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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

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20 July 2010

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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 Information derived from the published or unpublished work of others has been acknowledged in the text and a list of references is given.

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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).

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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.

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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.

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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

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