBS-Tween and a 1:1000 dilution of peroxidase conjugated anti-human IgG in PBS and incubated at room temperature for two hours. The plates were washed and the bound antibody detected with 100 µl TMB substrate. Reactions were stopped with 20 µl 2M H_2SO_4 and read at 450 nm (Dynex).

6.2.6 Western blot

Two hundred micrograms of E/S *N. americanus* antigen was loaded onto a 10% SDS-PAGE gel and separated by electrophoresis at 100 V for one hour and electrophoretically transferred to nitrocellulose. The nitrocellulose was dried and cut into strips then blocked with 5% milk. Serum specimens taken at baseline, six to seven weeks post-inoculation and the last specimen taken in the study were diluted 1:200 (for IgG detection) in or 1:20 (for IgE detection) in 5% milk-TrBS and incubated overnight at 4˚C. The strips were then washed three times in TrBS and incubated with either a antihuman IgG (1:500) peroxidase or IgE alkaline phosphatase conjugate (Binding Site) or two hours at room temperature. The strips were then washed another three times in

TrBS and blots developed with 4-choloro-1-napthol for the anti-IgG detection or BCIP/NBT and stopped with water.

6.2.7 Statistical analysis

Results were entered into Microsoft Excel (2003) and SPSS version 13 (SPSS Inc. Chicago, IL). For analysis of the lymphocyte proliferation stimulation and cytokine results probabilities were assigned using the Kreskas-Wallis test. Nonparametric multiple comparisons were performed on groups showing significant ($p<0.05$) differences as described by Zar (1999).

6.3 Results

6.3.1 Serum IgG levels

Overall, serum IgG levels to E/S antigens of *N. americanus* were significantly elevated ($p= 0.02$) 20 to 21 weeks post-inoculation compared to baseline levels in nine subjects who had samples taken at these time intervals. One subject of the ten CD patients inoculated did not have serum specimens to include in the analysis. The mean specific IgG level at baseline was 166 µg/ml (95% CI, 63–268 µg/ml) and after 20–21 weeks, 239 µg/ml (95% CI, 148–329 µg/ml). Antibody concentration for 11 subjects from serum samples taken at baseline and subsequent weeks over the course of the study is shown in Figure 6–1. There was a significant linear increase in antibody concentration over time ($p=0.007$).

Figure 6–1 Concentration of serum IgG (µg/ml) to E/S antigens of *N. americanus* post-inoculation

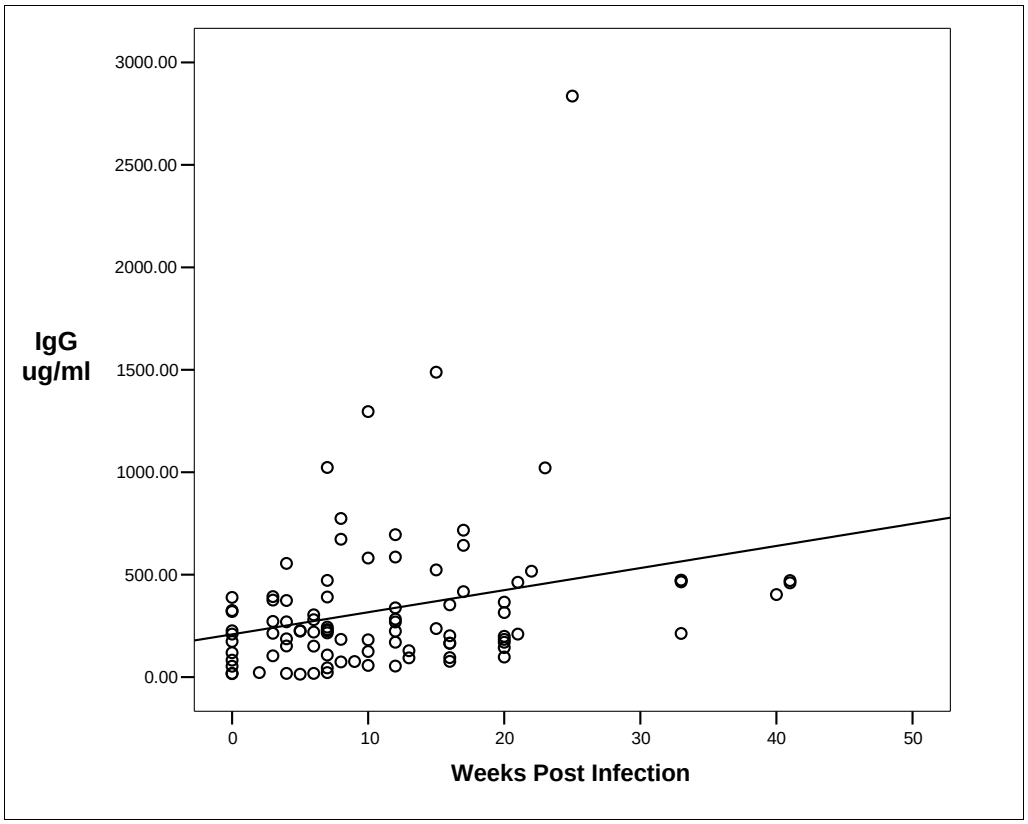


Figure 6–2 and Figure 6–3 show the individual change in specific IgG concentrations with the time, dose of L3 and medication subjects were on. Specific IgG rose steeply in two of the three control subjects with one control subject showing little change. The CD patient who also received 100 L3 also experienced a sharp rise in specific IgG, but this decreased over time.

Figure 6–2 Change in specific IgG concentration (µg/ml) over time in patients with CD (Number in bottom left corner patient identification)

Patient on: P → prednisone, M → methotrexate, A → azathioprine

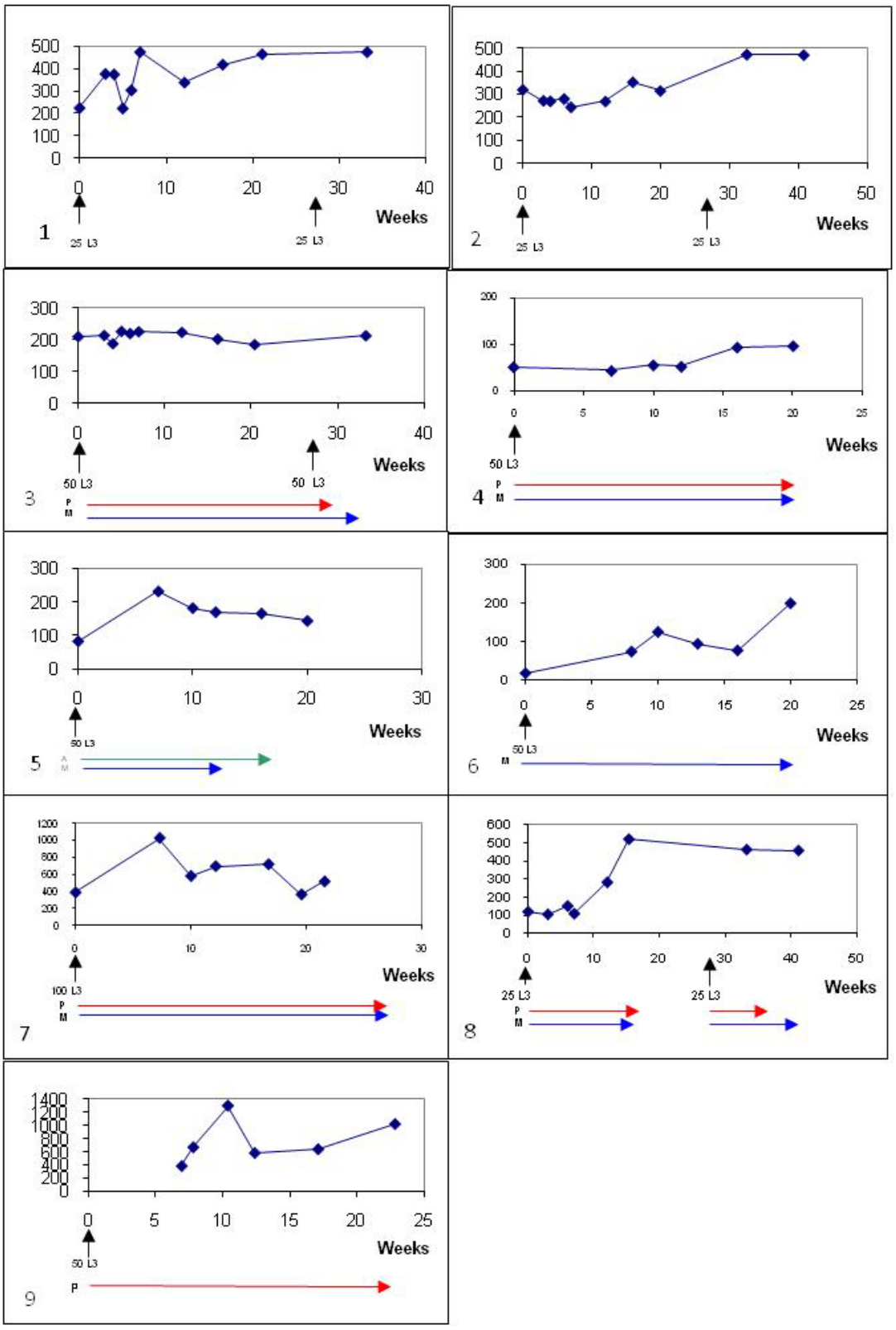
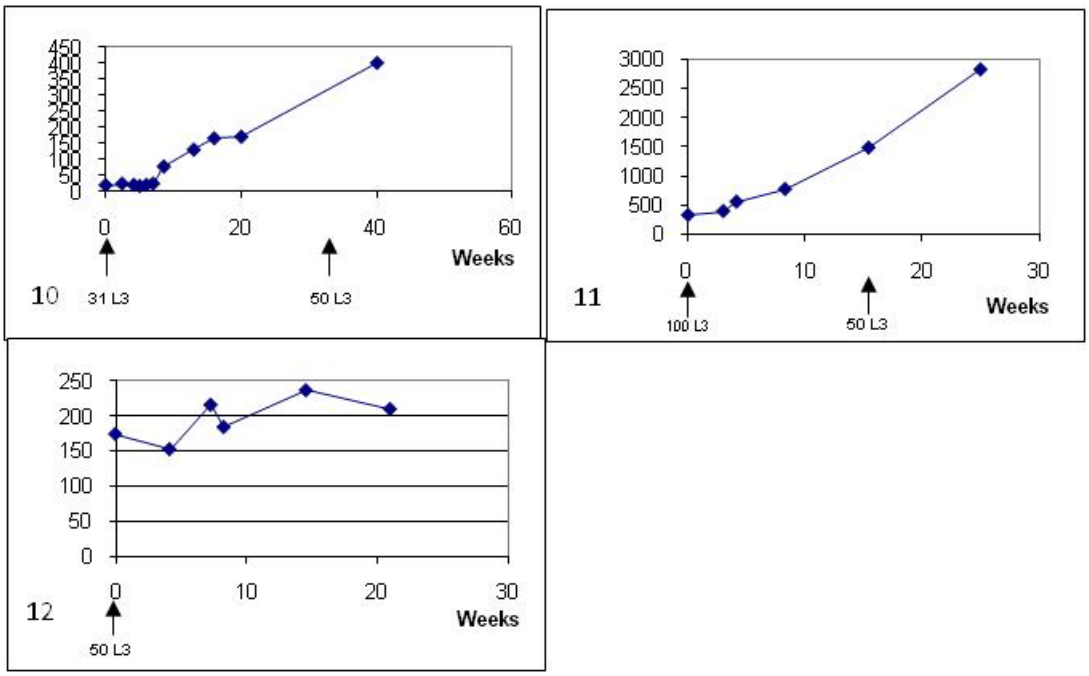

500
400
300
200
100
0
0 10 20 30 40
Weeks
1
25 L3
25 L3
500
400
300
200
100
0
0 10 20 30 40 50
Weeks
2
25 L3
25 L3
300
200
100
0
0 10 20 30 40
Weeks
3
50 L3
50 L3
P
M
200
100
0
0 5 10 15 20 25
Weeks
4
50 L3
P
M
300
200
100
0
0 10 20 30
Weeks
5
50 L3
M
300
200
100
0
0 5 10 15 20 25
Weeks
6
50 L3
M
1200
1000
800
600
400
200
0
0 10 20 30
Weeks
7
100 L3
P
M
600
500
400
300
200
100
0
0 10 20 30 40 50
Weeks
8
25 L3
25 L3
P
M
1400
1200
1000
800
600
400
200
0
0 5 10 15 20 25
Weeks
9
50 L3
P

Figure 6–3 Change in specific IgG concentration (µg/ml) over time in control subjects (Number in bottom left corner patient identification)

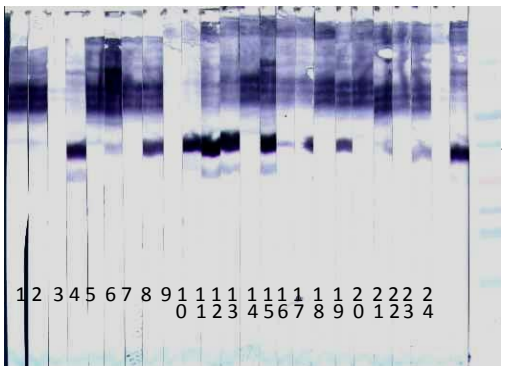


Seven of the 11 subjects with baseline values had a specific IgG peak concentration greater than double the baseline concentration. A negligible response occurred in both subjects who were inoculated with 50 L3 and were on methotrexate and prednisone. In contrast, the subject who had 25 L3 and receiving both these drugs had a good specific IgG response.

6.3.2 Western blot

There were two bands evident on the blots with sizes of 30 and 33 kDa as shown in Figure 6–4. The percentage of subjects who developed antibody to these proteins is shown in Table 6–1. One subject, in lanes 1 and 2, had not developed IgG antibody to the 33-kDa protein 33 weeks post-infection (see Figure 6–4). This was also evident in the IgG ELISA assay where there was no increase over time in IgG concentration. IgE to the 33-kDa protein was detected in one subject.

Figure 6–4 Immunoblot of ES products from adult worms of *N. americanus* probed with serum pre- and post-infection and identified for bound IgG



Odd number lanes have been probed using serum from pre inoculated subjects with the even number lanes probed with 20 – 41 weeks post inoculated serum from the same subject. Lanes 11 and 12 were probed using post inoculated serum from the same subject.

Table 6–1 Percentage of subjects recognising 30 and 33 kDa E/S proteins of *N. americanus* by Western Blot after specified periods

	Baseline (Week 0) (n =11)		**Weeks 6–7 Post-Inoculation** (n = 10)		**Weeks 20–41 Post-inoculation** (n =12)	
	30 kDa	33 kDa	30 kDa	33 kDa	30 kDa	33 kDa
IgG	0 %	0 %	10 %	60 %	42 %	92 %
IgE	0 %	0 %			0 %	8 %

6.3.3 Lymphocyte stimulation to antigens and mitogen

Figure 6–5 and Figure 6–6 show the median stimulation index to various antigens and the mitogen PHA with the vertical bars indicating the lower and upper quartile ranges.

The stimulation index was significantly different between the three groups reacting to crude soluble *B. fragilis* antigen and to whole dead *S. cerevisiae* cells. CD subjects with hookworm infection had a higher stimulation index ($p < 0.05$) to *B. fragilis* compared to controls while both CD/HW subjects and CD subjects had a higher stimulation to *S. cerevisiae* compared to controls ($p< 0.05$).

Figure 6–5 Lymphocyte stimulation to the mitogen, PHA (CD+HW, CD, Controls)

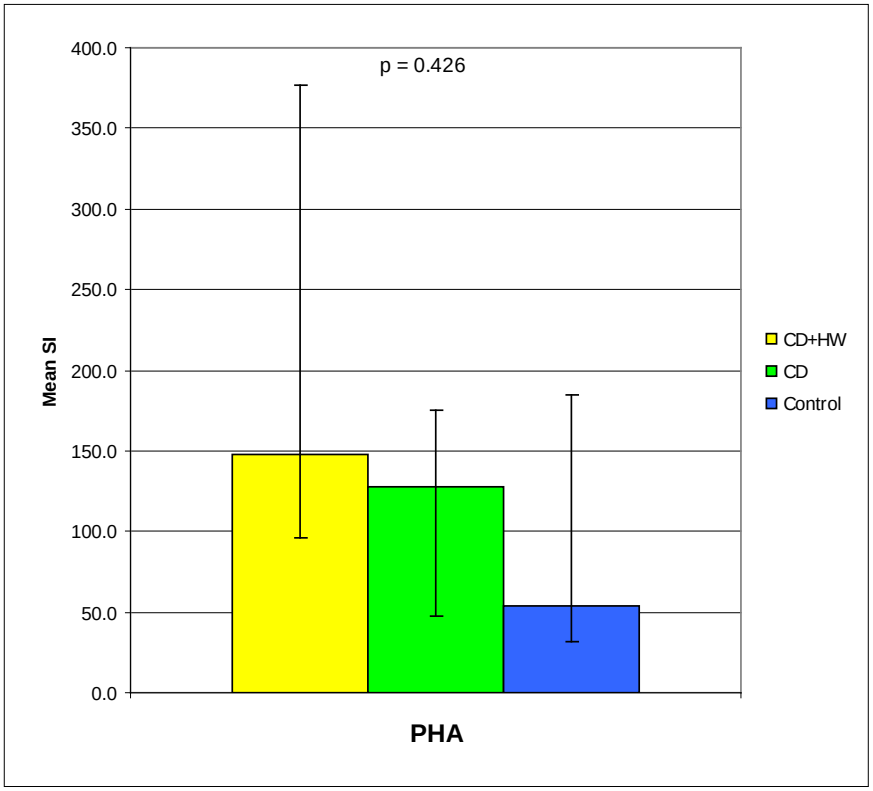
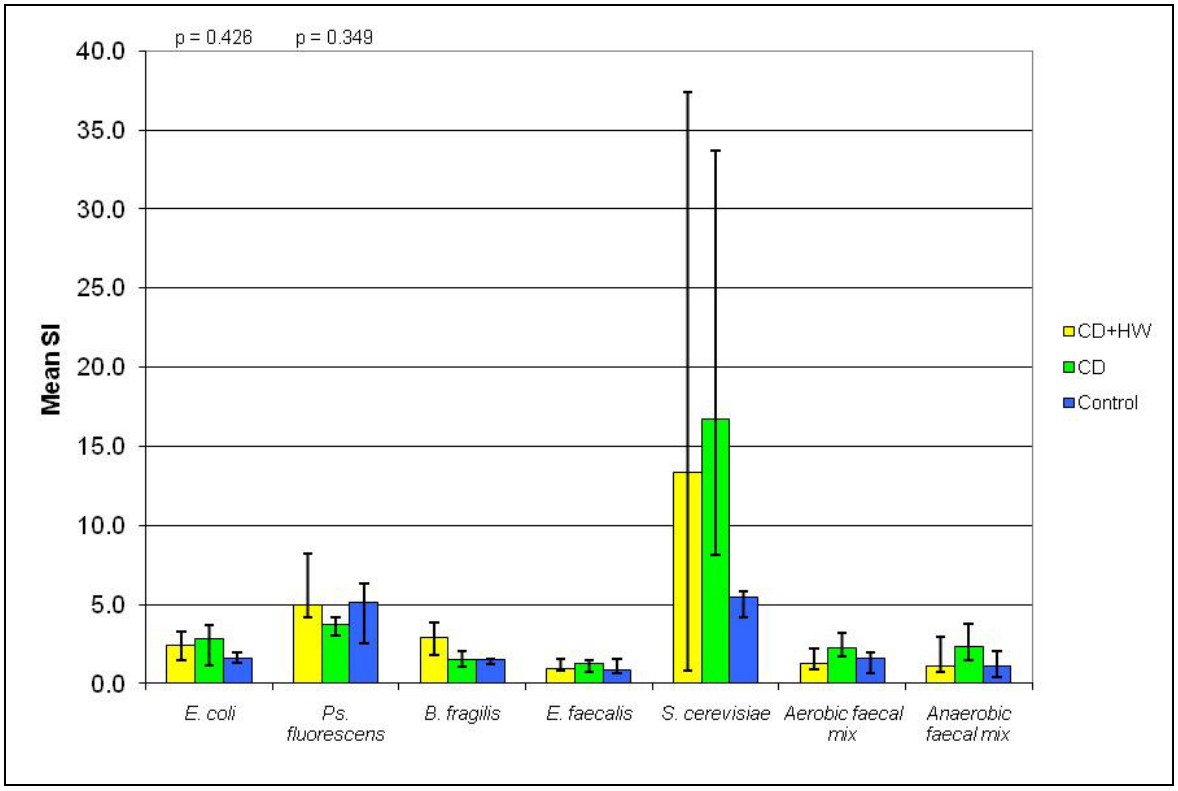


Figure 6–6 Lymphocyte stimulation to various antigens (CD+HW, CD, Controls)



6.3.4 Whole blood stimulated cytokine assays

Figure 6–7 to Figure 6–10 show the median IFN- γ, IL-6, IL-10 and IL-13 produced from one millilitre of whole or diluted blood after 24 or 48 hours from unstimulated cells and the net IFN-γ production from one millilitre of whole blood stimulated with PHA, various crude bacterial antigens and whole dead *S. cerevisiae* cells. Net cytokine is the concentration of cytokine from stimulated blood minus cytokine concentration from unstimulated blood. The error bars represent the lower and upper quartiles. CD subjects (n =20) were divided into those with hookworm infection and on methotrexate (CD/HW/M, n= 7), with hookworm infection and not taking methotrexate (CD/HW, n=3), without hookworm infection and taking methotrexate (CD/M, n=6) and those with no hookworm and not taking methotrexate (CD/A, n=4).

Figure 6–7 Net Interferon-γ production from whole blood stimulated with mitogen and various antigens

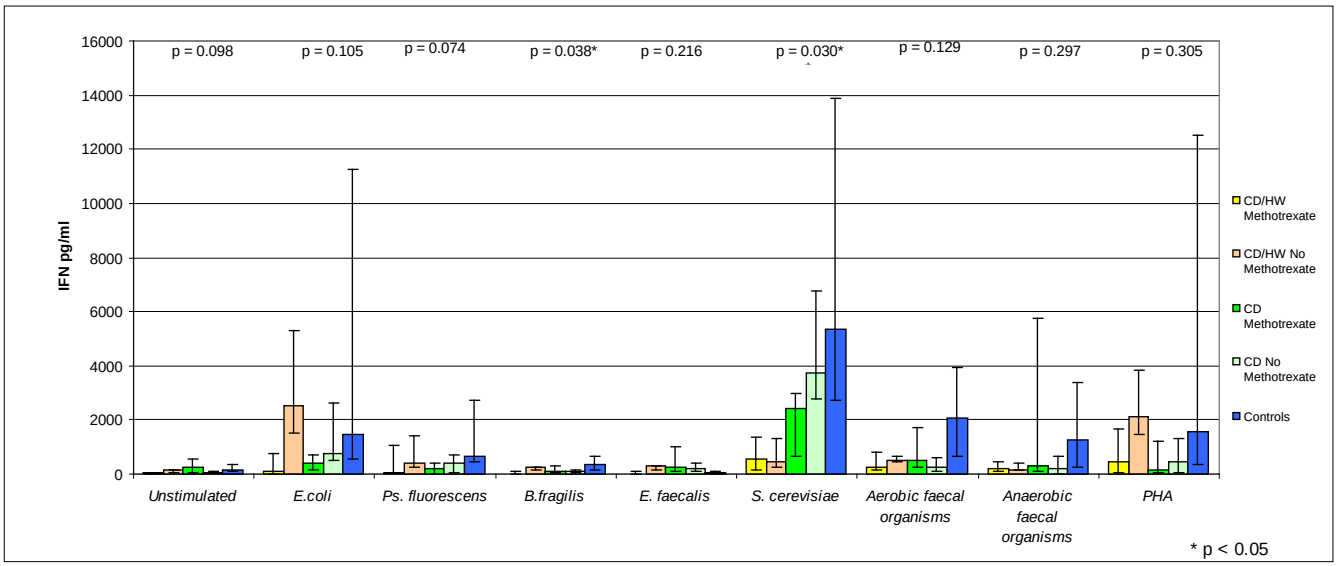


Figure 6–8 Net IL-6 production from diluted whole blood stimulated with PHA, crude *B. fragilis* antigen and whole dead *S. cerevisiae*

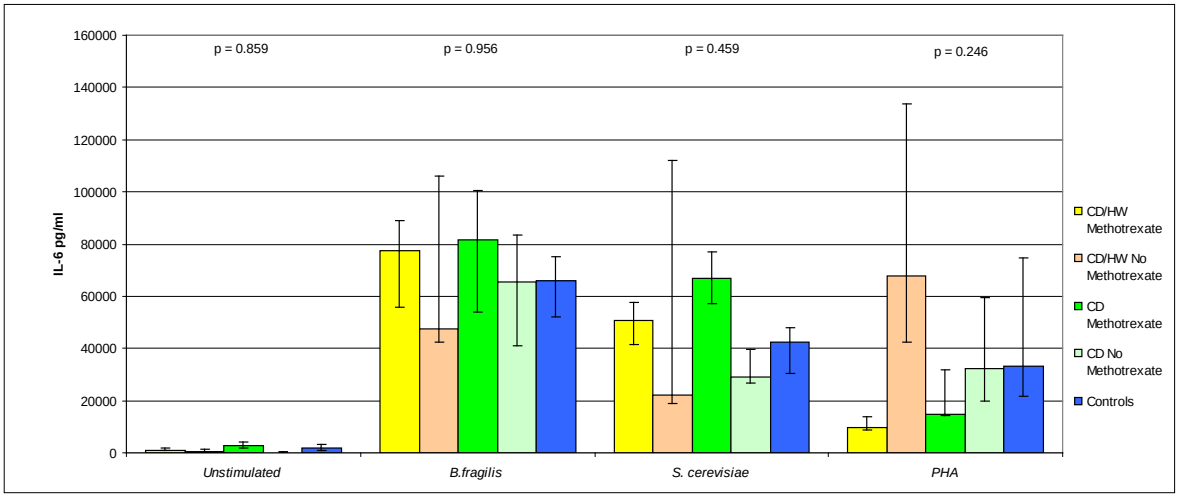


Figure 6–9 Net IL-10 production from diluted whole blood stimulated with PHA, crude *B. fragilis* antigen and whole dead *S. cerevisiae*

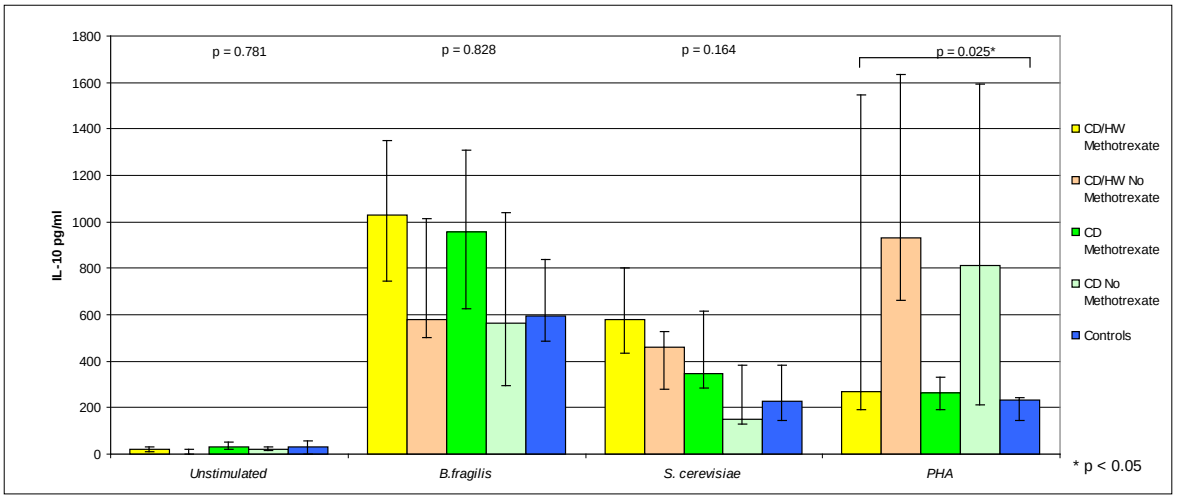


Figure 6–10 Net IL-13 production from diluted whole blood stimulated with PHA, crude *B. fragilis* antigen and whole dead *S. cerevisiae*

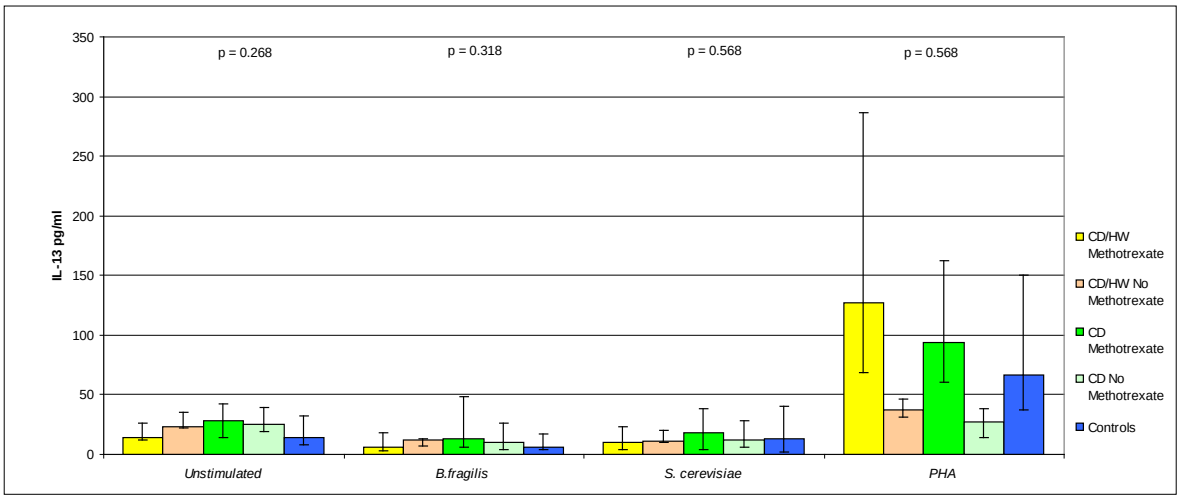
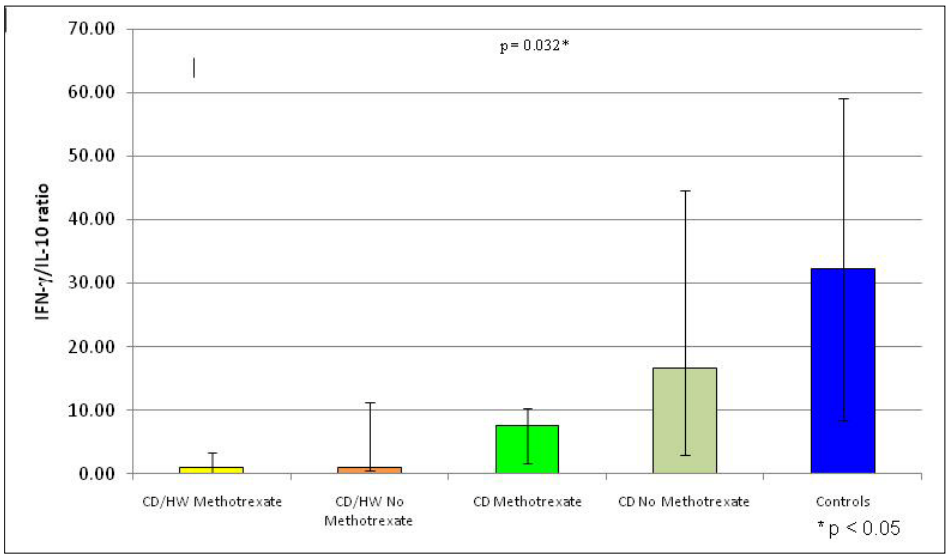


Figure 6–11 Net Interferon-γ/Net IL-10 ratio from diluted whole blood stimulated with *S. cerevisiae*



Median IFN- γ produced by controls was higher than the other groups for stimuli except PHA and crude *E. coli* antigen where CD/HW/M were higher. Net median IFN-γ was significantly different between the five groups when stimulated with crude antigen of *B. fragilis* (p = 0.038) and whole dead *S. cerevisiae* (p = 0.030). The median IFN- γ production to *S. cerevisiae* was lowest in those with CD who had hookworm infection. As only *B. fragilis* and *S. cerevisiae* showed significant results between groups in the proliferation assay and IFN- γ ELISA only additional cytokine levels in response to stimulation of these antigens were analysed.

IL-10 showed a significant difference between groups when stimulated with PHA (p=0.025) with a difference (0.10<p<0.050) between CD/A (811 pg/ml) and controls (231 pg/ml). For IL-10 production in response to *S. cerevisiae* showed greater production in CD/HW/M, followed by CD/HW, CD/M patients, control samples then finally CD/A.

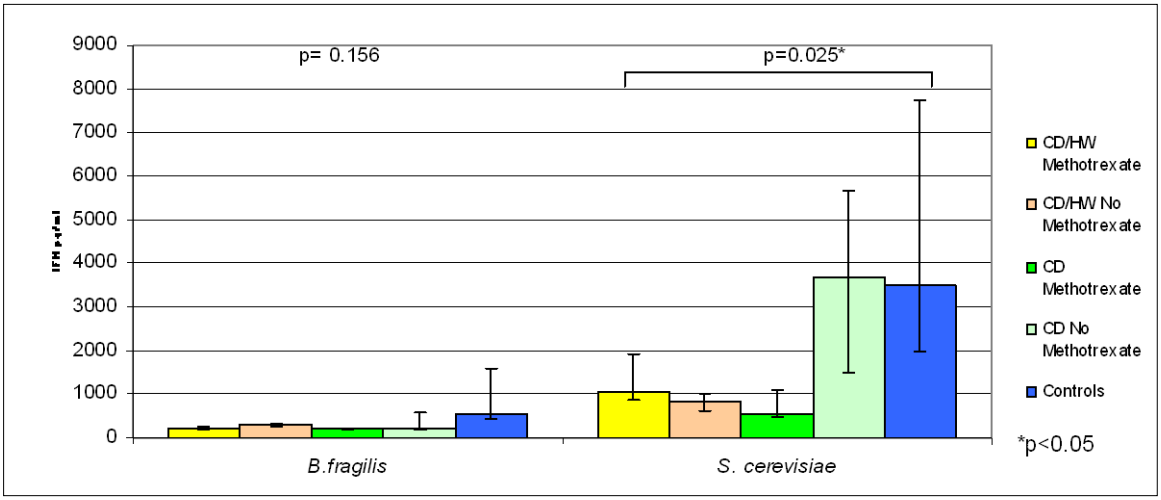
Figure 6–11 shows the ratio of net IFN- γ/IL-10 to *S. cerevisiae* and shows the lowest ratio for CD/HW/M patients followed by CD/HW patients, CD/M patients, CD/A patients then control samples.

There was no significant differences involving the other tested cytokines and the groups. IL-13 production was generally low except for stimulus by PHA. Interestingly, the pattern with low median levels for CD patients not on methotrexate was reversed in comparison to IL-10 levels.

Post-hoc analysis showed only a reduction for median IFN-γ ($p<0.05$) between CD/HW/M subjects (18 pg/ml) and controls (337 pg/ml) for *B. fragilis* stimulated whole blood cultures although absolute production of IFN- γ in response to *B. fragilis* was relatively low compared to PHA, gram negative stimuli and *S. cerevisiae*. The median IFN- γ production for this group (552 pg/ml) and controls (5371 pg/ml) also showed a difference in response to *S. cerevisiae* ($0.10<p<0.05$).

IFN- γ was also tested on the 48 hr incubated RPMI-1640 diluted samples stimulated with *B. fragilis* and *S. cerevisiae*. There was insufficient sample to test IFN-γ on unstimulated samples. However, gross production of IFN- γ also showed a significant difference between groups when stimulated with *S. cerevisiae* with post-hoc analysis showing a difference between CD/HW/M subjects having a lower median IFN-γ (540 pg/ml) compared to controls (3510 pg/ml).

Figure 6–12 Gross production of IFN- γ after stimulation with *B fragilis* and *S. cerevisiae in* RPMI diluted blood after 48 hr incubation



6.3.5 Serum cytokines

The cytokine levels in serum samples are shown in Table 6–2. TNF-α was also tested, but all samples were below the limit of detection. It was suspected that a benefit was experienced due to *N. americanus* infection by those with CD around week seven following infection based on responses to the IBD Questionnaire (Croese et al., 2006). No pattern in serum cytokines over time or difference at week seven was evident in either controls or CD patients.

Table 6–2 Serum cytokine levels in *N. americanus*-infected subjects

Weeks Post-Infection IL-10 pg/ml																
	0	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**10**	**15**	**16**	**17**	**20**	**21**	**23**	**25**
Control 1	1052			932			733			596				355		
Control 2	51		33	43				30		73						92
Control 3	13	32		< 2	40	21	< 2				< 2		< 2			
CD1	18		24				669					46		119		
CD2	191		161	446			184				232		74			
CD3							19		21			33			43	
CD4	10						303				312		175			
Weeks Post-Infection IFN pg/ml																
	0	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**10**	**15**	**16**	**17**	**20**	**21**	**23**	**25**
Control 1	43			41			40			73				95		
Control 2	331		162	227				630		832						661
Control 3	< 4	35		< 4	38	< 4	< 4				< 4		< 4			
CD1	37		36				74					82		84		
CD2	85		47	120			45				54		37			
CD3							33		< 4			47			36	
CD4	115						155				166		102			
Weeks Post-Infection IL-6 pg/ml																
	0	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**10**	**15**	**16**	**17**	**20**	**21**	**23**	**25**
Control 1	120			133			199			253				109		
Control 2	< 7		< 7	< 7				< 7	< 7	< 7	< 7					< 7
Control 3	< 7	< 7		< 7	< 7	< 7	< 7				< 7	< 7	< 7	< 7		
CD1	< 7	< 7	< 7	< 7			154					125		253		
CD2	157		< 7	< 7			< 7	< 7			< 7	< 7	< 7	< 7		
CD3							< 7		< 7	< 7		< 7	< 7		< 7	< 7
CD4	120						183				237		106			
Weeks Post-Infection IL-13 pg/ml																
	0	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**10**	**15**	**16**	**17**	**20**	**21**	**23**	**25**
Control 1	< 4			< 4			< 4			< 4				< 4		
Control 2	271		105	284				176		524						578
Control 3	< 4			< 4	< 4	< 4	< 4				< 4		< 4			
CD1	< 4		< 4				< 4					< 4		< 4		
CD2	< 4		< 4	< 4			< 4				< 4		< 4			
CD3							< 4		< 4			< 4			< 4	
CD4	< 4						< 4				< 4		< 4			

6.4 Discussion

Most subjects mounted a specific immune response to *N. americanus* manifested as a rise in specific IgG response despite many being on immunosuppressive therapy. Only one CD subject did not show a change in specific IgG, and no specific antibody was found in the Western blot. In a similar trial (Mortimer et al., 2006) with ten normal individuals inoculated with ten to 50 L3, the specific IgG antibody response increased

quickly over a 12-week period irrespective of hookworm dose. Median specific IgG concentration rose approximately five fold for doses of 25 and 50 L3 while about two fold for 10 L3. In case studies of individual experimental infection, one subject showed a strong IgG response (Maxwell et al., 1987) and the other a weak response (Wright and Bickle, 2005). In the current study, the overall rise in specific IgG was strong, greater than five times the baseline level, in three of the eleven total subjects who had IgG measured, two of whom were control subjects. Methotrexate and azathioprine, but not prednisones, are known to inhibit antibody response (Park et al., 1996; Kapetanovic et al., 2006; Vermeire et al., 2007). One control subject receiving 50 L3 also showed a weak IgG response indicating apart from dosage, an individual's innate or genetic factors control the level of immune response. Possibly, there is little benefit for individuals with hookworm infection who do not respond vigorously to infection. Allergic inflammation causes premature detachment of the hookworm, restricted feeding and expulsion (Croese et al., 2006b).The level of immune response may dictate to what extent it affects the mucosal inflammation in CD. In some individuals, the response to hookworm may be minimal and have little effect on gut immunology. Differences in naïve individual's cytokine responses to hookworm infection has been previously observed (Geiger et al., 2008)

Food allergies may contribute to the cause of IBD and *S. cerevisiae* found in baker's yeast has been implicated with intolerance to food containing raw yeast found more often in patients with CD than UC (Brunner et al., 2007). Yeast-like particles can also be found in granulation tissue of inflamed clonic mucosa (Oshitani et al., 2003). Determination of anti-*Saccharomyces cerevisiae* antibody directed against oligomannosides of *S. cerevisiae* (Sendid et al., 1996) is a diagnostic test for CD and is significantly associated with CD with ASCA having increased prevalence in CD

patients (Quinton et al., 1998; Glas et al., 2002). CD patients also have higher lymphocyte proliferative responses to baker's yeast compared to healthy controls (Young et al., 1994). In this study, median lymphocyte proliferative responses of both CD/HW and CD subjects towards the stimuli tended to be higher than control samples while significantly greater for *B. fragilis* and *S. cerevisiae.* This agrees with van den Bogaerde et al. (2001) who noted an increased PB lymphocyte reactivity in CD patients for a wide variety of food and microbial antigens compared to controls. Thirty-three per cent of the CD subjects in that study showed higher proliferative responses to a *Bacteroides* spp. compared to none of the control subjects and 19% had higher responses to baker's yeast compared to 3% of controls. Although the median proliferative response was greater in those without hookworm towards *S. cerevisiae,* this was not significant. The increased lymphocyte proliferative response to *B. fragilis* in CD could be due to a breakdown in the musoca allowing contact with stimulated T cells (Bogaerde et al., 2001). The initial study design compared proliferation responses of individuals pre- and post-hookworm inoculation to the various antigens but a technical fault was found with the cell harvester, which was deemed to have invalidated the results.

If the CD immunological paradigm is correct, it would be advantageous to suppress the Th1 inflammatory process though medication or other processes. Methotrexate is known to inhibit IL-6, IFN- γ, IL-13 production in stimulated whole blood cultures (Gerards et al., 2003; Kraan et al., 2004), increase IL-10 gene expression in PBMC after PHA stimulation (Constantin et al., 1998) and *in vivo* reduce a Th1 profile (Herman et al., 2008). Hookworm is an organism that appears to induce a Th2 environment within the gut, which may down regulate the Th1 inflammatory process in CD. If both methotrexate and hookworm act synergistically, it would be expected that there would

be a reduced Th1 profile when those two factors were in combination and a shift from this profile when either or both were absent. This was observed with CD/HW/M patients having the lowest IFN- γ/IL-10 ratio in response to *S. cerevisiae* followed by CD/HW, CD/M and then CD/A patients.

Even though hookworm may induce IL-10 and reduce IFN- γ, the role or effect of IL-10 in CD patients is unclear. Serum concentrations of IL-10 has shown to be higher in active CD (Kucharzik et al., 1995) as well as showing no difference between control subjects (Nielsen et al., 1996). Sventoraityte et al. (2008) found in CD patients in remission, an increased production of IL-10. In contrast, granulomas induce low IL-10 levels (Schreiber et al., 1995) and anti-CD3 stimulated, with or without PHA, PBMC or LPMC show no difference in IL-10 concentration in supernatants between CD and control subjects (Gasche et al., 2000) As a CD therapy, IL-10 does not induce remission (Siegmund and Zeitz, 2004). Rather than causing remission, the effect of the hookworm could be to control some inflammation associated with CD.

Capsule endoscopy shows visible current and post-feeding sites of hookworm in the distal duodenum and proximal jejunum (Croese et al., 2006b). E/S antigens from adult worms can down-regulate inflammatory TNFα (Geiger et al., 2007) and in the animal model, *Ancylostoma ceylanicum* is thought to produce many E/S molecules at the site of attachment (Bungiro and Cappello, 2005). It would be informative to analyse cell and cytokine type within these ulcerations to determine if these feeding sites can skew the immunological milieu of the mucosa tissue or draining lymph nodes to a Th2 profile. Tissue biopsies were analysed for cytokine mRNA of these subjects, but results not obtained due to sample degradation.

Conclusions from these experiments performed on PBMC rely on the assumption that the immune response from PBMC reflects what is occurring at the site of attachment. This relies on recirculation of lymphocytes through the body from the site of attachment. In mucosal immune responses, antigen presentation occurs in Peyer's patches. Here, they encounter foreign antigens, become activated, leave the Peyer's patch and pass through the mesenteric lymph nodes to the thoracic duct. They leave the thoracic duct and enter peripheral blood to circulate through the body to then renter mucosal tissue (Janeway, 2005). Using *Trichuris muris* in the mouse model for *T. trichiura* infection, the Th2 cytokine response occurring in the mesenteric lymph nodes of resistant mice or the Th1 cytokine response occurring in susceptible mice is also detectable in the peripheral blood lymphocytes. Using PBMC is likely to be a feasible alternative from using intestinal mucosal lymphocytes for monitoring human intestinal responses (Taylor et al., 2000).

The immunology of CD is yet to be fully elucidated. Immunological mechanisms may operate in patients differently whether the disease is in early or late stages (Kugathasan et al., 2007), active or inactive disease (Kucharzik et al., 1995) or of those in remission (Sventoraityte et al., 2008). Hookworm, as a therapy, may only benefit patients that are in one particular stage of CD. They may also only assist some patients who respond in a certain immunological way to hookworm infection. Proliferative responses to *N. americanus* antigen, PHA and tetanus toxoid in volunteers receiving under 50 L3 show variable responses with some volunteers showing no increase in response to stimulation (Maxwell et al., 1987). Hookworm may not be suitable in those who have low immunological responses to infection.

Alternatively, rather than curing established disease intestinal parasite infection may prevent conditions such as CD developing. The immunologic microenvironment at

the site of attachment of the hookworm may induce the gut mucosa into a state where it is difficult for CD to manifest. The absence of intestinal parasites, although not a cause of CD, may be a factor that allows its development. This has important implications for elimination programs that reduce the intestinal helminth burden. As discussed in Chapter Four, low to moderate burdens appear to have negligible detectible health effects. Does complete elimination of intestinal parasites or soil-transmitted helminths allow diseases such as CD to manifest? This is an important question that needs to be answered. Is the result of the cure worse than the disease? While Korea has almost successfully eliminated intestinal helminths (Hong et al., 2006), both CD (Yang et al., 2008) and asthma (Cho et al., 2006) have increased in incidence. Has the elimination of intestinal parasites contributed to the rises of these diseases? It must also be remembered the acquisition of intestinal parasites in the developing world is different from the current method of inoculation. Hookworm acquisition and loss is likely a continuous process in the developing world while in the laboratory large numbers are immediately introduced to the host. Does the continual acquisition to the individual confer some benefit? If allergy controls hookworm population numbers in the host (Croese et al., 2006), does the host response confer a benefit to the host such as reducing the likelihood of CD developing? It is important these questions be answered to determine the real health benefit to reducing and eliminating intestinal parasite loads. The same question should also be for lymphatic elimination programs. Will breaking the transmission of filariasis, eventually leading to its elimination cause the rise in incidence of another disease? Although there is a tremendous health burden on the individuals who have LF, is there some benefit not yet understood?

This study confirms that *S. cerevisiae* has some role in the etiology of CD. It suggests that hookworm may modify the immunological response to *S. cerevisiae*. If

hookworm can modify immunological responses to other antigens, it should be considered what effect this might have when parasites are eliminated.

Chapter Seven: Conclusion

Diagnostic testing is an important part of parasitic programs. There are various methods for detecting parasitic infections in humans, many are available commercially. When used in programs involving large populations it is important that the technique chosen is suitable not only for its sensitivity and specificity but also its usability. The importance of the choice of diagnostic test is exemplified by the incorporation of evaluating diagnostic tests as a priority in lymphatic filariasis research (Gyapong, 2004).

The literature was reviewed to compare diagnostic techniques for lymphatic filariasis, STH and NCC. It was determined that diagnostic tests using the recombinant antigen BmR1 were both sensitive and specific for Brugian filariasis and that there was a linear relationship between the prevalence of microfilaria and seropositivity to BmR1. This suggested that an elimination program would not be required with a prevalence of seropositivity to BmR1 below 1% and would be indicated above 10%.

The review in Chapter 2 showed that the filter-paper collection technique, for use with the TropBio assay to diagnose lymphatic filariasis, had several reports of poor sensitivity (Simonsen et al., 1997; Gyapong et al., 1998). However, when the ICT, the approved test for lymphatic filariasis control programs, was compared to the TropBio ELISA it too showed a poor sensitivity. The literature indicated that the boiling step in the TropBio ELISA may not be required (More and Copeland, 1990) leading to reformulation of the TropBio ELISA for trial under field conditions. The literature evaluation of techniques to microscopically examine faecal samples for STH showed poor sensitivity for single specimen examination and multiple examinations on multiple

specimens are required. This calls into question the results of studies that use single examinations of faecal specimens to examine the effects of giving anthelmintics. If a single specimen has poor sensitivity in detecting STH then this is even more likely following anthelmintic treatment. If it is not truly known if STH have been eliminated by the human host then it is difficult to determine the cause of an absence of changes in parameters such as weight gain or haemoglobin, following anthelmintic treatment. It could be due to the negligible effect of STH on the host or that the technique used has not detected STH though they are still present and having an influence. Serological tests for NCC were reviewed in the literature showing that the use of some recombinant antigens had acceptable sensitivity and specificity for diagnosing NCC.

The evaluation of diagnostic tests was an important part of the research component of this thesis. The *BRUGIArapid* cassette was evaluated for its usability, readability, robustness, stability of reaction, the clearness of the instruction sheet and the packaging of the kit contents. This evaluation found that the *BRUGIArapid* cassette was easy to use at field sites but determined there were problems with the reagent bottle lids and inaccurate instructions. The test pad within the cassette could not clear blood from the reading frame and hindered the determination of a result. The manufacturer rectified the leaking bottles and instruction sheet and were examining the clearing problem. This evaluation clearly shows the importance of evaluating diagnostic tests for parameters other than sensitivity and specificity. Following the presentation of this research, the *BRUGIArapid* cassette was accepted as a diagnostic tool in Brugian Elimination programs.

The filter paper collection technique was evaluated and compared with the serum TropBio ELISA and ICT. This collection technique was considered unsuitable as a collection method for further testing for lymphatic filariasis mainly due to its poor

sensitivity in the TropBio ELISA but also due to the cumbersomeness of the technique. The ICT also surprisingly had a poor sensitivity compared to the TropBio ELISA. The TropBio ELISA was used at ten locations within PNG to determine lymphatic filariasis prevalence prior to MDA. The survey showed areas with high prevalence among areas of low prevalence indicating the importance of mass administration coverage so these areas do not become focal points for further transmission. The data gathered will allow comparison of not only prevalence but also antigen titre during monitoring of the program. The TropBio ELISA with venous blood collection was successfully used in baseline surveys in the PNG Bancroftian Elimination of Lymphatic Filariasis Programs. It is recommended that this test be considered an approved test for use in these programs.

Timor-Leste filariasis elimination programs have an effect on reducing STH prevalence due to the use of albendazole. STH infection are considered to have public health significance contributing to both malnutrition and anaemia (Crompton, 2000; O'Lorcain and Holland, 2000). A baseline parasitological survey was conducted that determined prevalence of STH, which will be used to monitor the effects of MDA. This survey determined an overall prevalence among the three sites of 34.8%, 1.3% and 0.9% of hookworm, *Ascaris lumbricoides* and *Trichuris trichiura* respectively. Although it is anticipated the MDA program will reduce prevalence, the literature review showed other factors that contribute to prevalence (Bundy, 1988). Behaviour of the population such as children having contact with soil (Bundy and Cooper, 1988) and people not accessing latrines even if available (Chongsuvivatwong et al., 1996) may result in continuing transmission and need to be addressed to reduce prevalence. The literature indicates clustering of heavily infected individuals within households (Forrester et al., 1988; Killewo et al., 1991; Behnke et al., 2000). The WHO community

treatment strategy (WHO, 2006) could be improved by requiring treatment of a household if a child has been found to have a STH infection.

The parasitological survey in Timor-Leste also found the presence of *S. stercoralis,* previously unreported in Timor-Leste. *Taenia* spp was also found. Serological evidence for NCC in several residents in Timor-Leste and PNG was determined using purified *T. solium* metacestode glycoproteins and Ag1V1/AG2 recombinant antigen. NCC has not been previously reported in these countries. It is important to confirm these findings by a more wide spread surveys. Infection by *S. stercoralis* can cause acute gastrointestinal, respiratory and cutaneous syndromes and intermittent Gram-negative sepsis. It may also cause disseminated disease in immunosuppressed individuals where the case fatality rate is high (Johnston et al., 2005). NCC is the single most common cause of epilepsy (Garcia and Del Brutto, 2000) so further surveys in both humans and pigs for confirmation of this finding is needed to determine whether this disease has entered Timor-Leste and if control measures are needed.

This thesis reviewed the evidence for the use of albendazole in STH infection in relation to weight gain and found little evidence to support a beneficial effect. Additionally, antihelmintic treatment only had a negligible effect on haemoglobin levels (Gulani et al., 2007). The last chapter therefore considered whether the loss of helminth colonisation in humans could be responsible for immune related diseases developing (Elliott et al., 2007). Hookworm infections are thought to produce a biased Th2 cytokine pattern in the host (Quinnell et al., 2004) and were used in CD patients in an attempt to modify the immune response in this disease. Hookworm infection did appear to modify the immunological response to *S. cerevisiae* in CD. Serious consideration should be given to the impact of parasite reduction programs in terms of their impact on immune

related diseases and the possible negligible beneficial effects with mass anthelmintic treatment.

References

Abrahams, P.W. and Parsons, J.A. (1996) Geophagy in the tropics: a literature review. *Georg J/RGS* **162**: 63–73

Abu-Salem, O.T. and Hassan, M.A. (2003) Acute abdomen and *Taenia solium* peritonii. *KMJ* **35**: 299–300

Adams, E.J., Stephenson, L.S., Latham, M.C. and Kinoti, S.N. (1994) Physical activity and growth of Kenyan school children with hookworm, *Trichuris trichiura* and *Ascaris lumbricoides* infections are improved after treatment with albendazole. *J Nutr* **124**: 1199–206

Addiss, D.G. and Brady, M.A. (2007) Morbidity management in the Global Programme to Eliminate Lymphatic Filariasis: a review of the scientific literature. *Filaria J* **6**: 2

Albonico, M., Stoltzfus, R.J., Savioli, L., Tielsch, J.M., Chwaya, H.M., Ercole, E. and Cancrini, G. (1998) Epidemiological evidence for a differential effect of hookworm species, Ancylostoma duodenale or Necator americanus, on iron status of children. *Int J Epidemiol* **27**: 530–7

Albonico, M., Crompton, D.W. and Savioli, L. (1999) Control strategies for human intestinal nematode infections. *Adv Parasitol* **42**: 277–341

Alexander, N.D., Bockarie, M.J., Dimber, Z.B., Griffin, L., Kazura, J.W. and Alpers, M.P. (2001) Migration and dispersal of lymphatic filariasis in Papua New Guinea. *Trans R Soc Trop Med Hyg* **95**: 277–9

Amaral, F., Dreyer, G., Figueredo-Silva, J., Noroes, J., Cavalcanti, A., Samico, S.C., Santos, A. and Coutinho, A. (1994) Live adult worms detected by ultrasonography in human Bancroftian filariasis. *Am J Trop Med Hyg* **50**: 753–7

Anonymous. (2003) ICT card test for bancroftian filariasis: striving towards programmatic acceptability. *Mectizan Program Notes* **32**

Arambulo, P.V. 3rd, Walls, K.W., Bullock, S. and Kagan, I.G. (1978) Serodiagnosis of human cysticercosis by microplate enzyme-linked immunospecific assay (ELISA). *Acta Trop* **35**: 63–7

Araujo, M.I., Hoppe, B., Medeiros, M., Jr., Alcantara, L., Almeida, M.C., Schriefer, A., Oliveira, R.R., Kruschewsky, R., Figueiredo, J.P., Cruz, A.A. and Carvalho, E.M. (2004) Impaired T helper 2 response to aeroallergen in helminth-infected patients with asthma. *J Infect Dis* **190**: 1797–803

Arruda, G.C., da Silva, A.D., Quagliato, E.M., Maretti, M.A. and Rossi, C.L. (2005) Evaluation of *Taenia solium* and *Taenia crassiceps* cysticercal antigens for the serodiagnosis of neurocysticercosis. *Trop Med Int Health* **10**: 1005–12

Asaolu, S.O. and Ofoezie, I.E. (2003) The role of health education and sanitation in the control of helminth infections. *Acta Trop* **86**: 283–94

Asaolu, S.O., Ofoezie, I.E., Odumuyiwa, P.A., Sowemimo, O.A. and Ogunniyi, T.A.B. (2002) Effect of water supply and sanitation on the prevalence and intensity of *Ascaris lumbricoides* among pre-school-age children in Ajebandele and Ifewara, Osun State, Nigeria. *Trans R Soc Trop Med Hyg* **96**: 600–4

Awasthi, S., Pande, V.K. and Fletcher, R.H. (2000) Effectiveness and cost-effectiveness of albendazole in improving nutritional status of pre-school children in urban slums. *Indian Pediatr* **37**: 19–29

Babu, B.V. and Mishra, S. (2008) Mass drug administration under the programme to eliminate lymphatic filariasis in Orissa, India: a mixed-methods study to identify factors associated with compliance and non-compliance. *Trans R Soc Trop Med Hyg* **In Press, Corrected Proof**

Babu, B.V. and Satyanarayana, K. (2003) Factors responsible for coverage and compliance in mass drug administration during the programme to eliminate lymphatic filariasis in the East Godavari District, South India. *Trop Doct* **33**: 79–82

Bach, J.F. (2002) The effect of infections on susceptibility to autoimmune and allergic diseases. *N Engl J Med* **347**: 911–20

Backhouse, T.C. and Heydon, G.A. (1950) Filariasis in Melanesia: observations at Rabaul relating to incidence and vectors. *Trans R Soc Trop Med Hyg* **44**: 291–306

Bal, M.S., Beuria, M.K., Mandal, N.N. and Das, M.K. (2003a) Antigenemia is associated with low antibody response to carbohydrate determinants of a filarial surface antigen. *Parasite Immunol* **25**: 107–11

Bal, M.S., Beuria, M.K., Mandal, N.N. and Das, M.K. (2003b) Parasite antigenaemia and IgG4 antibodies to a filarial protease in an endemic human population in India. *J Helminthol* **77**: 287–90

Beaver, P.C. (1988) Light, long-lasting Necator infection in a volunteer. *Am J Trop Med Hyg* **39**: 369–72

Behnke, J.M. (1987) Do hookworms elicit protective immunity in man? *Parasitol Today* **3**: 200–6

Behnke, J.M., Clercq, D., Sacko, M., Gilbert, F.S., Ouattara, D.B. and Vercruysse, J. (2000) The epidemiology of human hookworm infections in the southern region of Mali. *Trop Med Int Health* **5**: 343–54

Berhe, N., Medhin, G., Erko, B., Smith, T., Gedamu, S., Bereded, D., Moore, R., Habte, E., Redda, A., Gebre-Michael, T. and Gundersen, S.G. (2004) Variations in

helminth faecal egg counts in Kato-Katz thick smears and their implications in assessing infection status with Schistosoma mansoni. *Acta Trop* **92**: 205–12

Beuria, M.K., Bal, M.S., Mandal, N.N. and Das, M.K. (2001) A long-term follow-up of asymptomatic amicrofilaraemic individuals living in a filaria-endemic region of Orissa, India: infection and disease status. *Trans R Soc Trop Med Hyg* **95**: 408–9

Beuria, M.K., Bal, M.S., Mandal, N.N. and Das, M.K. (2002) Antigenemia at 10 years after diethylcarbamazine treatment of asymptomatic microfilaraemic individuals: marginal conversion to infection-free state. *Parasite Immunol* **24**: 109–11

Bhumiratana, A., Koyadun, S., Suvannadabba, S., Karnjanopas, K., Rojanapremsuk, J., Buddhirakkul, P. and Tantiwattanasup, W. (1999) Field trial of the ICT filariasis for diagnosis of *Wuchereria bancrofti* infections in an endemic population of Thailand. *Southeast Asian J Trop Med Public Health* **30**: 562–8

Bhumiratana, A., Siriaut, C., Koyadun, S. and Satitvipawee, P. (2004) Evaluation of a single oral dose of diethylcarbamazine 300 mg as provocative test and simultaneous treatment in Myanmar migrant workers with *Wuchereria bancrofti* infection in Thailand. *Southeast Asian J Trop Med Public Health* **35**: 591–8

Biswas, R., Parija, S.C. and Narayan, S.K. (2004) Dot-ELISA for the diagnosis of neurocysticercosis. *Rev Inst Med Trop Sao Paulo* **46**: 249–52

Blumberg, R.S., Saubermann, L.J. and Strober, W. (1999) Animal models of mucosal inflammation and their relation to human inflammatory bowel disease. *Curr Opin Immunol* **11**: 648–56

Bockarie, M.J. (1994) Can lymphatic filariasis be eradicated in Papua New Guinea? *P N G Med J* **37**: 61–4

Bockarie, M.J., Jenkins, C., Blakie, W.M., Lagog, M. and Alpers, M.P. (2000) Control of lymphatic filariasis in a hunter-gatherer group in Madang Province. *P N G Med J* **43**: 196–202

Bockarie, M.J. and Kazura, J.W. (2003) Lymphatic filariasis in Papua New Guinea: prospects for elimination. *Med Microbiol Immunol* **192**: 9–14

Bockarie, M.J., Tavul, L., Ibam, I., Kastens, W., Hazlett, F., Tisch, D.J., Alpers, M.P. and Kazura, J.W. (2007) Efficacy of single-dose diethylcarbamazine compared with diethylcarbamazine combined with albendazole against *Wuchereria bancrofti* infection in Papua New Guinea. *Am J Trop Med Hyg* **76**: 62–6

Bockarie, M.J., Tisch, D.J., Kastens, W., Alexander, N.D., Dimber, Z., Bockarie, F., Ibam, E., Alpers, M.P. and Kazura, J.W. (2002) Mass treatment to eliminate filariasis in Papua New Guinea. *N Engl J Med* **347**: 1841–8

Booth, M., Vounatsou, P., N'Goran, E.K., Tanner, M. and Utzinger, J. (2003) The influence of sampling effort and the performance of the Kato-Katz technique in

diagnosing *Schistosoma mansoni* and hookworm co-infections in rural Côte d'Ivoire. *Parasitology* **127**: 525–31

Borkow, G., Leng, Q., Weisman, Z., Stein, M., Galai, N., Kalinkovich, A. and Bentwich, Z. (2000) Chronic immune activation associated with intestinal helminth infections results in impaired signal transduction and anergy. *J Clin Invest* **106**: 1053–60

Bowie, C., Purcell, B., Shaba, B., Makaula, P. and Perez, M. (2004) A national survey of the prevalence of schistosomiasis and soil transmitted helminths in Malawi. *BMC Infect Dis* **4**: 49

Bradley, M. and Chandiwana, S.K. (1990) Age-dependency in predisposition to hookworm infection in the Burma valley area of Zimbabwe. *Trans R Soc Trop Med Hyg* **84**: 826–8

Bradley, M., Chandiwana, S.K. and Bundy, D.A. (1993) The epidemiology and control of hookworm infection in the Burma Valley area of Zimbabwe. *Trans R Soc Trop Med Hyg* **87**: 145–7

Braga, C., Dourado, I., Ximenes, R., Miranda, J. and Alexander, N. (2005) Bancroftian filariasis in an endemic area of Brazil: differences between genders during puberty. *Rev Soc Bras Med Trop* **38**: 224–8

Braga, C., Dourado, M.I., Ximenes, R.A., Alves, L., Brayner, F., Rocha, A. and Alexander, N. (2003) Field evaluation of the whole blood immunochromatographic test for rapid bancroftian filariasis diagnosis in the northeast of Brazil. *Rev Inst Med Trop Sao Paulo* **45**: 125–9. Epub 2003 Jul 8

Brandt, J.R., Geerts, S., De Deken, R., Kumar, V., Ceulemans, F., Brijs, L. and Falla, N. (1992) A monoclonal antibody-based ELISA for the detection of circulating excretory-secretory antigens in *Taenia saginata* cysticercosis. *Int J Parasitol* **22**: 471–7

Brooker, S., Alexander, N., Geiger, S., Moyeed, R.A., Stander, J., Fleming, F., Hotez, P.J., Correa-Oliveira, R. and Bethony, J. (2006a) Contrasting patterns in the small-scale heterogeneity of human helminth infections in urban and rural environments in Brazil. *Int J Parasitol* **36**: 1143–51

Brooker, S., Beasley, M., Ndinaromtan, M., Madjiouroum, E.M., Baboguel, M., Djenguinabe, E., Hay, S.I. and Bundy, D.A. (2002a) Use of remote sensing and a geographical information system in a national helminth control programme in Chad. *Bull World Health Organ* **80**: 783–9

Brooker, S., Clements, A.C. and Bundy, D.A. (2006b) Global epidemiology, ecology and control of soil-transmitted helminth infections. *Adv Parasitol* **62**: 221–61

Brooker, S., Hay, S.I., Tcheum TchuenteÂ, L.A. and Ratard, R. (2002b) Using NOAA-AVHRR data to model helminth distributions for planning disease control in Cameroon, West Africa. *PE&RS* **68**: 175–9

Brooker, S., Peshu, N., Warn, P.A., Mosobo, M., Guyatt, H.L., Marsh, K. and Snow, R.W. (1999) The epidemiology of hookworm infection and its contribution to anaemia among pre-school children on the Kenyan coast. *Trans R Soc Trop Med Hyg* **93**: 240–6

Brunner, B., Scheurer, U. and Seibold, F. (2007) Differences in yeast intolerance between patients with Crohn's disease and ulcerative colitis. *Dis Colon Rectum* **50**: 83–8

Bueno, E.C., Scheel, C.M., Vaz, A.J., Machado, L.R., Livramento, J.A., Takayanagui, O.M., Tsang, V.C. and Hancock, K. (2005) Application of synthetic 8-kD and recombinant GP50 antigens in the diagnosis of neurocysticercosis by enzyme-linked immunosorbent assay. *Am J Trop Med Hyg* **72**: 278–83

Bueno, E.C., Vaz, A.J., Machado, L.D., Livramento, J.A. and Mielle, S.R. (2000) Specific *Taenia crassiceps and Taenia solium* antigenic peptides for neurocysticercosis immunodiagnosis using serum samples. *J Clin Microbiol* **38**: 146–51

Bundy, D.A. (1988) Population ecology of intestinal helminth infections in human communities. *Philos Trans R Soc Lond B Biol Sci* **321**: 405–20

Bundy, D.A. and Cooper, E.S. (1988) The evidence for predisposition to trichuriasis in humans: comparison of institutional and community studies. *Ann Trop Med Parasitol* **82**: 251–6

Bundy, D.A., Cooper, E.S., Thompson, D.E., Didier, J.M. and Simmons, I. (1987) Epidemiology and population dynamics of *Ascaris lumbricoides and Trichuris trichiura* infection in the same community. *Trans R Soc Trop Med Hyg* **81**: 987–93

Bundy, D.A., Wong, M.S., Lewis, L.L. and Horton, J. (1990) Control of geohelminths by delivery of targeted chemotherapy through schools. *Trans R Soc Trop Med Hyg* **84**: 115–20

Bungiro, R.D., Jr. and Cappello, M. (2005) Detection of excretory/secretory coproantigens in experimental hookworm infection. *Am J Trop Med Hyg* **73**: 915–20

Buning, J., Homann, N., von Smolinski, D., Borcherding, F., Noack, F., Stolte, M., Kohl, M., Lehnert, H. and Ludwig, D. (2008) Helminths as governors of inflammatory bowel disease. *Gut* **57**: 1182–3

Burkot, T. and Ichimori, K. (2002) The PacELF programme: will mass drug administration be enough? *Trends Parasitol* **18**: 109–15

Burkot, T.R., Taleo, G., Toeaso, V. and Ichimori, K. (2002) Progress towards, and challenges for, the elimination of filariasis from Pacific-island communities. *Ann Trop Med Parasitol* **96 Suppl 2**: S61–9

Cairncross, S., Blumenthal, U., Kolsky, P., Moraes, L. and Tayeh, A. (1996) The public and domestic domains in the transmission of disease. *Trop Med Int Health* **1**: 27–34

Cao, W., Van der Ploeg, C.P., Ren, Z. and Habbema, J.D. (1997) Success against lymphatic filariasis. *World Health Forum* **18**: 17–20

Carneiro, F.F., Cifuentes, E., Tellez-Rojo, M.M. and Romieu, I. (2002) The risk of *Ascaris lumbricoides* infection in children as an environmental health indicator to guide preventive activities in Caparao and Alto Caparao, Brazil. *Bull World Health Organ* **80**: 40–6

Carpio, A., Escobar, A. and Hauser, W.A. (1998) Cysticercosis and epilepsy: a critical review. *Epilepsia* **39**: 1025–40

Carr, A. and Pritchard, D.I. (1987) Antigen expression during development of the human hookworm, *Necator americanus* (Nematoda). *Parasite Immunol* **9**: 219–34

Casapia, M., Joseph, S.A., Nunez, C., Rahme, E. and Gyorkos, T.W. (2007) Parasite and maternal risk factors for malnutrition in preschool-age children in Belen, Peru using the new WHO Child Growth Standards. *Br J Nutr* **98**: 1259–66

Chan, M.S., Bradley, M. and Bundy, D.A. (1997) Transmission patterns and the epidemiology of hookworm infection. *Int J Epidemiol* **26**: 1392–400

Chan, M.S., Medley, G.F., Jamison, D. and Bundy, D.A. (1994) The evaluation of potential global morbidity attributable to intestinal nematode infections. *Parasitology* **109 (Pt 3)**: 373–87

Chandrasena, T.G., Premaratna, R., Abeyewickrema, W. and de Silva, N.R. (2002) Evaluation of the ICT whole-blood antigen card test to detect infection due to *Wuchereria bancrofti* in Sri Lanka. *Trans R Soc Trop Med Hyg* **96**: 60–3.

Chanteau, S., Glaziou, P., Luquiaud, P., Plichart, C., Moulia-Pelat, J.P. and Cartel, J.L. (1994a) Og4C3 circulating antigen, anti-*Brugia malayi* IgG and IgG4 titers in *Wuchereria bancrofti* infected patients, according to their parasitological status. *Trop Med Parasitol* **45**: 255–7

Chanteau, S., Glaziou, P., Plichart, C., Luquiaud, P., Moulia-Pelat, J.P., N'Guyen, L. and Cartel, J.L. (1995) *Wuchereria bancrofti* filariasis in French Polynesia: age-specific patterns of microfilaremia, circulating antigen, and specific IgG and IgG4 responses according to transmission level. *Int J Parasitol* **25**: 81–5

Chanteau, S., Moulia-Pelat, J.P., Glaziou, P., Nguyen, N.L., Luquiaud, P., Plichart, C., Martin, P.M. and Cartel, J.L. (1994b) Og4C3 circulating antigen: a marker of infection and adult worm burden in *Wuchereria bancrofti* filariasis. *J Infect Dis* **170**: 247–50

Chanteau, S., Plichart, R., Spiegel, A., Martin, P.M. and Cartel, J.L. (1991) Diagnostic values of ELISA-IgG4 as compared to ELISA-IgG and indirect immunofluorescence, for the routine diagnosis of bancroftian filariasis in the South Pacific. Application on capillary blood collected on filter paper. *Trop Med Parasitol* **42**: 339–42

Cho, S.H., Park, H.W. and Rosenberg, D.M. (2006) The current status of asthma in Korea. *J Korean Med Sci* **21**: 181–7

Chongsuvivatwong, V., Pas-Ong, S., McNeil, D., Geater, A. and Duerawee, M. (1996) Predictors for the risk of hookworm infection: experience from endemic villages in southern Thailand. *Trans R Soc Trop Med Hyg* **90**: 630–3

Chung, J.Y., Bahk, Y.Y., Huh, S., Kang, S.Y., Kong, Y. and Cho, S.Y. (1999) A recombinant 10-kDa protein of *Taenia solium* metacestodes specific to active neurocysticercosis. *J Infect Dis* **180**: 1307–15

Cline, B.L., Savioli, L. and Neira, M. (2000) Introduction: Opportunities to work together: intestinal helminth control and programmes to eliminate lymphatic filariasis. *Parasitology* **121 Suppl**: S3–4

Constantin, A., Loubet-Lescoulie, P., Lambert, N., Yassine-Diab, B., Abbal, M., Mazieres, B., de Preval, C. and Cantagrel, A. (1998) Antiinflammatory and immunoregulatory action of methotrexate in the treatment of rheumatoid arthritis: evidence of increased interleukin-4 and interleukin-10 gene expression demonstrated in vitro by competitive reverse transcriptase-polymerase chain reaction. *Arthritis Rheum* **41**: 48–57

Cooper, P.J., Chico, M.E., Bland, M., Griffin, G.E. and Nutman, T.B. (2003a) Allergic symptoms, atopy, and geohelminth infections in a rural area of Ecuador. *Am J Respir Crit Care Med* **168**: 313–7

Cooper, P.J., Chico, M.E., Rodrigues, L.C., Ordonez, M., Strachan, D., Griffin, G.E. and Nutman, T.B. (2003b) Reduced risk of atopy among school-age children infected with geohelminth parasites in a rural area of the tropics. *J Allergy Clin Immunol* **111**: 995–1000

Cooper, P.J., Chico, M.E., Rodrigues, L.C., Strachan, D.P., Anderson, H.R., Rodriguez, E.A., Gaus, D.P. and Griffin, G.E. (2004) Risk factors for atopy among school children in a rural area of Latin America. *Clin Exp Allergy* **34**: 845–52

Cort, W.W., Otto, G.F. and Spindler, L.A. (1930) Investigations on *Ascaris lumbricoides* and the associated intestinal helminths of man in southwestern Virginia. *Am J Epidemiol* **11**: 1–55

Croese, J., O'Neil, J., Masson, J., Cooke, S., Melrose, W., Pritchard, D. and Speare, R. (2006a) A proof of concept study establishing *Necator americanus* in Crohn's patients and reservoir donors. *Gut* **55**: 136–7

Croese, J., Wood, M.J., Melrose, W. and Speare, R. (2006b) Allergy controls the population density of *Necator americanus* in the small intestine. *Gastroenterology* **131**: 402–9

Croese, J. and Speare, R. (2006) Intestinal allergy expels hookworms: seeing is believing. *Trends Parasitol* **22**: 547–50

Crompton, D.W. (2000) The public health importance of hookworm disease. *Parasitology* **121 Suppl**: S39–50

Crompton, D.W. and Nesheim, M.C. (2002) Nutritional impact of intestinal helminthiasis during the human life cycle. *Annu Rev Nutr* **22**: 35–59

Crompton, D.W. and Savioli, L. (1993) Intestinal parasitic infections and urbanization. *Bull World Health Organ* **71**: 1–7

da Silva, A.D., Quagliato, E.M. and Rossi, C.L. (2000) A quantitative enzyme-linked immunosorbent assay (ELISA) for the immunodiagnosis of neurocysticercosis using a purified fraction from *Taenia solium* cysticerci. *Diagn Microbiol Infect Dis* **37**: 87–92

da Silva, M.R., Maia, A.A., Espindola, N.M., Machado Ldos, R., Vaz, A.J. and Henrique-Silva, F. (2006) Recombinant expression of *Taenia solium* TS14 antigen and its utilization for immunodiagnosis of neurocysticercosis. *Acta Trop* **100**: 192–8

Dacombe, R.J., Crampin, A.C., Floyd, S., Randall, A., Ndhlovu, R., Bickle, Q. and Fine, P.E.M. (2007) Time delays between patient and laboratory selectively affect accuracy of helminth diagnosis. *Trans R Soc Trop Med Hyg* **101**: 140–5

Dagoye, D., Bekele, Z., Woldemichael, K., Nida, H., Yimam, M., Hall, A., Venn, A.J., Britton, J.R., Hubbard, R. and Lewis, S.A. (2003) Wheezing, allergy, and parasite infection in children in urban and rural Ethiopia. *Am J Respir Crit Care Med* **167**: 1369–73. Epub 2003 Jan 24

Das, D., Kumar, S., Sahoo, P.K. and Dash, A.P. (2005) A survey of bancroftian filariasis for microfilariae & circulating antigenaemia in two villages of Madhya Pradesh. *Indian J Med Res* **121**: 771–5

Davey, G., Venn, A., Belete, H., Berhane, Y. and Britton, J. (2005) Wheeze, allergic sensitization and geohelminth infection in Butajira, Ethiopia. *Clin Exp Allergy* **35**: 301–7

David, H.L. and Edeson, J.F. (1965) Filariasis in Portuguese Timor, with observations on a new microfilaria found in man. *Ann Trop Med Parasitol* **59**: 193–204

David, H.L. and Edeson, J.F.B. (1964) Microfilariae from man in Portuguese Timor. *Trans R Soc Trop Med Hyg* **58**: 6–11

Day, K.P., Gregory, W.F. and Maizels, R.M. (1991) Age-specific acquisition of immunity to infective larvae in a bancroftian filariasis endemic area of Papua New Guinea. *Parasite Immunol* **13**: 277–90

de Silva, N.R., Brooker, S., Hotez, P.J., Montresor, A., Engels, D. and Savioli, L. (2003) Soil-transmitted helminth infections: updating the global picture. *Trends Parasitol* **19**: 547–51

de Silva, N.R., Guyatt, H.L. and Bundy, D.A. (1997) Morbidity and mortality due to Ascaris-induced intestinal obstruction. *Trans R Soc Trop Med Hyg* **91**: 31–6

de Silva, N.R., Pathmeswaran, A., Fernando, S.D., Weerasinghe, C.R., Selvaratnam, R.R., Padmasiri, E.A. and Montresor, A. (2003) Impact of mass chemotherapy for the control of filariasis on geohelminth infections in Sri Lanka. *Ann Trop Med Parasitol* **97**: 421–5

Dean, M. (2003) Pacific nations lead the way in fighting lymphatic filariasis. *Lancet* **362**: 1906

Dekumyoy, P., Anantaphruti, M.T., Nuamtanong, S., Watthanakulpanich, D., Waikagu, J. and Danis, M. (2000) Neurocysticercosis: utilizing the cystic fluid antigen from *Taenia solium* metacestodes for diagnosis by IgG-ELISA. *Southeast Asian J Trop Med Public Health* **31 Suppl 1**: 21–5

Del Brutto, O.H., Rajshekhar, V., White, A.C., Jr., Tsang, V.C., Nash, T.E., Takayanagui, O.M., Schantz, P.M., Evans, C.A., Flisser, A., Correa, D., Botero, D., Allan, J.C., Sarti, E., Gonzalez, A.E., Gilman, R.H. and Garcia, H.H. (2001) Proposed diagnostic criteria for neurocysticercosis. *Neurology* **57**: 177–83

Denham, D.A. (1975) The diagnosis of filariasis. *Ann Soc Belg Med Trop* **55**: 517

Dennis, D.T., McConnell, E. and White, G.B. (1976) Bancroftian filariasis and membrane filters: are night surveys necessary? *Am J Trop Med Hyg* **25**: 257–62

Desowitz, R.S., Jenkins, C. and Anian, G. (1993) Bancroftian filariasis in an isolated hunter-gatherer shifting horticulturist group in Papua New Guinea. *Bull World Health Organ* **71**: 55–8

Dickerson, J.W., Eberhard, M.L., Lammie, P.J. and Roberts, J.M. (1989) Further evidence of a skewed distribution of microfilariae in capillary blood. *Trop Med Parasitol* **40**: 472–3

Dickson, R., Awasthi, S., Williamson, P., Demellweek, C. and Garner, P. (2000) Effects of treatment for intestinal helminth infection on growth and cognitive performance in children: systematic review of randomised trials. *BMJ* **320**: 1697–701

Dissanayake, S. (2001) In *Wuchereria bancrofti* filariasis, asymptomatic microfilaraemia does not progress to amicrofilaraemic lymphatic disease. *Int J Epidemiol* **30**: 394–9

Dorny, P., Brandt, J. and Geerts, S. (2004) Immunodiagnostic approaches for detecting *Taenia solium*. *Trends Parasitol* **20**: 259–60; author reply 260–1

Dorny, P., Brandt, J., Zoli, A. and Geerts, S. (2003) Immunodiagnostic tools for human and porcine cysticercosis. *Acta Trop* **87**: 79–86

Dossa, R.A., Ategbo, E.A., de Koning, F.L., van Raaij, J.M. and Hautvast, J.G. (2001) Impact of iron supplementation and deworming on growth performance in preschool Beninese children. *Eur J Clin Nutr* **55**: 223–8

Drake, L.J., Jukes, M.C.H., Sternberg, R.J. and Bundy, D.A.P. (2000) Geohelminth Infections (Ascariasis, Trichuriasis, and Hookworm): Cognitive and Developmental Impacts. *Semin Pediatr Infect Dis* **11**: 245–51

Dreyer, G., Pimentael, A., Medeiros, Z., Beliz, F., Moura, I., Coutinho, A., de Andrade, L.D., Rocha, A., da Silva, L.M. and Piessens, W.F. (1996) Studies on the periodicity and intravascular distribution of *Wuchereria bancrofti* microfilariae in paired samples of capillary and venous blood from Recife, Brazil. *Trop Med Int Health* **1**: 264–72

Duchmann, R., Kaiser, I., Hermann, E., Mayet, W., Ewe, K. and Meyer zum Buschenfelde, K.H. (1995) Tolerance exists towards resident intestinal flora but is broken in active inflammatory bowel disease (IBD). *Clin Exp Immunol* **102**: 448–55

Duchmann, R., May, E., Heike, M., Knolle, P., Neurath, M. and Meyer zum Buschenfelde, K.H. (1999) T cell specificity and cross reactivity towards enterobacteria, bacteroides, bifidobacterium, and antigens from resident intestinal flora in humans. *Gut* **44**: 812–8

Duggan, A.E., Usmani, I., Neal, K.R. and Logan, R.F. (1998) Appendicectomy, childhood hygiene, Helicobacter pylori status, and risk of inflammatory bowel disease: a case control study. *Gut* **43**: 494–8

Durrheim, D.N., Govere, J., la Grange, J.J. and Mabuza, A. (2001) Rapid immunochromatographic diagnosis and Rolling Back Malaria—experiences from an African control program. *Afr J Med Med Sci* **30 Suppl**: 21–4

Dwyer, P.D. (2006) People, pigs and parasites in New Guinea: relational contexts and epidemiological possibilities. *Parasitol Int* **55 Suppl**: S167–73

Eberhard, M.L., Hightower, A.W., Addiss, D.G. and Lammie, P.J. (1997) Clearance of *Wuchereria bancrofti* antigen after treatment with diethylcarbamazine or ivermectin. *Am J Trop Med Hyg* **57**: 483–6

Eberhard, M.L., Roberts, I.M., Lammie, P.J. and Lowrie, R.C., Jr. (1988) Comparative densities of *Wuchereria bancrofti* microfilaria in paired samples of capillary and venous blood. *Trop Med Parasitol* **39**: 295–8

Eigege, A., Richards, F.O., Jr., Blaney, D.D., Miri, E.S., Gontor, I., Ogah, G., Umaru, J., Jinadu, M.Y., Mathai, W., Amadiegwu, S. and Hopkins, D.R. (2003) Rapid assessment for lymphatic filariasis in central Nigeria: a comparison of the immunochromatographic card test and hydrocele rates in an area of high endemicity. *Am J Trop Med Hyg* **68**: 643–6

Elias, D., Akuffo, H. and Britton, S. (2006a) Helminthes could influence the outcome of vaccines against TB in the tropics. *Parasite Immunol* **28**: 507–13

Elias, D., Mengistu, G., Akuffo, H. and Britton, S. (2006b) Are intestinal helminths risk factors for developing active tuberculosis? *Trop Med Int Health* **11**: 551–8

Elliott, D.E., Summers, R.W. and Weinstock, J.V. (2005) Helminths and the modulation of mucosal inflammation. *Curr Opin Gastroenterol* **21**: 51–8

Elliott, D.E., Summers, R.W. and Weinstock, J.V. (2007) Helminths as governors of immune-mediated inflammation. *Int J Parasitol* **37**: 457–64

Elliott, D.E., Urban, J.J., Argo, C.K. and Weinstock, J.V. (2000) Does the failure to acquire helminthic parasites predispose to Crohn's disease? *Faseb J* **14**: 1848–55

Ellis, M.K., Raso, G., Li, Y.-S., Rong, Z., Chen, H.-G. and McManus, D.P. (2007) Familial aggregation of human susceptibility to co- and multiple helminth infections in a population from the Poyang Lake region, China. *Int J Parasitol* **37**: 1153–61

Engelbrecht, F., Oettl, T., Herter, U., Link, C., Philipp, D., Edeghere, H., Kaliraj, P. and Enwezor, F. (2003) Analysis of *Wuchereria bancrofti* infections in a village community in northern Nigeria: increased prevalence in individuals infected with Onchocerca volvulus. *Parasitol Int* **52**: 13–20

Engels, D., Nahimana, S., De Vlas, S.J. and Gryseels, B. (1997) Variation in weight of stool samples prepared by the Kato-Katz method and its implications. *Trop Med Int Health* **2**: 265–71

Engels, D., Nahimana, S. and Gryseels, B. (1996) Comparison of the direct faecal smear and two thick smear techniques for the diagnosis of intestinal parasitic infections. *Trans R Soc Trop Med Hyg* **90**: 523–5

Erdman, D.D. (1981) Clinical comparison of ethyl acetate and diethyl ether in the formalin-ether sedimentation technique. *J Clin Microbiol* **14**: 483–5

Erhart, A., Dorny, P., Van De, N., Vien, H.V., Thach, D.C., Toan, N.D., Cong le, D., Geerts, S., Speybroeck, N., Berkvens, D. and Brandt, J. (2002) *Taenia solium* cysticercosis in a village in northern Vietnam: seroprevalence study using an ELISA for detecting circulating antigen. *Trans R Soc Trop Med Hyg* **96**: 270–2

Espindola, N.M., Iha, A.H., Fernandes, I., Takayanagui, O.M., Machado Ldos, R., Livramento, J.A., Mendes Maia, A.A., Peralta, J.M. and Vaz, A.J. (2005) Cysticercosis immunodiagnosis using 18- and 14-kilodalton proteins from

Taenia crassiceps cysticercus antigens obtained by immunoaffinity chromatography. *J Clin Microbiol* **43**: 3178–84

Esterre, P., Plichart, C., Sechan, Y. and Nguyen, N.L. (2001) The impact of 34 years of massive DEC chemotherapy on *Wuchereria bancrofti* infection and transmission: the Maupiti cohort. *Trop Med Int Health* **6**: 190–5

Ev, L.V., Maia, A.A., Pianetti, G. and Nascimento, E. (1999) Immunological evaluation of a 26-kDa antigen from *Taenia solium* larvae for specific immunodiagnosis of human neurocysticercosis. *Parasitol Res* **85**: 98–102

Faris, R., Hussain, O., El Setouhy, M., Ramzy, R.M. and Weil, G.J. (1998) Bancroftian filariasis in Egypt: visualization of adult worms and subclinical lymphatic pathology by scrotal ultrasound. *Am J Trop Med Hyg* **59**: 864–7

Faris, R., Ramzy, R.M., Gad, A.M., Weil, G.J. and Buck, A.A. (1993) Community diagnosis of Bancroftian filariasis. *Trans R Soc Trop Med Hyg* **87**: 659–61

Farrokhyar, F., Swarbrick, E.T. and Irvine, E.J. (2001) A critical review of epidemiological studies in inflammatory bowel disease. *Scand J Gastroenterol* **36**: 2–15

Feachem, R.G., Guy, M.W., Harrison, S., Iwugo, K.O., Marshall, T., Mbere, N., Muller, R. and Wright, A.M. (1983) Excreta disposal facilities and intestinal parasitism in urban Africa: preliminary studies in Botswana, Ghana and Zambia. *Trans R Soc Trop Med Hyg* **77**: 515–21

Feldmeier, H. and Poggensee, G. (1993) Diagnostic techniques in schistosomiasis control. A review. *Acta Trop* **52**: 205–20

Ferrer, E., Bonay, P., Foster-Cuevas, M., Gonzalez, L.M., Davila, I., Cortez, M.M., Harrison, L.J., Parkhouse, R.M. and Garate, T. (2007) Molecular cloning and characterisation of Ts8B1, Ts8B2 and Ts8B3, three new members of the *Taenia solium* metacestode 8 kDa diagnostic antigen family. *Mol Biochem Parasitol* **152**: 90–100

Ferrer, E., Cortez, M.M., Cabrera, Z., Rojas, G., Davila, I., Alarcon de Noya, B., Perez, H.A., Fernandez, I., Romero, H.U., Harrison, L.J., Parkhouse, R.M. and Garate, T. (2005) Oncospheral peptide-based ELISAs as potential seroepidemiological tools for *Taenia solium* cysticercosis/neurocysticercosis in Venezuela. *Trans R Soc Trop Med Hyg* **99**: 568–76

Fischer, P., Bonow, I., Supali, T., Ruckert, P. and Rahmah, N. (2005) Detection of filaria-specific IgG4 antibodies and filarial DNA, for the screening of blood spots for *Brugia timori*. *Ann Trop Med Parasitol* **99**: 53–60

Fischer, P., Supali, T. and Maizels, R.M. (2004) Lymphatic filariasis and *Brugia timori*: prospects for elimination. *Trends Parasitol* **20**: 351–5

Fleury, A., Beltran, C., Ferrer, E., Garate, T., Harrison, L.J., Parkhouse, R.M., Garcia, E., Fragoso, G., Costa-Cruz, J., Biondi, G., Agapejev, S. and Sciutto, E. (2003) Application of synthetic peptides to the diagnosis of neurocysticercosis. *Trop Med Int Health* **8**: 1124–30

Fleury, A., Hernandez, M., Avila, M., Cardenas, G., Bobes, R.J., Huerta, M., Fragoso, G., Uribe-Campero, L., Harrison, L.J., Parkhouse, R.M. and Sciutto, E. (2007) Detection of HP10 antigen in serum for diagnosis and follow-up of subarachnoidal and intraventricular human neurocysticercosis. *J Neurol Neurosurg Psychiatry* **78**: 970–4

Flisser, A. and Gyorkos, T.W. (2007) Contribution of immunodiagnostic tests to epidemiological/intervention studies of cysticercosis/taeniosis in Mexico. *Parasite Immunol* **29**: 637–49

Flohr, C., Quinnell, R.J. and Britton, J. (2009) Do helminth parasites protect against atopy and allergic disease? *Clin Exp Allergy* **39**: 20–32

Flohr, C., Tuyen, L.N., Lewis, S., Quinnell, R., Minh, T.T., Campbell, J., Pritchard, D., Hien, T.T., Farrar, J., Britton, J. and Williams, H.C. (2007) Regular anti-helminthic therapy increases allergen skin sensitisation: a randomised, double blind, placebo-controlled trial in Vietnam. *Clin Exp Allergy* **37**: 1880

Forrester, J.E., Scott, M.E., Bundy, D.A. and Golden, M.H. (1988) Clustering of *Ascaris lumbricoides* and *Trichuris trichiura* infections within households. *Trans R Soc Trop Med Hyg* **82**: 282–8

Forrester, J.E., Scott, M.E., Bundy, D.A. and Golden, M.H. (1990) Predisposition of individuals and families in Mexico to heavy infection with *Ascaris lumbricoides* and *Trichuris trichiura*. *Trans R Soc Trop Med Hyg* **84**: 272–6

Forsyth, K.P., Spark, R., Kazura, J., Brown, G.V., Peters, P., Heywood, P., Dissanayake, S. and Mitchell, G.F. (1985) A monoclonal antibody-based immunoradiometric assay for detection of circulating antigen in Bancroftian filariasis. *J Immunol* **134**: 1172–7

Fox, L.M., Wilson, S.F., Addiss, D.G., Louis-Charles, J., Beau de Rochars, M.V. and Lammie, P.J. (2005) Clinical correlates of filarial infection in Haitian children: an association with interdigital lesions. *Am J Trop Med Hyg* **73**: 759–65

Fraga de Azevedo, J., Gandara, A.F. and Ferreira, A.P. (1956) Estudo Sobre A Incidencia Das Parasitoses Intenstinais Na Provincia De Timor. *An Inst Med Trop (Lisbon)* **15**: 71–86

Freedman, D.O., de Almeida, A., Miranda, J., Plier, D.A. and Braga, C. (1997) Field trial of a rapid card test for *Wuchereria bancrofti*. *Lancet* **350**: 1681

Freedman, D.O., Plier, D.A., De Almeida, A.B., De Oliveira, A.L., Miranda, J. and Braga, C. (2001) Effect of aggressive prolonged diethylcarbamazine therapy on circulating antigen levels in bancroftian filariasis. *Trop Med Int Health* **6**: 37–41

Fritzsche, M., Gottstein, B., Wigglesworth, M.C. and Eckert, J. (1990) Serological survey of human cysticercosis in Irianese refugee camps in Papua New Guinea. *Acta Trop* **47**: 69–77

Fujiwara, R.T., Geiger, S.M., Bethony, J. and Mendez, S. (2006) Comparative immunology of human and animal models of hookworm infection. *Parasite Immunol* **28**: 285–93

Fuss, I.J., Neurath, M., Boirivant, M., Klein, J.S., de la Motte, C., Strong, S.A., Fiocchi, C. and Strober, W. (1996) Disparate CD4+ lamina propria (LP) lymphokine secretion profiles in inflammatory bowel disease. Crohn's disease LP cells manifest increased secretion of IFN-gamma, whereas ulcerative colitis LP cells manifest increased secretion of IL-5. *J Immunol* **157**: 1261–70

Gandhi, N.S., Jizhang, C., Khoshnood, K., Fuying, X., Shanwen, L., Yaoruo, L., Bin, Z., Haechou, X., Chongjin, T., Yan, W., Wensen, W., Dungxing, H., Chong, C., Shuhua, X., Hawdon, J.M. and Hotez, P.J. (2001) Epidemiology of *Necator americanus* hookworm infections in Xiulongkan Village, Hainan Province, China: high prevalence and intensity among middle-aged and elderly residents. *J Parasitol* **87**: 739–43

Garcia, H.H. and Del Brutto, O.H. (2000) *Taenia solium* cysticercosis. *Infect Dis Clin North Am* **14**: 97–119, ix

Garcia, H.H., Evans, C.A., Nash, T.E., Takayanagui, O.M., White, A.C., Jr., Botero, D., Rajshekhar, V., Tsang, V.C., Schantz, P.M., Allan, J.C., Flisser, A., Correa, D., Sarti, E., Friedland, J.S., Martinez, S.M., Gonzalez, A.E., Gilman, R.H. and Del Brutto, O.H. (2002a) Current consensus guidelines for treatment of neurocysticercosis. *Clin Microbiol Rev* **15**: 747–56

Garcia, H.H., Gonzalez, A.E., Gilman, R.H., Bernal, T., Rodriguez, S., Pretell, E.J., Azcurra, O., Parkhouse, R.M., Tsang, V.C. and Harrison, L.J. (2002b) Circulating parasite antigen in patients with hydrocephalus secondary to neurocysticercosis. *Am J Trop Med Hyg* **66**: 427–30

Garcia, H.H., Gonzalez, A.E., Gilman, R.H., Palacios, L.G., Jimenez, I., Rodriguez, S., Verastegui, M., Wilkins, P. and Tsang, V.C. (2001) Short report: transient antibody response in *Taenia solium* infection in field conditions—a major contributor to high seroprevalence. *Am J Trop Med Hyg* **65**: 31–2

Garcia, H.H., Harrison, L.J., Parkhouse, R.M., Montenegro, T., Martinez, S.M., Tsang, V.C. and Gilman, R.H. (1998) A specific antigen-detection ELISA for the diagnosis of human neurocysticercosis. The Cysticercosis Working Group in Peru. *Trans R Soc Trop Med Hyg* **92**: 411–4

Garcia, H.H., Parkhouse, R.M., Gilman, R.H., Montenegro, T., Bernal, T., Martinez, S.M., Gonzalez, A.E., Tsang, V.C. and Harrison, L.J. (2000) Serum antigen detection in the diagnosis, treatment, and follow-up of neurocysticercosis patients. *Trans R Soc Trop Med Hyg* **94**: 673–6

Garcia, L.S. and Shimizu, R. (1981) Comparison of clinical results for the use of ethyl acetate and diethyl ether in the formalin-ether sedimentation technique performed on polyvinyl alcohol-preserved specimens. *J Clin Microbiol* **13**: 709–13

Garg, R.K. and Nag, D. (1998) Single enhancing CT lesions in Indian patients with seizures: clinical and radiological evaluation and follow-up. *J Trop Pediatr* **44**: 204–10

Gasche, C., Bakos, S., Dejaco, C., Tillinger, W., Zakeri, S. and Reinisch, W. (2000) IL-10 secretion and sensitivity in normal human intestine and inflammatory bowel disease. *J Clin Immunol* **20**: 362–70

Geiger, S.M., Caldas, I.R., Mc Glone, B.E., Campi-Azevedo, A.C., De Oliveira, L.M., Brooker, S., Diemert, D., Correa-Oliveira, R. and Bethony, J.M. (2007) Stage-specific immune responses in human *Necator americanus* infection. *Parasite Immunol* **29**: 347–58

Geiger, S.M., Fujiwara, R.T., Santiago, H., Corrêa-Oliveira, R. and Bethony, J.M. (2008) Early stage-specific immune responses in primary experimental human hookworm infection. *Microbes Infect* **In Press, Uncorrected Proof**

Geiger, S.M., Massara, C.L., Bethony, J., Soboslay, P.T., Carvalho, O.S. and Correa-Oliveira, R. (2002) Cellular responses and cytokine profiles in *Ascaris lumbricoides* and *Trichuris trichiura* infected patients. *Parasite Immunol* **24**: 499–509

Geiger, S.M., Massara, C.L., Bethony, J., Soboslay, P.T. and Correa-Oliveira, R. (2004) Cellular responses and cytokine production in post-treatment hookworm patients from an endemic area in Brazil. *Clin Exp Immunol* **136**: 334–40

Geissler, P.W., Mwaniki, D., Thiong'o, F. and Friis, H. (1998) Geophagy as a risk factor for geohelminth infections: a longitudinal study of Kenyan primary schoolchildren. *Trans R Soc Trop Med Hyg* **92**: 7–11

Gent, A.E., Hellier, M.D., Grace, R.H., Swarbrick, E.T. and Coggon, D. (1994) Inflammatory bowel disease and domestic hygiene in infancy. *Lancet* **343**: 766–7

Gera, T., Sachdev, H.P., Nestel, P. and Sachdev, S.S. (2007) Effect of iron supplementation on haemoglobin response in children: systematic review of randomised controlled trials. *J Pediatr Gastroenterol Nutr* **44**: 468–86

Gerards, A.H., de Lathouder, S., de Groot, E.R., Dijkmans, B.A. and Aarden, L.A. (2003) Inhibition of cytokine production by methotrexate. Studies in healthy volunteers and patients with rheumatoid arthritis. *Rheumatology (Oxford)* **42**: 1189–96

Gibson, J. and Rozelle, S. (2004) Is it better to be a boy? A disaggregated outlay equivalent analysis of gender bias in Papua New Guinea. *J Dev Stud* **40**: 115–36

Glas, J., Torok, H.P., Vilsmaier, F., Herbinger, K.H., Hoelscher, M. and Folwaczny, C. (2002) Anti-saccharomyces cerevisiae antibodies in patients with inflammatory bowel disease and their first-degree relatives: potential clinical value. *Digestion* **66**: 173–7

Goodman, D., Haji, H.J., Bickle, Q.D., Stoltzfus, R.J., Tielsch, J.M., Ramsan, M., Savioli, L. and Albonico, M. (2007) A comparison of methods for detecting the eggs of *Ascaris, Trichuris*, and hookworm in infant stool, and the epidemiology of infection in Zanzibari infants. *Am J Trop Med Hyg* **76**: 725–31

Gottstein, B., Tsang, V.C. and Schantz, P.M. (1986) Demonstration of species-specific and cross-reactive components of *Taenia solium* metacestode antigens. *Am J Trop Med Hyg* **35**: 308–13

Grady, C.A., de Rochars, M.B., Direny, A.N., Orelus, J.N., Wendt, J., Radday, J., Mathieu, E., Roberts, J.M., Streit, T.G., Addiss, D.G. and Lammie, P.J. (2007) Endpoints for lymphatic filariasis programs. *Emerg Infect Dis* **13**: 608–10

Greene, R.M., Wilkins, P.P. and Tsang, V.C. (1999) Diagnostic glycoproteins of *Taenia solium* cysts share homologous 14- and 18-kDa subunits. *Mol Biochem Parasitol* **99**: 257–61

Gulani, A., Nagpal, J., Osmond, C. and Sachdev, H.P. (2007) Effect of administration of intestinal anthelmintic drugs on haemoglobin: systematic review of randomised controlled trials. *BMJ* **334**: 1095

Gunawardena, N.K., Amarasekera, N.D., Pathmeswaran, A. and de Silva, N.R. (2008) Effect of repeated mass chemotherapy for filariasis control on soil-transmitted helminth infections in Sri Lanka. *Ceylon Med J* **53**: 13–6

Gyapong, J.O. (1998) The relationship between infection and disease in *Wuchereria bancrofti* infection in Ghana. *Trans R Soc Trop Med Hyg* **92**: 390–2

Gyapong, J.O. (2004) 2.5 Monitoring and evaluation. *Am J Trop Med Hyg* **71**: 20–1

Gyapong, J.O., Omane-Badu, K. and Webber, R.H. (1998) Evaluation of the filter paper blood collection method for detecting Og4C3 circulating antigen in bancroftian filariasis. *Trans R Soc Trop Med Hyg* **92**: 407–10

Gyapong, J.O. and Twum-Danso, N.A. (2006) Editorial: Global elimination of lymphatic filariasis: fact or fantasy? *Trop Med Int Health* **11**: 125–8

Gyorkos, T.W. (2003) Monitoring and evaluation of large scale helminth control programmes. *Acta Trop* **86**: 275–82

Haider, B.A., Humayun, Q. and Bhutta, Z.A. (2009) Effect of administration of antihelminthics for soil transmitted helminths during pregnancy. *Cochrane Database Syst Rev*: CD005547

Hall, A., Anwar, K.S. and Tomkins, A.M. (1992) Intensity of reinfection with *Ascaris lumbricoides* and its implications for parasite control. *Lancet* **339**: 1253–7

Hancock, K., Khan, A., Williams, F.B., Yushak, M.L., Pattabhi, S., Noh, J. and Tsang, V.C. (2003) Characterization of the 8-kilodalton antigens of *Taenia solium* metacestodes and evaluation of their use in an enzyme-linked immunosorbent assay for serodiagnosis. *J Clin Microbiol* **41**: 2577–86

Handali, S., Liying, H., Lusikoy, C., Senis, J. and Sihombing, D. (1997) A survey report—July 1993: cysticercosis in the Grand Dani Valley, Jayawijaya District, Irian Jaya Province, Indonesia. *Southeast Asian J Trop Med Public Health* **28 Suppl 1**: 22–5

Harrison, L.J., Joshua, G.W., Wright, S.H. and Parkhouse, R.M. (1989) Specific detection of circulating surface/secreted glycoproteins of viable cysticerci in *Taenia saginata* cysticercosis. *Parasite Immunol* **11**: 351–70

Hartgers, F.C. and Yazdanbakhsh, M. (2006) Co-infection of helminths and malaria: modulation of the immune responses to malaria. *Parasite Immunol* **28**: 497–506

Hemer, S.R. (2005) Health care and illness in Lihir, New Ireland Province, in the context of the development of the Lihir gold mine. *P N G Med J* **48**: 188–95

Hendrickson, B.A., Gokhale, R. and Cho, J.H. (2002) Clinical aspects and pathophysiology of inflammatory bowel disease. *Clin Microbiol Rev* **15**: 79–94

Herman, S., Zurgil, N., Langevitz, P., Ehrenfeld, M. and Deutsch, M. (2008) Methotrexate selectively modulates TH1/TH2 balance in active rheumatoid arthritis patients. *Clin Exp Rheumatol* **26**: 317–23

Hernandez, M., Beltran, C., Garcia, E., Fragoso, G., Gevorkian, G., Fleury, A., Parkhouse, M., Harrison, L., Sotelo, J. and Sciutto, E. (2000) Cysticercosis: towards the design of a diagnostic kit based on synthetic peptides. *Immunol Lett* **71**: 13–7

Hesham Al-Mekhlafi, M., Surin, J., Atiya, A.S., Ariffin, W.A., Mohammed Mahdy, A.K. and Che Abdullah, H. (2008) Pattern and predictors of soil-transmitted helminth reinfection among aboriginal schoolchildren in rural Peninsular Malaysia. *Acta Trop* **107**: 200–4

Hii, J., Bockarie, M.J., Flew, S., Genton, B., Tali, A., Dagoro, H., Waulas, B., Samson, M. and Alpers, M.P. (2000) The epidemiology and control of lymphatic filariasis on Lihir Island, New Ireland Province. *P N G Med J* **43**: 188–95

Hlaing, T., Saw, T. and Lwin, M. (1987) Reinfection of people with *Ascaris lumbricoides* following single, 6-month and 12-month interval mass chemotherapy in Okpo village, rural Burma. *Trans R Soc Trop Med Hyg* **81**: 140–6

Holland, C.V., Taren, D.L., Crompton, D.W., Nesheim, M.C., Sanjur, D., Barbeau, I., Tucker, K., Tiffany, J. and Rivera, G. (1988) Intestinal helminthiases in relation to the socioeconomic environment of Panamanian children. *Soc Sci Med* **26**: 209–13

Hong, S.T., Chai, J.Y., Choi, M.H., Huh, S., Rim, H.J. and Lee, S.H. (2006) A successful experience of soil-transmitted helminth control in the Republic of Korea. *Korean J Parasitol* **44**: 177–85

Horton, J. (2003) Global anthelmintic chemotherapy programs: learning from history. *Trends Parasitol* **19**: 405–9

Hoti, S.L., Elango, A., Radjame, K., Yuvaraj, J. and Pani, S.P. (2002) Detection of day blood filarial antigens by Og4C3 ELISA test using filter paper samples. *Natl Med J India* **15**: 263–6

Hsieh, G.C., Loukas, A., Wahl, A.M., Bhatia, M., Wang, Y., Williamson, A.L., Kehn, K.W., Maruyama, H., Hotez, P.J., Leitenberg, D., Bethony, J. and Constant, S.L. (2004) A secreted protein from the human hookworm *Necator americanus* binds selectively to NK cells and induces IFN-gamma production. *J Immunol* **173**: 2699–704

Hugot, J.P., Chamaillard, M., Zouali, H., Lesage, S., Cezard, J.P., Belaiche, J., Almer, S., Tysk, C., O'Morain, C.A., Gassull, M., Binder, V., Finkel, Y., Cortot, A., Modigliani, R., Laurent-Puig, P., Gower-Rousseau, C., Macry, J., Colombel, J.F., Sahbatou, M. and Thomas, G. (2001) Association of NOD2 leucine-rich repeat variants with susceptibility to Crohn's disease. *Nature* **411**: 599–603

Huppatz, C., Durrheim, D., Lammie, P., Kelly, P. and Melrose, W. (2008) Eliminating lymphatic filariasis—the surveillance challenge. *Trop Med Int Health* **13**: 292–4

Inohara, N., Ogura, Y., Fontalba, A., Gutierrez, O., Pons, F., Crespo, J., Fukase, K., Inamura, S., Kusumoto, S., Hashimoto, M., Foster, S.J., Moran, A.P., Fernandez-Luna, J.L. and Nunez, G. (2003) Host recognition of bacterial muramyl dipeptide mediated through NOD2. Implications for Crohn's disease. *J Biol Chem* **278**: 5509–12

International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use. (1995) Validation of analytical procedures, Q2A definitions and terminology, guidelines prepared within the International Conference on Harmonization of Technical Requirements for the Registration of Pharmaceuticals for Human Use, pp. 1–5.

Iqbal, J. and Sher, A. (2006) Determination of the prevalence of lymphatic filariasis among migrant workers in Kuwait by detecting circulating filarial antigen. *J Med Microbiol* **55**: 401–5

Ishida, M.M., Rubinsky-Elefant, G., Ferreira, A.W., Hoshino-Shimizu, S. and Vaz, A.J. (2003) Helminth antigens (*Taenia solium, Taenia crassiceps, Toxocara canis*,

Schistosoma mansoni and *Echinococcus granulosus*) and cross-reactivities in human infections and immunized animals. *Acta Trop* **89**: 73–84

Ito, A., Nakao, M., Ito, Y., Yuzawa, I., Morishima, H., Kawano, N. and Fujii, K. (1999) Neurocysticercosis case with a single cyst in the brain showing dramatic drop in specific antibody titers within 1 year after curative surgical resection. *Parasitol Int* **48**: 95–9

Ito, A., Plancarte, A., Ma, L., Kong, Y., Flisser, A., Cho, S.Y., Liu, Y.H., Kamhawi, S., Lightowlers, M.W. and Schantz, P.M. (1998) Novel antigens for neurocysticercosis: simple method for preparation and evaluation for serodiagnosis. *Am J Trop Med Hyg* **59**: 291–4

Ito, A., Sako, Y., Nakao, M. and Nakaya, K. (2002) Neurocysticercosis in Asia: serology/seroepidemiology in humans and pigs. In: P.C.Z. Pawlowski (Ed), Cestode Zoonoses: Echinococcosis and Cysticercosis: An Emergent and Global Problem. IOS Press, Amsterdam

Ito, A., Wandra, T., Yamasaki, H., Nakao, M., Sako, Y., Nakaya, K., Margono, S.S., Suroso, T., Gauci, C. and Lightowlers, M.W. (2004) Cysticercosis/taeniasis in Asia and the Pacific. *Vector Borne Zoonotic Dis* **4**: 95–107

Itoh, M., Gunawardena, N.K., Qiu, X.G., Weerasooriya, M.V. and Kimura, E. (1998) The use of whole blood absorbed on filter paper to detect *Wuchereria bancrofti* circulating antigen. *Trans R Soc Trop Med Hyg* **92**: 513–5

Itoh, M., Weerasooriya, M.V., Gunawardena, N.K., Mudalige, M.P., Samarawickrema, W.A. and Kimura, E. (1999) *Wuchereria bancrofti* antigenaemia in Sri Lanka. *Trop Med Int Health* **4**: 207–10

Jamail, M., Andrew, K., Junaidi, D., Krishnan, A.K., Faizal, M. and Rahmah, N. (2005) Field validation of sensitivity and specificity of rapid test for detection of Brugia malayi infection. *Trop Med Int Health* **10**: 99–104

Janeway, C.A., Jr., Travers, P., Walport, M. Shlomchik, M.J. (2005) Immunobiology. 6th ed. Garland Science Publishing.

Jaoko, W.G., Simonsen, P.E., Meyrowitsch, D.W., Estambale, B.B., Malecela-Lazaro, M.N. and Michael, E. (2006) Filarial-specific antibody response in East African bancroftian filariasis: effects of host infection, clinical disease, and filarial endemicity. *Am J Trop Med Hyg* **75**: 97–107

Jaoko, W.G., Simonsen, P.E., Meyrowitsch, D.W., Pedersen, E.M., Rwegoshora, R.T. and Michael, E. (2001) *Wuchereria bancrofti* in a community with seasonal transmission: stability of microfilaraemia, antigenaemia and filarial-specific antibody concentrations. *Ann Trop Med Parasitol* **95**: 253–61

Jihui, D. (2005) Filariasis control and elimination activities. In: E. Kimura, H. Rim, S. Dejian and M. Weerasooriya (Eds), Filariasis in Asian and Western Pacific Islands. AAA Committee–The Federation of Asian Parasitologists

Johnston, F.H., Morris, P.S., Speare, R., McCarthy, J., Currie, B., Ewald, D., Page, W. and Dempsey, K. (2005) Strongyloidiasis: a review of the evidence for Australian practitioners. *Aust J Rural Health* **13:** 247-54

Jones, H.I. (1976) Human intestinal helminthiases in East Timor. *Southeast Asian J Trop Med Public Health* **7**: 523–9

Joseph, S., Jones, F.M., Kimani, G., Mwatha, J.K., Kamau, T., Kazibwe, F., Kemijumbi, J., Kabatereine, N.B., Booth, M., Kariuki, H.C., Ouma, J.H., Vennervald, B.J. and Dunne, D.W. (2004) Cytokine production in whole blood cultures from a fishing community in an area of high endemicity for *Schistosoma mansoni* in Uganda: the differential effect of parasite worm and egg antigens. *Infect Immun* **72**: 728–34

Kakazu, T., Hara, J., Matsumoto, T., Nakamura, S., Oshitani, N., Arakawa, T., Kitano, A., Nakatani, K., Kinjo, F. and Kuroki, T. (1999) Type 1 T-helper cell predominance in granulomas of Crohn's disease. *Am J Gastroenterol* **94**: 2149–55

Kapetanovic, M.C., Saxne, T., Sjoholm, A., Truedsson, L., Jonsson, G. and Geborek, P. (2006) Influence of methotrexate, TNF blockers and prednisolone on antibody responses to pneumococcal polysaccharide vaccine in patients with rheumatoid arthritis. *Rheumatology (Oxford)* **45**: 106–11

Kapil, U. (2000) Albendazole-the magic bullet for prevention of stunting in Indian children. *Indian Pediatr* **37**: 804–6

Kato, K. and Miura, M. (1954) Comparative examinations. *Jap J Parasit* **3**: 35

Katz, N., Chaves, A. and Pellegrino, J. (1972) A simple device for quantitative stool thick-smear technique in Schistosomiasis mansoni. *Rev Inst Med Trop Sao Paulo* **14**: 397–400

Kazura, J.W., Bockarie, M., Alexander, N., Perry, R., Bockarie, F., Dagoro, H., Dimber, Z., Hyun, P. and Alpers, M.P. (1997) Transmission intensity and its relationship to infection and disease due to *Wuchereria bancrofti* in Papua New Guinea. *J Infect Dis* **176**: 242–6

Kazura, J.W. and Bockarie, M.J. (2003) Lymphatic filariasis in Papua New Guinea: interdisciplinary research on a national health problem. *Trends Parasitol* **19**: 260–3

Kazura, J.W., Spark, R., Forsyth, K., Brown, G., Heywood, P., Peters, P. and Alpers, M. (1984) Parasitologic and clinical features of bancroftian filariasis in a community in East Sepik Province, Papua New Guinea. *Am J Trop Med Hyg* **33**: 1119–23

Keiser, J. and Utzinger, J. (2008) Efficacy of current drugs against soil-transmitted helminth infections: systematic review and meta-analysis. *JAMA* **299**: 1937–48

Kightlinger, L.K., Seed, J.R. and Kightlinger, M.B. (1998) *Ascaris lumbricoides* intensity in relation to environmental, socioeconomic, and behavioral determinants of exposure to infection in children from southeast Madagascar. *J Parasitol* **84**: 480–4

Killewo, J.Z.J., Cairncross, S., Smet, J.E.M., Maikwano, L.F. and van Asten, H. (1991) Patterns of hookworm and Ascaris infection in Dar es Salaam. *Acta Trop* **48**: 247–9

Kimura, E., Penaia, L. and Spears, G.F. (1985) The efficacy of annual single-dose treatment with diethylcarbamazine citrate against diurnally subperiodic bancroftian filariasis in Samoa. *Bull World Health Organ* **63**: 1097–106

Kiyu, A. and Hardin, S. (1993) Latrine use in rural Sarawak, Malaysia. *Southeast Asian J Trop Med Public Health* **24**: 40–2

Knight, R., McAdam, K.P., Matola, Y.G. and Kirkham, V. (1979) Bancroftian filariasis and other parasitic infections in the Middle Fly River region of Western Papua New Guinea I. Clinical, parasitooogical and serological studies. *Ann Trop Med Parasitol* **73**: 563–76

Knopp, S., Mgeni, A.F., Khamis, I.S., Steinmann, P., Stothard, J.R., Rollinson, D., Marti, H. and Utzinger, J. (2008a) Diagnosis of soil-transmitted helminths in the era of preventive chemotherapy: effect of multiple stool sampling and use of different diagnostic techniques. *PLoS Negl Trop Dis* **2**: e331

Knopp, S., Mohammed, K.A., Simba Khamis, I., Mgeni, A.F., Stothard, J.R., Rollinson, D., Marti, H. and Utzinger, J. (2008b) Spatial distribution of soil-transmitted helminths, including Strongyloides stercoralis, among children in Zanzibar. *Geospat Health* **3**: 47–56

Ko, R.C. and Ng, T.F. (1998a) Purification of larval *Taenia solium* antigens by isoelectric focusing. *Vet Parasitol* **74**: 191–202

Ko, R.C. and Ng, T.F. (1998b) Specificity of isoelectric focusing-purified antigens in the diagnosis of human cysticercosis. *Parasitol Res* **84**: 565–9

Komiya, Y. and Kobayashi, A. (1966) Evaluation of Kato's thick smear technic with a cellophane cover for helminth eggs in feces. *Jpn J Med Sci Biol* **19**: 59–64

Kongs, A., Marks, G., Verle, P. and Van der Stuyft, P. (2001) The unreliability of the Kato-Katz technique limits its usefulness for evaluating *S. mansoni* infections. *Trop Med Int Health* **6**: 163–9

Koyadun, S., Bhumiratana, A. and Prikchu, P. (2003) *Wuchereria bancrofti* antigenemia clearance among Myanmar migrants after biannual mass treatments with diethylcarbamazine, 300 mg oral-dose FILADEC tablet, in Southern Thailand. *Southeast Asian J Trop Med Public Health* **34**: 758–67

Kraan, M.C., Smeets, T.J., van Loon, M.J., Breedveld, F.C., Dijkmans, B.A. and Tak, P.P. (2004) Differential effects of leflunomide and methotrexate on cytokine production in rheumatoid arthritis. *Ann Rheum Dis* **63**: 1056–61

Kradin, R.L., Badizadegan, K., Auluck, P., Korzenik, J. and Lauwers, G.Y. (2006) Iatrogenic *Trichuris suis* infection in a patient with Crohn disease. *Arch Pathol Lab Med* **130**: 718–20

Krecek, R.C., Michael, L.M., Schantz, P.M., Ntanjana, L., Smith, M.F., Dorny, P., Harrison, L.J., Grimm, F., Praet, N. and Willingham, A.L., 3rd. (2008) Prevalence of *Taenia solium* cysticercosis in swine from a community-based study in 21 villages of the Eastern Cape Province, South Africa. *Vet Parasitol* **154:** 38-47

Kruger, M., Badenhorst, C.J., Mansvelt, E.P.G., Laubscher, J.A. and Benade, A.J.S. (1996) The effect of iron fortification in a school feeding scheme and anthelminthic therapy on the iron status and growth of 6–8 year old school children. *Food Nutr Bull* **17**: 11–21

Kucharzik, T., Stoll, R., Lugering, N. and Domschke, W. (1995) Circulating antiinflammatory cytokine IL-10 in patients with inflammatory bowel disease (IBD). *Clin Exp Immunol* **100**: 452–6

Kugathasan, S., Saubermann, L.J., Smith, L., Kou, D., Itoh, J., Binion, D.G., Levine, A.D., Blumberg, R.S. and Fiocchi, C. (2007) Mucosal T-cell immunoregulation varies in early and late inflammatory bowel disease. *Gut* **56**: 1696–705

Kumar, A., Khan, S.A., Khan, S., Das, S., Anurag and Negi, K.S. (2006) A study of neurocysticercosis in the foothills of the Himalayas. *Int J Infect Dis* **10**: 79–82

Kvalsvig, J.D., Cooppan, R.M. and Connolly, K.J. (1991) The effects of parasite infections on cognitive processes in children. *Ann Trop Med Parasitol* **85**: 551–68

Kwan-Lim, G.E., Forsyth, K.P. and Maizels, R.M. (1990) Filarial-specific IgG4 response correlates with active *Wuchereria bancrofti* infection. *J Immunol* **145**: 4298–305

Lal, R.B., Paranjape, R.S., Briles, D.E., Nutman, T.B. and Ottesen, E.A. (1987) Circulating parasite antigen(s) in lymphatic filariasis: use of monoclonal antibodies to phosphocholine for immunodiagnosis. *J Immunol* **138**: 3454–60

Lalitha, P., Ravichandran, M., Suba, S., Kaliraj, P., Narayanan, R.B. and Jayaraman, K. (1998) Quantitative assessment of circulating antigens in human lymphatic filariasis: a field evaluation of monoclonal antibody-based ELISA using blood collected on filter strips. *Trop Med Int Health* **3**: 41–5

Lammie, P.J., Hightower, A.W. and Eberhard, M.L. (1994) Age-specific prevalence of antigenemia in a *Wuchereria bancrofti*-exposed population. *Am J Trop Med Hyg* **51**: 348–55

Lammie, P.J., Weil, G., Noordin, R., Kaliraj, P., Steel, C., Goodman, D., Lakshmikanthan, V.B. and Ottesen, E. (2004) Recombinant antigen-based antibody assays for the diagnosis and surveillance of lymphatic filariasis—a multicenter trial. *Filaria J* **3**: 9

Larralde, C., Sotelo, J., Montoya, R.M., Palencia, G., Padilla, A., Govezensky, T., Diaz, M.L. and Sciutto, E. (1990) Immunodiagnosis of human cysticercosis in cerebrospinal fluid. Antigens from murine *Taenia crassiceps* cysticerci effectively substitute those from porcine Taenia solium. *Arch Pathol Lab Med* **114**: 926–8

Lashner, B.A. and Loftus, E.V., Jr. (2006) True or false? The hygiene hypothesis for Crohn's disease. *Am J Gastroenterol* **101**: 1003–4

Lee, E.G., Bae, Y.A., Jeong, Y.T., Chung, J.Y., Je, E.Y., Kim, S.H., Na, B.K., Ju, J.W., Kim, T.S., Ma, L., Cho, S.Y. and Kong, Y. (2005a) Proteomic analysis of a 120 kDa protein complex in cyst fluid of *Taenia solium* metacestode and preliminary evaluation of its value for the serodiagnosis of neurocysticercosis. *Parasitology* **131**: 867–79

Lee, E.G., Lee, M.Y., Chung, J.Y., Je, E.Y., Bae, Y.A., Na, B.K., Kim, T.S., Eom, K.S., Cho, S.Y. and Kong, Y. (2005b) Feasibility of baculovirus-expressed recombinant 10-kDa antigen in the serodiagnosis of *Taenia solium* neurocysticercosis. *Trans R Soc Trop Med Hyg* **99**: 919–26

Leonardi-Bee, J., Pritchard, D. and Britton, J. (2006) Asthma and current intestinal parasite infection: systematic review and meta-analysis. *Am J Respir Crit Care Med* **174**: 514–23

Lim, B.H., Noordin, R., Nor, Z.M., Rahman, R.A., Abdullah, K.A. and Sinnadurai, S. (2004) *Brugia malayi* infection in *Meriones unguiculatus*: antibody response to recombinant BmR1. *Exp Parasitol* **108**: 1–6

Lim, B.H., Rahmah, N., Afifi, S.A., Ramli, A. and Mehdi, R. (2001) Comparison of *Brugia*-Elisa and thick blood smear examination in a prevalence study of brugian filariasis in Setiu, Terengganu, Malaysia. *Med J Malaysia* **56**: 491–6

Lopez, J.A., Garcia, E., Cortes, I.M., Sotelo, J., Tato, P. and Molinari, J.L. (2004) Neurocysticercosis: relationship between the developmental stage of metacestode present and the titer of specific IgG in the cerebrospinal fluid. *Ann Trop Med Parasitol* **98**: 569–79

Luoba, A.I., Wenzel Geissler, P., Estambale, B., Ouma, J.H., Alusala, D., Ayah, R., Mwaniki, D., Magnussen, P. and Friis, H. (2005) Earth-eating and reinfection with intestinal helminths among pregnant and lactating women in western Kenya. *Trop Med Int Health* **10**: 220–7

Macdonald, T.T. and Monteleone, G. (2005) Immunity, inflammation, and allergy in the gut. *Science* **307**: 1920–5

Mandal, J., Singhi, P.D., Khandelwal, N. and Malla, N. (2006) Evaluation of ELISA and dot blots for the serodiagnosis of neurocysticercosis, in children found to have single or multiple enhancing lesions in computerized tomographic scans of the brain. *Ann Trop Med Parasitol* **100**: 39–48

Marchi Blatt, J. and Cantos, G.A. (2003) Evaluation of techniques for the diagnosis of Strongyloides stercoralis in human immunodeficiency virus (HIV) positive and HIV negative individuals in the city of Itajai, Brazil. *Braz J Infect Dis* **7**: 402–8

Margono, S.S., Ito, A., Sato, M.O., Okamoto, M., Subahar, R., Yamasaki, H., Hamid, A., Wandra, T., Purba, W.H., Nakaya, K., Ito, M., Craig, P.S. and Suroso, T. (2003) *Taenia solium* taeniasis/cysticercosis in Papua, Indonesia in 2001: detection of human worm carriers. *J Helminthol* **77**: 39–42

Margono, S.S., Wandra, T., Swasono, M.F., Murni, S., Craig, P.S. and Ito, A. (2006) Taeniasis/cysticercosis in Papua (Irian Jaya), Indonesia. *Parasitol Int* **55 Suppl**: S143–8

Markell, E.K., John, D.T. and Krotoski, W.A. (1999) Markell and Voge's Medical Parasitology. 8th ed. W.B Saunders Company, Philadelphia.

Marley, S.E., Lammie, P.J., Eberhard, M.L. and Hightower, A.W. (1995) Reduced antifilarial IgG4 responsiveness in a subpopulation of microfilaremic persons. *J Infect Dis* **172**: 1630–3

Marti, H. and Koella, J.C. (1993) Multiple stool examinations for ova and parasites and rate of false-negative results. *J Clin Microbiol* **31**: 3044–5

Martin, L.K. and Beaver, P.C. (1968) Evaluation of Kato thick-smear technique for quantitative diagnosis of helminth infections. *Am J Trop Med Hyg* **17**: 382–91

Maxwell, C., Hussain, R., Nutman, T.B., Poindexter, R.W., Little, M.D., Schad, G.A. and Ottesen, E.A. (1987) The clinical and immunologic responses of normal human volunteers to low dose hookworm (*Necator americanus*) infection. *Am J Trop Med Hyg* **37**: 126–34

McCarthy, J.S., Guinea, A., Weil, G.J. and Ottesen, E.A. (1995) Clearance of circulating filarial antigen as a measure of the macrofilaricidal activity of diethylcarbamazine in *Wuchereria bancrofti* infection. *J Infect Dis* **172**: 521–6

McCormack, G., Moriarty, D., O'Donoghue, D.P., McCormick, P.A., Sheahan, K. and Baird, A.W. (2001) Tissue cytokine and chemokine expression in inflammatory bowel disease. *Inflamm Res* **50**: 491–5

Melmed, G.Y. and Abreu, M.T. (2004) New insights into the pathogenesis of inflammatory bowel disease. *Curr Gastroenterol Rep* **6**: 474–81

Melrose, W. (2002) A Survey of Glucose 6 Phosphate Dehydrogenase (G6PD) Deficiency, Lymphatic Filariasis and Intestinal parasites in School-Age Children in the Republic of East Timor. WHO

Melrose, W., Pisters, P., Turner, P., Kombati, Z., Selve, B.P., Hii, J. and Speare, R. (2000) Prevalence of filarial antigenaemia in Papua New Guinea: results of surveys by the School of Public Health and Tropical Medicine, James Cook University, Townsville, Australia. *P N G Med J* **43**: 161–5

Methanitikorn, R., Sukontason, K., Sukontason, K.L. and Piangjai, S. (2003) Evaluation of the formalin-tween concentration technique for parasitic detection. *Rev Inst Med Trop Sao Paulo* **45**: 289–91

Meyrowitsch, D.W., Simonsen, P.E. and Magesa, S.M. (2004) Long-term effect of three different strategies for mass diethylcarbamazine administration in bancroftian filariasis: follow-up at 10 years after treatment. *Trans R Soc Trop Med Hyg* **98**: 627–34

Meyrowitsch, D.W., Simonsen, P.E. and Makunde, W.H. (1995) A 16-year follow-up study on bancroftian filariasis in three communities of north-eastern Tanzania. *Ann Trop Med Parasitol* **89**: 665–75

Michael, E., Bhargava, A., Bundy, D., Cooper, E., Savioli, L., Neira, M., Albonico, M., Beach, M.J., Chwaya, H.M., Crompton, D.W.T., Dunne, J., Ehrenberg, J.P., Gyorkos, T., Kvalsvig, J., Taylor, M.G., Urbani, C.I.e., Zheng, F., Garner, P., Dickson, R., Demellweek, C., Williamson, P. and Awasthi, S. (2000) Treatment for intestinal helminth infection.(Letter to the Editor). *BMJ* **321**: 1224

Miguel, E. and Kremer, M. (2004) Worms: identifying impacts on education and health in the presence of treatment externalities. *Econometrica* **72**: 159–217

Ministry of Health and National Statistics Office, T.L., University of Newcastle and The Australian National University. (2004) Timor-Leste 2003 Demographic and Health Survey. Ministry of Health. Dili

Montano, S.M., Villaran, M.V., Ylquimiche, L., Figueroa, J.J., Rodriguez, S., Bautista, C.T., Gonzalez, A.E., Tsang, V.C., Gilman, R.H. and Garcia, H.H. (2005) Neurocysticercosis: association between seizures, serology, and brain CT in rural Peru. *Neurology* **65**: 229–33

Montresor, A. and Palmer, K. (2006) Taeniasis/cysticercosis trend worldwide and rationale for control. *Parasitol Int* **55 Suppl**: S301–3

Moraes, L.R.S. and Cairncross, S. (2004) Environmental interventions and the pattern of geohelminth infections in Salvador, Brazil. *Parasitology* **129**: 223–32

Moraes, L.R.S., Cancio, J.A. and Cairncross, S. (2004) Impact of drainage and sewerage on intestinal nematode infections in poor urban areas in Salvador, Brazil. *Trans R Soc Trop Med Hyg* **98**: 197–204

More, S.J. and Copeman, D.B. (1990) A highly specific and sensitive monoclonal antibody-based ELISA for the detection of circulating antigen in bancroftian filariasis. *Trop Med Parasitol* **41**: 403–6

Morimoto, M., Whitmire, J., Xiao, S., Anthony, R.M., Mirakami, H., Star, R.A., Urban, J.F., Jr. and Gause, W.C. (2004) Peripheral CD4 T cells rapidly accumulate at the host: parasite interface during an inflammatory Th2 memory response. *J Immunol* **172**: 2424–30

Mortimer, K., Brown, A., Feary, J., Jagger, C., Lewis, S., Antoniak, M., Pritchard, D. and Britton, J. (2006) Dose-ranging study for trials of therapeutic infection with *Necator americanus* in humans. *Am J Trop Med Hyg* **75**: 914–20

Moulia-Pelat, J.P., Glaziou, P., Chanteau, S., Nguyen-Ngoc, L., Marcet, Y., Gardines, R., Martin, P.M. and Cartel, J.L. (1993) Periodicity of *Wuchereria bancrofti* var. *pacifica* filariasis in French Polynesia. *Trop Med Parasitol* **44**: 83–5

Moulia-Pelat, J.P., Glaziou, P., Nguyen-Ngoc, L., Cardines, D., Spiegel, A. and Cartel, J.L. (1992) A comparative study of detection methods for evaluation of microfilaremia in lymphatic filariasis control programmes. *Trop Med Parasitol* **43**: 146–8

Moulia-Pelat, J.P., Glaziou, P., Weil, G.J., Nguyen, L.N., Gaxotte, P. and Nicolas, L. (1995) Combination ivermectin plus diethylcarbamazine, a new effective tool for control of lymphatic filariasis. *Trop Med Parasitol* **46**: 9–12

Muck, A.E., Pires, M.L. and Lammie, P.J. (2003) Influence of infection with non-filarial helminths on the specificity of serological assays for antifilarial immunoglobulin G4. *Trans R Soc Trop Med Hyg* **97**: 88–90

Naish, S., McCarthy, J. and Williams, G.M. (2004) Prevalence, intensity and risk factors for soil-transmitted helminth infection in a South Indian fishing village. *Acta Trop* **91**: 177–87

National Statistical Office of Papua New Guinea. (2003) Population and Social Statistics. Retrieved 7/5/2009, from http://www.spc.int/prism/country/pg/stats/Pop_Soc_%20Stats/popsoc.htm

Ng, T.F. and Ko, R.C. (1994) Serodiagnosis of cysticercosis: specificity of different antigens and enzyme-linked immunosorbent assays. *Trans R Soc Trop Med Hyg* **88**: 421–2

Nguyen, N.L., Plichart, C. and Esterre, P. (1999) Assessment of immunochromatographic test for rapid lymphatic filariasis diagnosis. *Parasite* **6**: 355–8

Nguyen, P.H., Nguyen, K.C., Nguyen, T.D., Le, M.B. and Al, E. (2006) Intestinal helminth infections among reproductive age women in Vietnam: prevalence, co-infection and risk factors. *Southeast Asian J Trop Med Public Health and Public Health* **37**: 865

Ngwira, B.M., Jabu, C.H., Kanyongoloka, H., Mponda, M., Crampin, A.C., Branson, K., Alexander, N.D. and Fine, P.E. (2002) Lymphatic filariasis in the Karonga district of northern Malawi: a prevalence survey. *Ann Trop Med Parasitol* **96**: 137–44

Nicolas, L., Plichart, C., Nguyen, L.N. and Moulia-Pelat, J.P. (1997) Reduction of *Wuchereria bancrofti* adult worm circulating antigen after annual treatments of diethylcarbamazine combined with ivermectin in French Polynesia. *J Infect Dis* **175**: 489–92

Nielsen, N.O., Makaula, P., Nyakuipa, D., Bloch, P., Nyasulu, Y. and Simonsen, P.E. (2002) Lymphatic filariasis in Lower Shire, southern Malawi. *Trans R Soc Trop Med Hyg* **96**: 133–8

Nielsen, O.H., Koppen, T., Rudiger, N., Horn, T., Eriksen, J. and Kirman, I. (1996) Involvement of interleukin-4 and -10 in inflammatory bowel disease. *Dig Dis Sci* **41**: 1786–93

Njenga, S.M. and Wamae, C.N. (2001) Evaluation of ICT filariasis card test using whole capillary blood: comparison with Knott's concentration and counting chamber methods. *J Parasitol* **87**: 1140–3

Noordin, R., Aziz, R.A. and Ravindran, B. (2004a) Homologs of the *Brugia malayi* diagnostic antigen BmR1 are present in other filarial parasites but induce different humoral immune responses. *Filaria J* **3**: 10

Noordin, R., Shenoy, R.K. and Rahman, R.A. (2003) Comparison of two IgG4 assay formats (ELISA and rapid dipstick test) for detection of brugian filariasis. *Southeast Asian J Trop Med Public Health* **34**: 768–70

Noordin, R., Wahyuni, S., Mangali, A., Huat, L.B., Yazdanbakhsh, M. and Sartono, E. (2004b) Comparison of IgG4 assays using whole parasite extract and BmR1 recombinant antigen in determining antibody prevalence in brugian filariasis. *Filaria J* **3**: 8

Nuchprayoon, S., Porksakorn, C., Junpee, A., Sanprasert, V. and Poovorawan, Y. (2003) Comparative assessment of an Og4C3 ELISA and an ICT filariasis test: a study of Myanmar migrants in Thailand. *Asian Pac J Allergy Immunol* **21**: 253–7

Nuchprayoon, S., Yentakam, S., Sangprakarn, S. and Junpee, A. (2001) Endemic bancroftian filariasis in Thailand: detection by Og4C3 antigen capture ELISA and the polymerase chain reaction. *J Med Assoc Thai* **84**: 1300–7

Nyan, O.A., Walraven, G.E., Banya, W.A., Milligan, P., Van Der Sande, M., Ceesay, S.M., Del Prete, G. and McAdam, K.P. (2001) Atopy, intestinal helminth infection and total serum IgE in rural and urban adult Gambian communities. *Clin Exp Allergy* **31**: 1672–8

Obregon-Henao, A., Gil, D.L., Gomez, D.I., Sanzon, F., Teale, J.M. and Restrepo, B.I. (2001) The role of N-linked carbohydrates in the antigenicity of *Taenia solium* metacestode glycoproteins of 12, 16 and 18 kD. *Mol Biochem Parasitol* **114**: 209–15

Odashima, N.S., Takayanagui, O.M. and Figueiredo, J.F. (2002) Enzyme linked immunosorbent assay (ELISA) for the detection of IgG, IgM, IgE and IgA against Cysticercus cellulosae in cerebrospinal fluid of patients with neurocysticercosis. *Arq Neuropsiquiatr* **60**: 400–5

Odermatt, P., Leang, R., Bin, B., Bunkea, T. and Socheat, D. (2008) Prevention of lymphatic filariasis with insecticide-treated bednets in Cambodia. *Ann Trop Med Parasitol* **102**: 135–42

Odongo-Aginya, E.I., Taylor, M.G., Sturrock, R.F., Ackers, J.P. and Doehring, E. (1995) Field evaluation of an improved Kato-Katz thick smear technique for quantitative determination of helminth eggs in faeces. *Trop Med Parasitol* **46**: 275–7

Ogilvie, B.M., Bartlett, A., Godfrey, R.C., Turton, J.A., Worms, M.J. and Yeates, R.A. (1978) Antibody responses in self-infections with *Necator americanus*. *Trans R Soc Trop Med Hyg* **72**: 66–71

Ogura, Y., Bonen, D.K., Inohara, N., Nicolae, D.L., Chen, F.F., Ramos, R., Britton, H., Moran, T., Karaliuskas, R., Duerr, R.H., Achkar, J.P., Brant, S.R., Bayless, T.M., Kirschner, B.S., Hanauer, S.B., Nunez, G. and Cho, J.H. (2001a) A frameshift mutation in NOD2 associated with susceptibility to Crohn's disease. *Nature* **411**: 603–6

Ogura, Y., Inohara, N., Benito, A., Chen, F.F., Yamaoka, S. and Nunez, G. (2001b) Nod2, a Nod1/Apaf-1 family member that is restricted to monocytes and activates NF-kappaB. *J Biol Chem* **276**: 4812–8

Ogura, Y., Lala, S., Xin, W., Smith, E., Dowds, T.A., Chen, F.F., Zimmermann, E., Tretiakova, M., Cho, J.H., Hart, J., Greenson, J.K., Keshav, S. and Nunez, G. (2003) Expression of NOD2 in Paneth cells: a possible link to Crohn's ileitis. *Gut* **52**: 1591–7

O'Lorcain, P. and Holland, C.V. (2000) The public health importance of *Ascaris lumbricoides*. *Parasitology* **121 Suppl**: S51–71

Omar, M.S., Sheikha, A.K., Al-Amari, O.M., Abdalla, S.E. and Musa, R.A. (2000) Field evaluation of two diagnostic antigen tests for *Wuchereria bancrofti* infection among Indian expatriates in Saudi Arabia. *Southeast Asian J Trop Med Public Health* **31**: 415–8

Onapa, A.W., Simonsen, P.E., Pedersen, E.M. and Okello, D.O. (2001) Lymphatic filariasis in Uganda: baseline investigations in Lira, Soroti and Katakwi districts. *Trans R Soc Trop Med Hyg* **95**: 161–7

Oshitani, N., Hato, F., Suzuki, K., Sawa, Y., Matsumoto, T., Maeda, K., Higuchi, K., Kitagawa, S. and Arakawa, T. (2003) Cross-reactivity of yeast antigens in human colon and peripheral leukocytes. *J Pathol* **199**: 361–7

Ott, S.J., Musfeldt, M., Ullmann, U., Hampe, J. and Schreiber, S. (2004) Quantification of intestinal bacterial populations by real-time PCR with a universal primer set and minor groove binder probes: a global approach to the enteric flora. *J Clin Microbiol* **42**: 2566–72

Ottesen, E.A., Hooper, P.J., Bradley, M. and Biswas, G. (2008) The global programme to eliminate lymphatic filariasis: health impact after 8 years. *PLoS Negl Trop Dis* **2**: e317

Ottesen, E.A., Skvaril, F., Tripathy, S.P., Poindexter, R.W. and Hussain, R. (1985) Prominence of IgG4 in the IgG antibody response to human filariasis. *J Immunol* **134**: 2707–12.

Owen, I.L. (2006) Current status of *Taenia solium* and cysticercosis in Papua New Guinea. *Parasitol Int* **55 Suppl**: S149–53

Owen, I.L., Pozio, E., Tamburrini, A., Danaya, R.T., Bruschi, F. and Gomez Morales, M.A. (2001) Focus of human trichinellosis in Papua New Guinea. *Am J Trop Med Hyg* **65**: 553–7

PacELF Monitoring and Analysis Network. (2004) PacMAN Book. PacELF Manual Part 2 ed. Pac ELF Home Office

Palmer, L.J., Celedon, J.C., Weiss, S.T., Wang, B., Fang, Z. and Xu, X. (2002) *Ascaris lumbricoides* infection is associated with increased risk of childhood asthma and atopy in rural China. *Am J Respir Crit Care Med* **165**: 1489–93

Palupi, L., Schultink, W., Achadi, E. and Gross, R. (1997) Effective community intervention to improve hemoglobin status in preschoolers receiving once-weekly iron supplementation. *Am J Clin Nutr* **65**: 1057–61

Pani, S.P., Hoti, S.L., Elango, A., Yuvaraj, J., Lall, R. and Ramaiah, K.D. (2000) Evaluation of the ICT whole blood antigen card test to detect infection due to nocturnally periodic *Wuchereria bancrofti* in South India. *Trop Med Int Health* **5**: 359–63

Pani, S.P., Hoti, S.L., Vanamail, P. and Das, L.K. (2004) Comparison of an immunochromatographic card test with night blood smear examination for detection of *Wuchereria bancrofti* microfilaria carriers. *Natl Med J India* **17**: 304–6

Parija, S.C., Bhattacharya, S., Padhan, P. and Shivaprakash, M.R. (2003) Evaluation of formalin-acetone sedimentation in the concentration of stool for intestinal parasites. *Trop Doct* **33**: 163–4

Park, C.L., Frank, A.L., Sullivan, M., Jindal, P. and Baxter, B.D. (1996) Influenza vaccination of children during acute asthma exacerbation and concurrent prednisone therapy. *Pediatrics* **98**: 196–200

Parronchi, P., Romagnani, P., Annunziato, F., Sampognaro, S., Becchio, A., Giannarini, L., Maggi, E., Pupilli, C., Tonelli, F. and Romagnani, S. (1997) Type 1 T-helper cell predominance and interleukin-12 expression in the gut of patients with Crohn's disease. *Am J Pathol* **150**: 823–32

Pattison, D.A. and Speare, R. (2008) Strongyloidiasis in personnel of the Regional Assistance Mission to Solomon Islands (RAMSI). *Med J Aust* **189**: 203–6

Peralta, R.H., Vaz, A.J., Pardini, A., Macedo, H.W., Machado, L.R., De Simone, S.G. and Peralta, J.M. (2002) Evaluation of an antigen from *Taenia crassiceps* cysticercus for the serodiagnosis of neurocysticercosis. *Acta Trop* **83**: 159–68

Perera, M., Whitehead, M., Molyneux, D., Weerasooriya, M. and Gunatilleke, G. (2007) Neglected patients with a neglected disease? A qualitative study of lymphatic filariasis. *PLoS Negl Trop Dis* **1**: e128

Peters, P.A., El Alamy, M., Warren, K.S. and Mahmoud, A.A. (1980) Quick Kato smear for field quantification of Schistosoma mansoni eggs. *Am J Trop Med Hyg* **29**: 217–9

Phantana, S., Sensathein, S., Songtrus, J., Klagrathoke, S. and Phongnin, K. (1999) ICT filariasis test: a new screening test for Bancroftian filariasis. *Southeast Asian J Trop Med Public Health* **30**: 47–51

Pineton de Chambrun, G., Colombel, J.F., Poulain, D. and Darfeuille-Michaud, A. (2008) Pathogenic agents in inflammatory bowel diseases. *Curr Opin Gastroenterol* **24**: 440–7

Pit, D.S., Polderman, A.M., Baeta, S., Schulz-Key, H. and Soboslay, P.T. (2001) Parasite-specific antibody and cellular immune responses in human infected with *Necator americanus* and *Oesophagostomum bifurcum*. *Parasitol Res* **87**: 722–9

Pit, D.S., Polderman, A.M., Schulz-Key, H. and Soboslay, P.T. (2000) Prenatal immune priming with helminth infections: parasite-specific cellular reactivity and Th1 and Th2 cytokine responses in neonates. *Allergy* **55**: 732–9

Prabhakaran, V., Rajshekhar, V., Murrell, K.D. and Oommen, A. (2004) *Taenia solium* metacestode glycoproteins as diagnostic antigens for solitary cysticercus granuloma in Indian patients. *Trans R Soc Trop Med Hyg* **98**: 478–84

Prabhakaran, V., Rajshekhar, V., Murrell, K.D. and Oommen, A. (2007) Conformation-sensitive immunoassays improve the serodiagnosis of solitary cysticercus granuloma in Indian patients. *Trans R Soc Trop Med Hyg* **101**: 570–7

Pritchard, D.I. and Brown, A. (2001) Is *Necator americanus* approaching a mutualistic symbiotic relationship with humans? *Trends Parasitol* **17**: 169–72

Pritchard, D.I., Quinnell, R.J., Slater, A.F., McKean, P.G., Dale, D.D., Raiko, A. and Keymer, A.E. (1990) Epidemiology and immunology of *Necator americanus* infection in a community in Papua New Guinea: humoral responses to excretory-secretory and cuticular collagen antigens. *Parasitology* **100 (Pt 2)**: 317–26

Pritchard, D.I., Quinnell, R.J. and Walsh, E.A. (1995) Immunity in humans to *Necator americanus*: IgE, parasite weight and fecundity. *Parasite Immunol* **17**: 71–5

Pritchard, D.I., Walsh, E.A., Quinell, R.J., Raiko, A., Edmonds, P. and Keymer, A.E. (1992) Isotypic variation in antibody responses in a community in Papua New Guinea to larval and adult antigens during infection, and following reinfection, with the hookworm *Necator americanus*. *Parasite Immunol* **14**: 617–31

Proano-Narvaez, J.V., Meza-Lucas, A., Mata-Ruiz, O., Garcia-Jeronimo, R.C. and Correa, D. (2002) Laboratory diagnosis of human neurocysticercosis: double-blind comparison of enzyme-linked immunosorbent assay and electroimmunotransfer blot assay. *J Clin Microbiol* **40**: 2115–8

Prybylski, D., Alto, W.A., Mengeap, S. and Odaibaiyue, S. (1994) Introduction of an integrated community-based bancroftian filariasis control program into the Mt Bosavi region of the Southern Highlands of Papua New Guinea. *P N G Med J* **37**: 82–9

Quinnell, R., Pritchard, D., Raiko, A., Brown, A. and Shaw, M. (2004) Immune responses in human necatoriasis: association between interleukin-5 responses and resistance to reinfection. *J Infect Dis* **190**: 430–8

Quinton, J.F., Sendid, B., Reumaux, D., Duthilleul, P., Cortot, A., Grandbastien, B., Charrier, G., Targan, S.R., Colombel, J.F. and Poulain, D. (1998) Anti-*Saccharomyces cerevisiae* mannan antibodies combined with antineutrophil cytoplasmic autoantibodies in inflammatory bowel disease: prevalence and diagnostic role. *Gut* **42**: 788–91

Rahmah, N., Anuar, A.K., Ariff, R.H., Zurainee, M.N., A'Shikin A, N., Fadzillah, A., Maimunah, A. and Haq, J.A. (1998) Use of antifilarial IgG4-ELISA to detect *Brugia malayi* infection in an endemic area of Malaysia. *Trop Med Int Health* **3**: 184–8

Rahmah, N., Lim, B.H., Azian, H., Ramelah, T.S. and Rohana, A.R. (2003a) Short communication: use of a recombinant antigen-based ELISA to determine prevalence of brugian filariasis among Malaysian schoolchildren near Pasir Mas, Kelantan-Thailand border. *Trop Med Int Health* **8**: 158–63

Rahmah, N., Lim, B.H., Khairul Anuar, A., Shenoy, R.K., Kumaraswami, V., Lokman Hakim, S., Chotechuang, P., Kanjanopas, K. and Ramachandran, C.P. (2001a) A

recombinant antigen-based IgG4 ELISA for the specific and sensitive detection of *Brugia malayi* infection. *Trans R Soc Trop Med Hyg* **95**: 280–4

Rahmah, N., Shenoy, R.K., Nutman, T.B., Weiss, N., Gilmour, K., Maizels, R.M., Yazdanbakhsh, M. and Sartono, E. (2003b) Multicentre laboratory evaluation of Brugia Rapid dipstick test for detection of brugian filariasis. *Trop Med Int Health* **8**: 895–900

Rahmah, N., Taniawati, S., Shenoy, R.K., Lim, B.H., Kumaraswami, V., Anuar, A.K., Hakim, S.L., Hayati, M.I., Chan, B.T., Suharni, M. and Ramachandran, C.P. (2001b) Specificity and sensitivity of a rapid dipstick test (Brugia Rapid) in the detection of *Brugia malayi* infection. *Trans R Soc Trop Med Hyg* **95**: 601–4

Rajan, T.V. (2005) Natural course of lymphatic filariasis: insights from epidemiology, experimental human infections, and clinical observations. *Am J Trop Med Hyg* **73**: 995–8

Rajgor, D., Gogtay, N.J., Garg, B.S., Deshmukh, P.R. and Kshirsagar, N.A. (2002) Reading ICT filariasis rapid diagnostic card tests under field conditions and issues of good clinical practice in clinical trials. *Trans R Soc Trop Med Hyg* **96**: 574–5

Ramaiah, K.D., Vanamail, P. and Das, P.K. (2007) Changes in *Wuchereria bancrofti* infection in a highly endemic community following 10 rounds of mass administration of diethylcarbamazine. *Trans R Soc Trop Med Hyg* **101**: 250–5

Ramos-Kuri, M., Montoya, R.M., Padilla, A., Govezensky, T., Diaz, M.L., Sciutto, E., Sotelo, J. and Larralde, C. (1992) Immunodiagnosis of neurocysticercosis. Disappointing performance of serology (enzyme–linked immunosorbent assay) in an unbiased sample of neurological patients. *Arch Neurol* **49**: 633–6

Ramzy, R.M., Gad, A.M., Faris, R. and Weil, G.J. (1991) Evaluation of a monoclonal-antibody based antigen assay for diagnosis of *Wuchereria bancrofti* infection in Egypt. *Am J Trop Med Hyg* **44**: 691–5

Ramzy, R.M., Helmy, H., el-Lethy, A.S., Kandil, A.M., Ahmed, E.S., Weil, G.J. and Faris, R. (1999) Field evaluation of a rapid-format kit for the diagnosis of bancroftian filariasis in Egypt. *East Mediterr Health J* **5**: 880–7

Raso, G., Utzinger, J., Silue, K.D., Ouattara, M., Yapi, A., Toty, A., Matthys, B., Vounatsou, P., Tanner, M. and N'Goran, E.K. (2005) Disparities in parasitic infections, perceived ill health and access to health care among poorer and less poor schoolchildren of rural C d'Ivoire. *Trop Med Int Health* **10**: 42–57

Raso, G., Vounatsou, P., Gosoniu, L., Tanner, M., N'Goran, E.K. and Utzinger, J. (2006) Risk factors and spatial patterns of hookworm infection among schoolchildren in a rural area of western Cote d'Ivoire. *Int J Parasitol* **36**: 201–10

Ravindran, B. (2003) Aping Jane Goodall: insights into human lymphatic filariasis. *Trends Parasitol* **19**: 105–9

Ravindran, B., Satapathy, A.K., Sahoo, P.K. and Babu Geddam, J.J. (2000) Protective immunity in human Bancroftian filariasis: inverse relationship between antibodies to microfilarial sheath and circulating filarial antigens. *Parasite Immunol* **22**: 633–7

Recht, M. (2009) Thrombocytopenia and anemia in infants and children. *Emerg Med Clin North Am* **27**: 505–23

Reeder, J.C. (2003) Health research in Papua New Guinea. *Trends Parasitol* **19**: 241–5

Ridley, D.S. and Hawgood, B.C. (1956) The value of formol-ether concentration of faecal cysts and ova. *J Clin Pathol* **9**: 74–6

Ritchie, L.S. (1948) An ether sedimentation technique for routine stool examinations. *Bull U S Army Med Dep* **8**: 326

Rocha, A., Addiss, D., Ribeiro, M.E., Noroes, J., Baliza, M., Medeiros, Z. and Dreyer, G. (1996) Evaluation of the Og4C3 ELISA in *Wuchereria bancrofti* infection: infected persons with undetectable or ultra-low microfilarial densities. *Trop Med Int Health* **1**: 859–64

Rocha, A., Lima, G., Medeiros, Z., Aguiar-Santos, A., Alves, S., Montarroyos, U., Oliveira, P., Beliz, F., Netto, M.J. and Furtado, A. (2004) Circulating filarial antigen in the hydrocele fluid from individuals living in a bancroftian filariasis area—Recife, Brazil: detected by the monoclonal antibody Og4C3-assay. *Mem Inst Oswaldo Cruz* **99**: 101–5

Rodriguez, S., Dorny, P., Tsang, V.C., Pretell, E.J., Brandt, J., Lescano, A.G., Gonzalez, A.E., Gilman, R.H. and Garcia, H.H. (2009) Detection of *Taenia solium* antigens and anti-*T. solium* antibodies in paired serum and cerebrospinal fluid samples from patients with intraparenchymal or extraparenchymal neurocysticercosis. *J Infect Dis* **199**: 1345–52

Rose, G. (1985) Sick individuals and sick populations. *Int J Epidemiol* **14**: 32–8

Saathoff, E., Olsen, A., Kvalsvig, J.D. and Geissler, P.W. (2002) Geophagy and its association with geohelminth infection in rural schoolchildren from northern KwaZulu-Natal, South Africa. *Trans R Soc Trop Med Hyg* **96**: 485–90

Saathoff, E., Olsen, A., Kvalsvig, J.D., Appleton, C.C., Sharp, B. and Kleinschmidt, I. (2005) Ecological covariates of Ascaris lumbricoides infection in schoolchildren from rural KwaZulu-Natal, South Africa. *Trop Med Int Health* **10:** 412–2

Sahoo, P.K., Babu Geddam, J.J., Satapathy, A.K., Mohanty, M.C., Das, B.K., Acharya, A.S., Mishra, N. and Ravindran, B. (2002) Bancroftian filariasis: a 13-year follow-up study of asymptomatic microfilariae carriers and endemic normals in Orissa, India. *Parasitology* **124**: 191–201

Sako, Y., Nakao, M., Ikejima, T., Piao, X.Z., Nakaya, K. and Ito, A. (2000) Molecular characterization and diagnostic value of *Taenia solium* low-molecular-weight antigen genes. *J Clin Microbiol* **38**: 4439–44

Sako, Y., Nakao, M., Nakaya, K., Yamasaki, H. and Ito, A. (2006) Recombinant antigens for serodiagnosis of cysticercosis and echinococcosis. *Parasitol Int* **55 Suppl**: S69–73

Sanchez-Munoz, F., Dominguez-Lopez, A. and Yamamoto-Furusho, J.K. (2008) Role of cytokines in inflammatory bowel disease. *World J Gastroenterol* **14**: 4280–8

Sandars, D. (1951) Viability of *Ascaris lumbricoides* Eggs 'Preserved' in Formalin. *Nature* **167**: 730

Santhanam, S., Kumar, H., Sethumadhavan, K.V., Chandrasekharan, A., Jain, D.C., Malhotra, A., Ghosh, T.K. and Weil, G.J. (1989) Detection of *Wuchereria bancrofti* antigen in serum and finger prick blood samples by enzyme immunoassay: field evaluation. *Trop Med Parasitol* **40**: 440–4

Santos, F.L., Cerqueira, E.J. and Soares, N.M. (2005) Comparison of the thick smear and Kato-Katz techniques for diagnosis of intestinal helminth infections. *Rev Soc Bras Med Trop* **38**: 196–8

Sapak, P., Williams, G., Bryan, J. and Riley, I. (2000) Efficacy of mass single-dose diethylcarbamazine and DEC-fortified salt against bancroftian filariasis in Papua New Guinea six months after treatment. *P N G Med J* **43**: 213–20

Sato, M.O., Sako, Y., Nakao, M., Yamasaki, H., Nakaya, K. and Ito, A. (2006) Evaluation of purified *Taenia solium* glycoproteins and recombinant antigens in the serologic detection of human and swine cysticercosis. *J Infect Dis* **194**: 1783–90

Savioli, L., Albonico, M., Engels, D. and Montresor, A. (2004) Progress in the prevention and control of schistosomiasis and soil-transmitted helminthiasis. *Parasitol Int* **53**: 103–13

Savioli, L., Bundy, D. and Tomkins, A. (1992) Intestinal parasitic infections: a soluble public health problem. *Trans R Soc Trop Med Hyg* **86**: 353–4

Schad, G.A. and Anderson, R.M. (1985) Predisposition to hookworm infection in humans. *Science* **228**: 1537–40

Schantz, P.M., Sarti, E., Plancarte, A., Wilson, M., Criales, J.L., Roberts, J. and Flisser, A. (1994) Community-based epidemiological investigations of cysticercosis due to *Taenia solium*: comparison of serological screening tests and clinical findings in two populations in Mexico. *Clin Infect Dis* **18**: 879–85

Scheel, C.M., Khan, A., Hancock, K., Garcia, H.H., Gonzalez, A.E., Gilman, R.H. and Tsang, V.C. (2005) Serodiagnosis of neurocysticercosis using synthetic 8-kD proteins: comparison of assay formats. *Am J Trop Med Hyg* **73**: 771–6

Schopf, L.R., Hoffmann, K.F., Cheever, A.W., Urban, J.F., Jr. and Wynn, T.A. (2002) IL-10 is critical for host resistance and survival during gastrointestinal helminth infection. *J Immunol* **168**: 2383–92

Schreiber, S., Heinig, T., Thiele, H.G. and Raedler, A. (1995) Immunoregulatory role of interleukin 10 in patients with inflammatory bowel disease. *Gastroenterology* **108**: 1434–44

Schuetz, A., Addiss, D.G., Eberhard, M.L. and Lammie, P.J. (2000) Evaluation of the whole blood filariasis ICT test for short-term monitoring after antifilarial treatment. *Am J Trop Med Hyg* **62**: 502–3

Schuurkamp, G.J., Kereu, R.K., Bulungol, P.K., Kawereng, A. and Spicer, P.E. (1994) Diethylcarbamazine in the control of bancroftian filariasis in the Ok Tedi area of Papua New Guinea: phase 2—annual single-dose treatment. *P N G Med J* **37**: 65–81

Scrivener, S., Yemaneberhan, H., Zebenigus, M., Tilahun, D., Girma, S., Ali, S., McElroy, P., Custovic, A., Woodcock, A., Pritchard, D., Venn, A. and Britton, J. (2001) Independent effects of intestinal parasite infection and domestic allergen exposure on risk of wheeze in Ethiopia: a nested case-control study. *Lancet* **358**: 1493–9

Sehgal, S.C., Vinayak, V.K. and Gupta, U. (1977) Evaluation of Kato thick smear technique for the detection of helminthic ova in faeces. *Indian J Med Res* **65**: 509–12

Selve, B.P., Bwadua, S., Misa, M., James, K., Usurup, J.P., Turner, P., Melrose, W., Yad, W., Samuel, R. and Eddie, C. (2000) Community empowerment in the control of lymphatic filariasis in Misima, Milne Bay Province using diethylcarbamazine in combination with albendazole. *P N G Med J* **43**: 183–7

Senanayake, N. and Roman G.C. (1993) Epidemiology of epilepsy in developing countries. *Bull World Health Organ* **71(2):** 247-58

Sendid, B., Colombel, J.F., Jacquinot, P.M., Faille, C., Fruit, J., Cortot, A., Lucidarme, D., Camus, D. and Poulain, D. (1996) Specific antibody response to oligomannosidic epitopes in Crohn's disease. *Clin Diagn Lab Immunol* **3**: 219–26

Sherchand, J.B., Obsomer, V., Thakur, G.D. and Hommel, M. (2003) Mapping of lymphatic filariasis in Nepal. *Filaria J* **2**: 7

Shivananda, S., Lennard-Jones, J., Logan, R., Fear, N., Price, A., Carpenter, L. and van Blankenstein, M. (1996) Incidence of inflammatory bowel disease across

Europe: is there a difference between north and south? Results of the European Collaborative Study on Inflammatory Bowel Disease (EC-IBD). *Gut* **39**: 690–7

Siegmund, B. and Zeitz, M. (2004) Therapeutic approaches in inflammatory bowel disease based on the immunopathogenesis. *Rocz Akad Med Bialymst* **49**: 22–30

Simanjuntak, G.M., Margono, S.S., Okamoto, M. and Ito, A. (1997) Taeniasis/cysticercosis in Indonesia as an emerging disease. *Parasitol Today* **19**: 321–3

Simeon, D.T., Grantham-McGregor, S.M., Callender, J.E. and Wong, M.S. (1995) Treatment of *Trichuris trichiura* infections improves growth, spelling scores and school attendance in some children. *J Nutr* **125**: 1875–83

Simonsen, P., Pedersen, E., Lemnge, M. and Michael, E. (1997) Lymphatic filariasis research and control in Africa. *Parasitol Today* **13**: 413–5

Simonsen, P.E., Bernhard, P., Jaoko, W.G., Meyrowitsch, D.W., Malecela-Lazaro, M.N., Magnussen, P. and Michael, E. (2002) Filaria dance sign and subclinical hydrocoele in two east African communities with bancroftian filariasis. *Trans R Soc Trop Med Hyg* **96**: 649–53

Simonsen, P.E. and Dunyo, S.K. (1999) Comparative evaluation of three new tools for diagnosis of bancroftian filariasis based on detection of specific circulating antigens. *Trans R Soc Trop Med Hyg* **93**: 278–82

Simonsen, P.E., Lemnge, M.M., Msangeni, H.A., Jakobsen, P.H. and Bygbjerg, I.C. (1996) Bancroftian filariasis: the patterns of filarial-specific immunoglobulin G1 (IgG1), IgG4, and circulating antigens in an endemic community of northeastern Tanzania. *Am J Trop Med Hyg* **55**: 69–75

Simonsen, P.E. and Magesa, S.M. (2004) Observations on false positive reactions in the rapid NOW Filariasis card test. *Trop Med Int Health* **9**: 1200–2

Simonsen, P.E., Magesa, S.M., Meyrowitsch, D.W., Malecela-Lazaro, M.N., Rwegoshora, R.T., Jaoko, W.G. and Michael, E. (2005) The effect of eight half–yearly single-dose treatments with DEC on *Wuchereria bancrofti* circulating antigenaemia. *Trans R Soc Trop Med Hyg* **99**: 541–7

Sonnenberg, A., McCarty, D.J. and Jacobsen, S.J. (1991) Geographic variation of inflammatory bowel disease within the United States. *Gastroenterology* **100**: 143–9

Sotelo, J., Guerrero, V. and Rubio, F. (1985) Neurocysticercosis: a new classification based on active and inactive forms. A study of 753 cases. *Arch Intern Med* **145**: 442–5

Southgate, B.A. (1974) A quantitative approach to parasitological techniques in Bancroftian filariasis and its effect on epidemiological understanding. *Trans R Soc Trop Med Hyg* **68**: 177–86

Speare, R. (1988) Strongyloides in Africa: the need to identify the species. *Postgrad Doctor* **10**: 164–71

Speare, R., Latasi, F.F., Nelesone, T., Harmen, S., Melrose, W., Durrheim, D. and Heukelbach, J. (2006) Prevalence of soil transmitted nematodes on Nukufetau, a remote Pacific island in Tuvalu. *BMC Infect Dis* **6**: 110

Steel, C. and Ottesen, E.A. (2001) Evolution of immunologic responsiveness of persons living in an area of endemic bancroftian filariasis: a 17-year follow-up. *J Infect Dis* **184**: 73–9

Steel, C., Ottesen, E.A., Weller, P.F. and Nutman, T.B. (2001) Worm burden and host responsiveness in *Wuchereria bancrofti* infection: use of antigen detection to refine earlier assessments from the South Pacific. *Am J Trop Med Hyg* **65**: 498–503

Steinmann, P., Zhou, X.-N., Li, Y.-L., Li, H.-J., Chen, S.-R., Yang, Z., Fan, W., Jia, T.-W., Li, L.-H., Vounatsou, P. and Utzinger, J. (2007) Helminth infections and risk factor analysis among residents in Eryuan county, Yunnan province, China. *Acta Trop* **104**: 38–51

Stephenson, L.S., Holland, C.V. and Cooper, E.S. (2000) The public health significance of *Trichuris trichiura*. *Parasitology* **121 Suppl**: S73–95

Stephenson, L.S., Latham, M.C., Adams, E.J., Kinoti, S.N. and Pertet, A. (1993a) Physical fitness, growth and appetite of Kenyan school boys with hookworm, *Trichuris trichiura* and *Ascaris lumbricoides* infections are improved four months after a single dose of albendazole. *J Nutr* **123**: 1036–46

Stephenson, L.S., Latham, M.C., Adams, E.J., Kinoti, S.N. and Pertet, A. (1993b) Weight gain of Kenyan school children infected with hookworm, *Trichuris trichiura* and *Ascaris lumbricoides* is improved following once- or twice-yearly treatment with albendazole. *J Nutr* **123**: 656–65

Stephenson, L.S., Latham, M.C., Kurz, K.M., Kinoti, S.N. and Brigham, H. (1989) Treatment with a single dose of albendazole improves growth of Kenyan schoolchildren with hookworm, *Trichuris trichiura*, and *Ascaris lumbricoides* infections. *Am J Trop Med Hyg* **41**: 78–87

Stoll, N.R. (1947) This wormy world. *J Parasitol* **31**: 1–18

Stoltzfus, R.J., Albonico, M., Chwaya, H.M., Savioli, L., Tielsch, J., Schulze, K. and Yip, R. (1996) Hemoquant determination of hookworm-related blood loss and its role in iron deficiency in African children. *Am J Trop Med Hyg* **55**: 399–404

Stothard, J.R., Imison, E., French, M.D., Sousa-Figueiredo, J.C., Khamis, I.S. and Rollinson, D. (2008) Soil-transmitted helminthiasis among mothers and their pre-school children on Unguja Island, Zanzibar with emphasis upon ascariasis. *Parasitology* **135**: 1447–55

Strachan, D.P. (1989) Hay fever, hygiene, and household size. *BMJ* **299**: 1259–60

Strachan, D.P. (2000) Family size, infection and atopy: the first decade of the 'hygiene hypothesis'. *Thorax* **55 Suppl 1**: S2–10

Streit, T. and Lafontant, J.G. (2008) Eliminating lymphatic filariasis: a view from the field. *Ann N Y Acad Sci* **1136**: 53–63

Subahar, R., Hamid, A., Purba, W., Wandra, T., Karma, C., Sako, Y., Margono, S.S., Craig, P.S. and Ito, A. (2001) *Taenia solium* infection in Irian Jaya (west Papua), Indonesia: a pilot serological survey of human and porcine cysticercosis in Jayawijaya district. *Trans R Soc Trop Med Hyg* **95**: 388–90

Summers, R.W., Elliott, D.E., Qadir, K., Urban, J.F., Jr., Thompson, R. and Weinstock, J.V. (2003) *Trichuris suis* seems to be safe and possibly effective in the treatment of inflammatory bowel disease. *Am J Gastroenterol* **98**: 2034–41

Summers, R.W., Elliott, D.E., Urban, J.F., Jr., Thompson, R. and Weinstock, J.V. (2005a) *Trichuris suis* therapy in Crohn's disease. *Gut* **54**: 87–90

Summers, R.W., Elliott, D.E., Urban, J.F., Jr., Thompson, R.A. and Weinstock, J.V. (2005b) *Trichuris suis* therapy for active ulcerative colitis: a randomized controlled trial. *Gastroenterology* **128**: 825–32

Sunish, I.P., Rajendran, R., Satyanarayana, K., Munirathinam, A. and Gajanana, A. (2001) Immunochromatographic test (ICT) for estimation of true prevalence of bancroftian filariasis in an endemic area in southern India. *Trans R Soc Trop Med Hyg* **95**: 607–9

Supali, T., Rahmah, N., Djuardi, Y., Sartono, E., Ruckert, P. and Fischer, P. (2004) Detection of filaria-specific IgG4 antibodies using Brugia Rapid test in individuals from an area highly endemic for Brugia timori. *Acta Trop* **90**: 255–61

Suzuki, L.A., Arruda, G.C., Quagliato, E.M. and Rossi, Q.L. (2007) Evaluation of *Taenia solium* and Taenia crassiceps cysticercal antigens for immunodiagnosis of neurocysticercosis using ELISA on cerebrospinal fluid samples. *Rev Soc Bras Med Trop* **40**: 152–5

Sventoraityte, J., Zvirbliene, A., Kiudelis, G., Zalinkevicius, R., Praskevicius, A., Kupcinskas, L. and Tamosiunas, V. (2008) Immune system alterations in patients with inflammatory bowel disease during remission. *Medicina (Kaunas)* **44**: 27–33

Takayanagui, O.M. and Odashima, N.S. (2006) Clinical aspects of neurocysticercosis. *Parasitol Int* **55 Suppl**: S111–5

Talbot, J.T., Viall, A., Direny, A., de Rochars, M.B., Addiss, D., Streit, T., Mathieu, E. and Lammie, P.J. (2008) Predictors of compliance in mass drug administration

for the treatment and prevention of lymphatic filariasis in Leogane, Haiti. *Am J Trop Med Hyg* **78**: 283–8

Talla, I., Kongs, A. and Verle, P. (1992) Preliminary study of the prevalence of human schistosomiasis in Richard-Toll (the Senegal river basin). *Trans R Soc Trop Med Hyg* **86**: 182

Taylor, M.D., Betts, C.J. and Else, K.J. (2000) Peripheral cytokine responses to *Trichuris muris* reflect those occurring locally at the site of infection. *Infect Immun* **68**: 1815–9

Taylor-Robinson, D.C., Jones, A.P. and Garner, P. (2007) Deworming drugs for treating soil-transmitted intestinal worms in children: effects on growth and school performance. *Cochrane Database Syst Rev*: CD000371

Teesdale, C.H. and Amin, M.A. (1976a) Comparison of the Bell technique, a modified Kato thick smear technique, and a digestion method for the field diagnosis of schistosomiasis mansoni. *J Helminthol* **50**: 17–20

Teesdale, C.H. and Amin, M.A. (1976b) A simple thick-smear technique for the diagnosis of Schistosoma mansoni infection. *Bull World Health Organ* **54**: 703–5

Teesdale, C.H., Fahringer, K. and Chitsulo, L. (1985) Egg count variability and sensitivity of a thin smear technique for the diagnosis of Schistosoma mansoni. *Trans R Soc Trop Med Hyg* **79**: 369–73

Terhell, A.J., Haarbrink, M., Abadi, K., Bronneberg, D.C., Tieleman, M.C., Asri, M. and Yazdanbakhsh, M. (1996) A filter paper technique for the detection of anti-filarial IgG4 in lymphatic filariasis. *Trans R Soc Trop Med Hyg* **90**: 196–8

Thompson, R.C., Owen, I.L., Puana, I., Banks, D., Davis, T.M. and Reid, S.A. (2003) Parasites and biosecurity—the example of Australia. *Trends Parasitol* **19**: 410–6

Tisch, D.J., Bockarie, M.J., Dimber, Z., Kiniboro, B., Tarongka, N., Hazlett, F.E., Kastens, W., Alpers, M.P. and Kazura, J.W. (2008) Mass drug administration trial to eliminate lymphatic filariasis in Papua New Guinea: changes in microfilaremia, filarial antigen, and Bm14 antibody after cessation. *Am J Trop Med Hyg* **78**: 289–93

Tisch, D.J., Hazlett, F.E., Kastens, W., Alpers, M.P., Bockarie, M.J. and Kazura, J.W. (2001) Ecologic and biologic determinants of filarial antigenemia in bancroftian filariasis in Papua New Guinea. *J Infect Dis* **184**: 898–904

Tomono, N., Anantaphruti, M.T., Jongsuksuntigul, P., Thongthien, P., Leerapan, P., Silapharatsamee, Y., Kojima, S. and Looareesuwan, S. (2003) Risk factors of helminthiases among schoolchildren in southern Thailand. *Southeast Asian J Trop Med Public Health* **34**: 264–8

Tripathy, K., Duque, E., Bolanos, O., Lotero, H. and Mayoral, L.G. (1972) Malabsorption syndrome in ascariasis. *Am J Clin Nutr* **25**: 1276–81

Truant, A.L., Elliott, S.H., Kelly, M.T. and Smith, J.H. (1981) Comparison of formalin-ethyl ether sedimentation, formalin-ethyl acetate sedimentation, and zinc sulfate flotation techniques for detection of intestinal parasites. *J Clin Microbiol* **13**: 882–4

Tsang, V.C., Brand, J.A. and Boyer, A.E. (1989) An enzyme-linked immunoelectrotransfer blot assay and glycoprotein antigens for diagnosing human cysticercosis (Taenia solium). *J Infect Dis* **159**: 50–9

Tsironi, E., Feakins, R.M., Probert, C.S., Rampton, D.S. and Phil, D. (2004) Incidence of inflammatory bowel disease is rising and abdominal tuberculosis is falling in Bangladeshis in East London, United Kingdom. *Am J Gastroenterol* **99**: 1749–55

Turner, J.D., Faulkner, H., Kamgno, J., Cormont, F., Van Snick, J., Else, K.J., Grencis, R.K., Behnke, J.M., Boussinesq, M. and Bradley, J.E. (2003) Th2 cytokines are associated with reduced worm burdens in a human intestinal helminth infection. *J Infect Dis* **188**: 1768–75

Turner, P., Copeman, B., Gerisi, D. and Speare, R. (1993) A comparison of the Og4C3 antigen capture ELISA, the Knott test, an IgG4 assay and clinical signs, in the diagnosis of Bancroftian filariasis. *Trop Med Parasitol* **44**: 45–8.

US Federal Drug and Administration. (2009). IA 57-21: Imported Trichuris Suis Ova (TSO) Pig Whipworm Eggs. Retrieved 20/12/2009, from http://www.accessdata.fda.gov/cms_ia/importalert_159.html

Utzinger, J., Rinaldi, L., Lohourignon, L.K., Rohner, F., Zimmermann, M.B., Tschannen, A.B., N'Goran, E.K. and Cringoli, G. (2008) FLOTAC: a new sensitive technique for the diagnosis of hookworm infections in humans. *Trans R Soc Trop Med Hyg* **102**: 84–90

Utzinger, J., Vounatsou, P., N'Goran, E.K., Tanner, M. and Booth, M. (2002) Reduction in the prevalence and intensity of hookworm infections after praziquantel treatment for schistosomiasis infection. *Int J Parasitol* **32**: 759–65

van den Biggelaar, A.H., Rodrigues, L.C., van Ree, R., van der Zee, J.S., Hoeksma-Kruize, Y.C., Souverijn, J.H., Missinou, M.A., Borrmann, S., Kremsner, P.G. and Yazdanbakhsh, M. (2004) Long-term treatment of intestinal helminths increases mite skin-test reactivity in Gabonese schoolchildren. *J Infect Dis* **189**: 892–900

van den Bogaerde, J., Kamm, M. and Knight, S. (2001) Immune sensitization to food, yeast and bacteria in Crohn's disease. *Aliment Pharmacol Ther* **15**: 1647–53

Van Kruiningen, H.J.V. and West, A.B. (2005) Potential danger in the medical use of *Trichuris suis* for the treatment of inflammatory bowel disease. *Inflamm. Bowel Dis*. **11**: 515

Van Kruiningen, H.J. and West, A.B. (2007) Iatrogenic *Trichuris suis* infection. *Arch Pathol Lab Med* **131**: 180

Vanamail, P., Ramaiah, K.D., Pani, S.P., Das, P.K., Grenfell, B.T. and Bundy, D.A. (1996) Estimation of the fecund life span of *Wuchereria bancrofti* in an endemic area. *Trans R Soc Trop Med Hyg* **90**: 119–21

Vermeire, S., Noman, M., Van Assche, G., Baert, F., D'Haens, G. and Rutgeerts, P. (2007) Effectiveness of concomitant immunosuppressive therapy in suppressing the formation of antibodies to infliximab in Crohn's disease. *Gut* **56**: 1226–31

Vinayak, V.K., Sehgal, S.C., Gupta, U. and Chhuttani, P.N. (1978) Evaluation of Kato thick smear technique for quantitative estimation of helminthic infections. *Indian J Med Res* **67**: 231–3

Walker, S.P., Wachs, T.D., Gardner, J.M., Lozoff, B., Wasserman, G.A., Pollitt, E. and Carter, J.A. (2007) Child development: risk factors for adverse outcomes in developing countries. *Lancet* **369**: 145–57

Wallin, M.T. and Kurtzke, J.F. (2004) Neurocysticercosis in the United States: review of an important emerging infection. *Neurology* **63**: 1559–64

Walson, J.L., Herrin, B.R. and John-Stewart, G. (2009) Deworming helminth co-infected individuals for delaying HIV disease progression. *Cochrane Database Syst Rev:* CD006419

Wandra, T., Subahar, R., Simanjuntak, G.M., Margono, S.S., Suroso, T., Okamoto, M., Nakao, M., Sako, Y., Nakaya, K., Schantz, P.M. and Ito, A. (2000) Resurgence of cases of epileptic seizures and burns associated with cysticercosis in Assologaima, Jayawijaya, Irian Jaya, Indonesia, 1991–95. *Trans R Soc Trop Med Hyg* **94**: 46–50

Watkins, W.E., Cruz, J. and Pollitt, E. (1996) The effects of deworming on indicators of school performance in Guatemala. *Trans R Soc Trop Med Hyg* **90**: 156–61

Watkins, W.E. and Pollitt, E. (1996) Effect of removing Ascaris on the growth of Guatemalan schoolchildren. *Pediatrics* **97**: 871–6

Wattal, S., Dhariwal, A.C., Ralhan, P.K., Tripathi, V.C., Regu, K., Kamal, S. and Lal, S. (2007) Evaluation of Og4C3 antigen ELISA as a tool for detection of bancroftian filariasis under lymphatic filariasis elimination programme. *J Commun Dis* **39**: 75–84

Weerasooriya, M.V., Gunawardena, N.K., Itoh, M., Qiu, X.G. and Kimura, E. (2002) Prevalence and intensity of *Wuchereria bancrofti* antigenaemia in Sri Lanka by

Og4C3 ELISA using filter paper-absorbed whole blood. *Trans R Soc Trop Med Hyg* **96**: 41-5

Weerasooriya, M.V., Itoh, M., Mudalige, M.P., Qiu, X.G., Kimura, E., Gunawardena, N.K. and Fujimaki, Y. (2003) Human infection with *Wuchereria bancrofti* in Matara, Sri Lanka: the use, in parallel, of an ELISA to detect filaria-specific IgG4 in urine and of ICT card tests to detect filarial antigen in whole blood. *Ann Trop Med Parasitol* **97**: 179–85

Weil, G. (2000) Letters to the Editor. *Trop Med Int Health* **5**: 832–3

Weil, G. and Liftis, F. (1987) Identification and partial characterization of a parasite antigen in sera from humans infected with *Wuchereria bancrofti*. *J Immunol* **138**: 3035–41

Weil, G.J., Chandrashekar, R., Liftis, F., McVay, C.S., Bosshardt, S.C. and Klei, T.R. (1990) Circulating parasite antigen in *Brugia pahangi*-infected jirds. *J Parasitol* **76**: 78–84

Weil, G.J., Jain, D.C., Santhanam, S., Malhotra, A., Kumar, H., Sethumadhavan, K.V., Liftis, F. and Ghosh, T.K. (1987) A monoclonal antibody-based enzyme immunoassay for detecting parasite antigenemia in bancroftian filariasis. *J Infect Dis* **156**: 350–5

Weil, G.J., Lammie, P.J. and Weiss, N. (1997) The ICT Filariasis Test: A rapid-format antigen test for diagnosis of bancroftian filariasis. *Parasitol Today* **13**: 401–4

Weil, G.J., Malane, M.S., Powers, K.G. and Blair, L.S. (1985) Monoclonal antibodies to parasite antigens found in the serum of *Dirofilaria immitis*-infected dogs. *J Immunol* **134**: 1185–91

Weil, G.J. and Ramzy, R.M. (2007) Diagnostic tools for filariasis elimination programs. *Trends Parasitol* **23**: 78–82

Weil, G.J., Ramzy, R.M., Chandrashekar, R., Gad, A.M., Lowrie, R.C., Jr. and Faris, R. (1996) Parasite antigenemia without microfilaremia in bancroftian filariasis. *Am J Trop Med Hyg* **55**: 333–7

Williams-Blangero, S., Blangero, J. and Bradley, M. (1997) Quantitative genetic analysis of susceptibility to hookworm infection in a population from rural Zimbabwe. *Hum Biol* **69**: 201–8

Williams-Blangero, S., Subedi, J., Upadhayay, R.P., Manral, D.B., Rai, D.R., Jha, B., Robinson, E.S. and Blangero, J. (1999) Genetic analysis of susceptibility to infection with *Ascaris lumbricoides*. *Am J Trop Med Hyg* **60**: 921–6

Wilson, M., Bryan, R.T., Fried, J.A., Ware, D.A., Schantz, P.M., Pilcher, J.B. and Tsang, V.C. (1991) Clinical evaluation of the cysticercosis enzyme-linked immunoelectrotransfer blot in patients with neurocysticercosis. *J Infect Dis* **164**: 1007–9

Wong, M.S. and Bundy, D.A. (1990) Quantitative assessment of contamination of soil by the eggs of *Ascaris lumbricoides* and *Trichuris trichiura*. *Trans R Soc Trop Med Hyg* **84**: 567–70

Wong, M.S., Bundy, D.A. and Golden, M.H. (1988) Quantitative assessment of geophagous behaviour as a potential source of exposure to geohelminth infection. *Trans R Soc Trop Med Hyg* **82**: 621–5

World Health Organisation. (1987) Prevention and Control of Intestinal Parasitic Infections. Report of a WHO Expert Committee.Technical Report Series 749. World Health Organisation.

World Health Organization. (1992) Global Health Situation and Projections Estimates. Vol. 2007. Division of Epidemiological Surveillance and Health Situation and Trend Assessment, Geneva.

World Health Organization. (1997) WHA50.29 Elimination of Lymphatic Filariasis as a Public Health Problem

World Health Organization. (2001) Schistosomiasis and Soil Transmitted Helminth Infections WHA 54.19. Vol. 2007. World Health Organization, Geneva

World Health Organization. (2002) Prevention and Control of Schistosomiasis and Soil-transmitted Helminthiasis: Report of a WHO Expert committee. World Health Organization WHO technical report series no. 912. 8–14 October 2001. Geneva

World Health Organization. (2005) Deworming: The Millennium Development Goals. The Evidence Is in: Deworming Helps Neet the Millennium Development Goals. World Health Organization WHO/CDS/CPE/PVC/2005.12. Geneva

World Health Organization. (2006) Preventive Chemotherapy in Human Helminthiasis. Preventive Chemotherapy and Transmission Control, Department of Control and Neglected Tropical Diseases, World Health Organization, Geneva.

World Health Organization. (2007) Global Programme to Eliminate Lymphatic Filariasis. *Wkly Epidemiol Rec* **82**: 361–80

World Health Organization, Montresor, A., Crompton, D.W., Hall, A., Bundy, D. and Savioli, L. (1998) Guidelines for the Evaluation of Soil-transmitted Helminthiasis and Schistosomiasis at Community Level. World Health Organization WHO/CTD/SIP/98.1. Geneva

Wright, V. and Bickle, Q. (2005) Immune responses following experimental human hookworm infection. *Clin Exp Immunol* **142**: 398–403

Wynd, S., Carron, J., Selve, B., Leggat, P.A., Melrose, W. and Durrheim, D.N. (2007a) Qualitative analysis of the impact of a lymphatic filariasis elimination programme using mass drug administration on Misima Island, Papua New Guinea. *Filaria J* **6**: 1

Wynd, S., Durrheim, D.N., Carron, J., Selve, B., Chaine, J.P., Leggat, P.A. and Melrose, W. (2007b) Socio-cultural insights and lymphatic filariasis control—lessons from the Pacific. *Filaria J* **6**: 3

Wynd, S., Melrose, W.D., Durrheim, D.N., Carron, J. and Gyapong, M. (2007c) Understanding the community impact of lymphatic filariasis: a review of the sociocultural literature. *Bull World Health Organ* **85**: 493–8

Yajima, A., Jouquet, P., Trung, D.D., Cam, T.D.T., Cong, D.T., Orange, D. and Montresor, A. (2008) High latrine coverage is not reducing the prevalence of soil-transmitted helminthiasis in Hoa Binh province, Vietnam. *Trans R Soc Trop Med Hyg* **In Press, Corrected Proof**

Yang, S.K., Yun, S., Kim, J.H., Park, J.Y., Kim, H.Y., Kim, Y.H., Chang, D.K., Kim, J.S., Song, I.S., Park, J.B., Park, E.R., Kim, K.J., Moon, G. and Yang, S.H. (2008) Epidemiology of inflammatory bowel disease in the Songpa-Kangdong district, Seoul, Korea, 1986–2005: a KASID study. *Inflamm Bowel Dis* **14**: 542–9

Young, C.A., Sonnenberg, A. and Burns, E.A. (1994) Lymphocyte proliferation response to baker's yeast in Crohn's disease. *Digestion* **55**: 40–3

Young, K.H., Bullock, S.L., Melvin, D.M. and Spruill, C.L. (1979) Ethyl acetate as a substitute for diethyl ether in the formalin-ether sedimentation technique. *J Clin Microbiol* **10**: 852–3

Young, S.L., Goodman, D., Farag, T.H., Ali, S.M., Khatib, M.R., Khalfan, S.S., Tielsch, J.M. and Stoltzfus, R.J. (2007) Geophagia is not associated with *Trichuris* or hookworm transmission in Zanzibar, Tanzania. *Trans R Soc Trop Med Hyg* **101**: 766–72

Zamen, V. and Cheong, C.H. (1967) A comparison of Kato thick smear technique with zinc sulphate flotation method, for the detection of helminth ova in faeces. *Trans R Soc Trop Med Hyg* **61**: 751

Zar, J.H. (1999) Biostatistical analysis. 4th ed. Prentice Hall, Upper Saddle River, N.J.

Zini, D., Farrell, V.J. and Wadee, A.A. (1990) The relationship of antibody levels to the clinical spectrum of human neurocysticercosis. *J Neurol Neurosurg Psychiatry* **53**: 656–61

Zoli, A.P., Nguekam, Shey-Njila, O., Nsame Nforninwe, D., Speybroeck, N., Ito, A., Sato, M.O., Dorny, P., Brandt, J. and Geerts, S. (2003) Neurocysticercosis and epilepsy in Cameroon. *Trans R Soc Trop Med Hyg* **97**: 683–6

Appendices

Appendix 1: Materials

1.3.3 Collection and Test Method

3 % Giemsa Stain

Giemsa powder	3.8 g
Glycerol	250 ml
Methanol	250 ml

For use dilute 1.5 ml of prepared stain in 50 ml of buffered water pH 7.1-7.2

Buffered Water pH 7.1-7.2

Stock phosphate solution A

Sodium dihydrogen phosphate 1-hydrate	27.6 g
Deionised water	1000 ml

Stock phosphate solution B

di-Sodium hydrogen phosphate anhydrous	28.39 g
Deionised water	1000 ml

Stock phosphate solution A	140 ml
Stock phosphate solution B	360 ml
Deionised water	500 ml

4.2.3 Parasitology Field Procedures

SAF fixative (X5 concentrate)

Sodium acetate	75 g
Acetic acid, glacial	100 ml
Formaldehyde	200 ml
Deionised water	625 ml

For use 1 part concentrate is diluted with 4 parts water

6.4.4 Parasitology laboratory procedures

D'Antoni's iodine solution

Potassium iodide	1 g

Potassium iodine crystals	1.5 g
Deionised water	100 ml
Store in brown bottle	

Kinyoun's carbolfuchsin

Basic fuchsin	4 g
Phenol (liquefied)	8 g
Ethanol	20 ml
Deionised water	100 ml

1% Sulphuric acid

Sulphuric acid	1 ml
Deionised water	99 ml

Methylene blue

Methylene blue	0.3 g
Ethanol (95%)	30 ml
0.01% Potassium hydroxide	100 ml

0.01% Potassium hydroxide

Potassium hydroxide	0.1 g
Deionised water	1 L

5.2.2 ELISA for NCC

Phosphate Buffered Saline

Sodium chloride	8 g
di-Sodium hydrogen phosphate.12 H_2O	1.44 g
Potassium chloride	0.2 g
Potassium dihydrogen phosphate	0.24 g
Deionised water	1000 ml

Adjust to pH 7.4 with HCl/NaOH

PBS-Tween 20 washing buffer

PBS	1000 ml
Tween 20	0.5 ml

Casein Blocking Buffer

Casein	10 g
Sodium chloride	8.8 g
Tris	2.42 g

Deionised water	1000 ml

Adjust to pH 7.6 with HCl/NaOH

Coating Buffer

Sodium carbonate	1.59 g
Sodium bicarbonate	2.93 g
Sodium azide	0.2 g
Deionised water	1000 ml

Adjust to pH 9.6 with HCl/NaOH

0.1 M Citrate buffer

Citric acid	8.4 g
Deionised water	400 ml

Adjust to pH 4.7 with sodium hydroxide

ABTS Substrate

2.2-azino-di(3-ethyle-benzthiazoline-6-sulphonate)	9 mg
0.1 M Citrate Buffer	30 ml
Hydrogen peroxide (30%)	10 ul

5.2.3 SDS-PAGE and Immunoblot

30% Acrylamide

Acrylamide	29.2 g
Bis-acrylamide	0.8 g
Deionised water	100 ml

1.5 M Tris-HCl pH 8.8

Tris	18.17 g
SDS	0.4 g
Deionised water	100 ml

Adjust to pH 8.8

0.5 M Tris-HCL pH 6.8

Tris	6.06 g
SDS	0.4 g
Deionised water	100 ml

Adjust to pH 6.8

12.5 % Acrylamide gel (Running gel)

30% acrylamide	3 ml
1.5 M Tris-HCl 0.4% SDS pH 8.8	1.8 ml
Deionised water	2.4 ml
Ammonium persulphate	28 ul
TEMED	4 ul

4 % Acrylamide gel (Stacking gel)

30% acrylamide	0.36 ml
0.5 M Tris-HCL 0.4%, SDS pH 6.8	0.6 ml
Deionised water	1.44 ml
Ammonium persulphate	7.2 ul
TEMED	4 ul

Running Buffer

Tris	15 g
Glycine	72 g
SDS	5 g
Deionised water	5 l

Blotting Buffer

Tris	15 g
Glycine	72 g
Methanol	1000 ml

Sample Buffer

0.5 M Tris	20 ml
Glycerol	20 ml
10% Sodium Dodecyl Sulphate	20 ml
2-Mercaptoethanol	2 ml
0.05% Bromophenol blue	10 ml
Deionised water	8 ml

4-Chloro-1-Napthol

Ethanol (100%)	160 ml
Deionised water	40 ml
4-Chloro-1-Napththol	0.4 g

0.1 M Phosphate Buffer

Sodium dihydrogen phosphate 1-hydrate	14.19 g
Deionised water	1000 ml

Staining Buffer

0.1 M Phosphate Buffer	4 ml
4CN	1 ml
Hydrogen peroxide	4 ul

6.2.3 Lymphocyte Proliferation Assay

100 mM L-glutamine

L-glutamine	292.2 mg
Deionised water	20 ml
Filter through 0.2 µm filter	

1.25 M Hepes

Hepes	5.96 g
Deionised water	20 ml

Filter through 0.2 µm filter

ds-RPMI-1640

Foetal Horse Serum	1 ml
100 mM L-glutamine	0.2 ml
1.25 M Hepes	0.2 ml
RPMI-1640	3.6 ml

6.2.4 Whole Blood Cytokine Assays

PBS-0.1 BSA

Phosphate Buffered Saline	100 ml
Bovine Serum Albumin	0.1 g

Phosphate Buffered Saline (PBS)-Tween

PBS	1000 ml
Tween 20	0.5 ml

0.5 M Sulphuric Acid

Sulphuric Acid	2.7 ml
Deionised water	97.3 ml

6.2.5 IgG ELISA assay to Excretory/Secretory *N. americanus* antigen

0.1 M Citric Acid

Citric acid	19.21 g
Deionised water	1000 ml

0.2 M dibasic Sodium Phosphate

Sodium phosphate.$7H_20$	53.65 g
Deionised water	1000 ml

Citrate Phosphate Buffer pH 5.0

0.1 M Citric acid	24.3 ml
0.2 M dibasic sodium phosphate	25.7 ml
Deionised water	50 ml

TMB Substrate

1 tablet TMP (Sigma)	
Citrate Phosphate Buffer pH 5.0	10 ml
Hydrogen peroxide 30%	2 ul

2 M Sulphuric Acid

Sulphuric Acid	11 ml
Deionised water	89 ml

6.2.6 Western Blot

3 M Tris-HCl 0.5 % SDS pH 8.5

Tris	36.34 g
SDS	0.5 g
Deionised water	100 ml

10 % Acrylamide gel (Running gel)

30% acrylamide	3.3 ml
3 M Tris-HCl 0.5 % SDS pH 8.5	3.3 ml
Deionised water	3.4 ml
Ammonium persulphate	100 ul
TEMED	10 ul

4% Acrylamide gel (Stacking gel)

30% acrylamide	0.67 ml
3 M Tris-HCl 0.5 % SDS pH 8.5	1.25 ml
Deionised water	3.1 ml
Ammonium persulphate	50 ul
TEMED	5 ul

0.1M Tris-HCl (Running Buffers) Bottom Reservoir

Tris-HCl	12.1 g
Deionised water	1000 ml

Adjust to pH 8.9 with HCl

Top Reservoir

Tris	12.1 g
Tricine	17.9 g
SDS	10 g
Deionised water	1000 ml

Transfer Buffer

Glycine	14.41 g
Tris	3.025 g
SDS	1 g
Methanol	200 ml
Deionised water	800 ml

Tris Buffered Saline

Tris	3 g
Sodium Chloride	8 g
Potassium Chloride	0.2 g

Adjust to pH 7.4 with HCl

Blocking Buffer

TrBS	100 ml
Milk Powder	5 g

Wash Solution

TrBS	1000 ml
Tween-20	0.5 ml

4-chloro-1-napthol (4CN Substrate)

4CN	50 mg
Ethanol	10 ml
TrBS	40 ml
Hydrogen peroxide	30 ul

Nitro blue Tetrazolium

NBT	75 mg
Dimethylformamide	1 ml

5-Bromo-4 Chloro-3'Indoylphosphate p-Toluidine

BCIP	50 mg
Dimethyl formamide	1 ml

Substrate Buffer

Tris	45.41 g
Deionised water	500 ml
Adjust to pH 9.5 with HCl	

BCIP/NBT Substrate

Substrate Buffer	20 ml
NBT	44 ul
BCIP	33 ul

Appendix 2: Checklist of Materials required for Baseline Survey

Item	Number	√	Item	Number	√
Needles	1000 (21 boxes)		Pencils	5	
SST II BD Vacutainer Tubes	1000 (10 packets)		Pens	10	
EDTA BD Vacutainer Tubes	1000 (10 packets)		ICT kits: From Ministry of Health, PNG	1000 (2 boxes)	
Glass slides	1000 (20 packets)		Parafilm	1 roll	
Cotton wool *	1050 pieces		Labels	1000	
Alcohol swabs *	1050 pieces (10 packets)		Gloves*	1 box	
Capillary tubes	1000 (10 containers)		Biohazard bags	5	
Faeces containers	1000 (2 boxes)		Sharps containers*	2	
Terri Wipes	12		Watch	1	
SAF concentrate	2 L		Slide boxes	10	
Exercise book	2		Masking Tap	2 rolls	
Packing Slip	1		Tourniquets	10	
Receipt book	1		Ice packs	5	
Torch	1		Anti malarial	Enough	
Lancets*	200				

Appendix 3: Survey Questions for Timor-Leste

Vizita fatin Sentinel 2006

Aldeia _____

GPS group _____ GPS point _____

Naran _____

Loron moris _____ Mane / Feto

Idade _____

Relasaun ho Chefe familia _____

Tratamento ba Lumbriga Simu ona Seidauk

Data tratamento _____

Ai-moruk nia naran _____

Perguntas

1. Hela iha aldeia ne'e tinan hira ona?
2. Ema nain hira mak hela iha uma ida ne'e?
3. Imi iha sintina ka lae? Iha La iha
4. Imio ba sintina iha ne'ebe? Uza hamutuk sintina isa/ railuan / kintal / mota
5. Iha kolesaun lixo publiku Iha La iha
6. Bele lee? Bele Labele Ble hakerek? Bele Labele
7. Imi iha? Fahi/Karau timor ka Vaka/Bibi/Manu/Asu/Busa

Observasaun

Matrial hodi halo uma Ai/ kalen/ Tali-tahan / Du'ut/ Au/ Sementi/Semi Sementi/

Eletrisidade solar panel Iha la iha

Bee Posu/ Pia/ mota

Uza bee hamutuk ho vizinho sira? Loos Lae