LYMPHATIC FILARIASIS ELIMINATION: RESIDUAL ENDEMNICITY, SPATIAL CLUSTERING AND FUTURE SURVEILLANCE USING THE NEW FILARIASIS CELISA DIAGNOSTIC ASSAY

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 Information derived from the published or unpublished work of others has been acknowledged in the text and a list of references is given.

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Signature Date

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PREFACE

The research was made possible by ongoing financial support from GlaxoSmithKline (GSK). By using the statistical software, SaTScan, I am obligated to note: "SaTScan™ is a trademark of Martin Kulldorff. The SaTScan™ software was developed under the joint auspices of (i) Martin Kulldorff, (ii) the National Cancer Institute, and (iii) Farzad Mostashari of the New York City Department of Health and Mental Hygiene".

The research undertaken as part of the thesis was a collaborative effort with the Pacific Programme for the Elimination of Lymphatic Filariasis (PacELF), based in Fiji, and the Centers for Disease Control and Prevention (CDC), based in USA. The bulk of the research from Samoa was a collaborative effort with the World Health Organization (Apia, Samoa) and the Ministry of Health in Samoa.

In general, editorial assistance was provided by my supervisors including advice on data interpretation. Advice on statistical analysis is described in detail below.

Base Shape Files for ArcGIS Version 9.0
Phil Bright (Secretariat to the Pacific Community (SPC), New Caledonia).

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the ground…… oh by the way, do not forget the emergency beacon on my desk – you may need it….." And believe me, there were times when I felt like flicking that switch! Running my own field teams in various countries across the Pacific has provided me with priceless life lessons and memories and I only have Wayne to thank for providing me with this opportunity.

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PRESENTATIONS

Joseph, H., and Melrose, W. Laboratory Diagnosis of Lymphatic Filariasis. Australian Institute of Medical Scientists (AIMS), Cairns, Australia, June 2007

Joseph, H., Lymphatic Filariasis in Samoa; project proposal. Ministry of Health, Samoa, May 2008

Joseph, H., Lammie, P., McClintock, S., Maiava, F., and Melrose, W. Spatial Analysis of Lymphatic Filariasis in Samoa. American Society of Tropical Medicine and Hygiene (ASTMH) conference, New Orleans, USA, Dec 2008

Future Presentations:

Joseph, H., Lammie, P., McClintock, S., Maiava, F., and Melrose, W. First Evidence of Spatial Clustering of Lymphatic Filariasis in an ***Aedes polynesiensis*** Endemic Area. American Society of Tropical Medicine and Hygiene (ASTMH) conference, Atlanta, USA, Nov 2010

PUBLICATIONS

Joseph, H., and Melrose, W. D. (2010) Applicability of the filter paper technique for detection of antifilarial IgG_4 antibodies using the Bm14 Filariasis CELISA. ***Journal of Parasitology Research*** doi:10.1155/2010/594687: 6 pages

Weil, G.J., Curtis, K.C., Fischer, P.U., Won, K.Y., Lammie, P.J., **Joseph, H.M.,** Melrose, W.D., and Brattig, N.W., (2010). A multi-centre evaluation of a new antibody test kit for lymphatic filariasis employing recombinant ***Brugia malayi*** antigen BM-14. ***Acta Tropica*** [Epub ahead of print]

Dos Santos, M., Armaral, S., Harmen, S., **Joseph, H.,** Fernandes, J. and Counahan, M. (2010). The prevalence of common skin infections in Timor-Leste: A cross sectional survey. ***BMC Infectious Diseases*** **10**: 61

Future Publications

Joseph, H., Maiava, F., Lammie, P. and Melrose, W. Evaluation of continuing transmission and clustering of residual infection of lymphatic filariasis in Samoa. Part I: epidemiological assessment ***In Submission Acta Tropica***

Joseph, H., Moloney, J., Maiava, F., McClintock, S., Lammie, P. and Melrose, W. Evaluation of continuing transmission and clustering of residual infection of lymphatic filariasis in Samoa. Part II: spatial clustering ***In Submission Acta Tropica***

Joseph, H., Clough, A., Maiava, F and Melrose, W. Exploratory Study Investigating Factors Influencing Mass Drug Administration (MDA) Compliance for Lymphatic Filariasis in Samoa, ***Manuscript in preparation***

Joseph, H., Moloney, J., Maiava, F., Taleo, F., 'Ake, M., Capuano, C. and Melrose, W. Application of the Filariasis CELISA anti-filarial IgG_4 antibody assay in LF surveillance in the South Pacific. ***Manuscript in preparation***

ABSTRACT

Lymphatic Filariasis (LF) is a mosquito-transmitted parasitic disease caused by the filarial nematodes ***Wuchereria bancrofti***, ***Brugia malayi*** and ***Brugia timori***. In 1997, the 50th World Health Assembly approved a resolution calling for the elimination of LF as a public health problem (WHA50.29). This was deemed achievable with a regime of annual Mass Drug Administrations (MDAs) and, where appropriate, vector control for a minimum of four to six years. The Pacific counterpart was named the Pacific Programme to Eliminate Lymphatic Filariasis (PacELF). In the Pacific, countries which have reached the threshold of < 0.1% circulating filarial antigen (CFA) prevalence in children entered surveillance mode until 2012, whereas countries with persistent transmission planned further MDAs. Successful elimination of LF requires:

1) Accurate identification of residual foci of transmission (in countries with persistent transmission);
2) Comprehensive surveillance strategies to detect and combat potential resurgence (in countries entering surveillance mode); and,
3) Culturally appropriate education campaigns to encourage MDA compliance, as systematic non-compliers become reservoirs of infection.

It is crucial to apply sensitive diagnostic tools which are capable of identifying these areas of residual endemnicity or resurgence early. This phase of low prevalence poses particular challenges: “hot spots” may be scattered and ill-

defined and the diagnostic tools measuring microfilaraemia (Mf) and CFA that were successful in the earlier phase of the programme may no longer be adequate because of issues with sensitivity, the requirement for larger sampling sizes, and lag phases before Mf or CFA are detectable in newly infected persons. The addition of antibody serology as a complementary diagnostic tool would provide an earlier warning system, since children born after the interruption of transmission would be antibody negative.

In order to incorporate serology into the LF programme, use of a standardised commercial assay must be used, such as the Filariasis Cellabs Enzyme-Linked Immunosorbent Assay (CELISA). Although the Filariasis CELISA has been manufactured since 2006, it is yet to be investigated for its potential use in large scale sampling. It was the aim of this research to determine:

1) The efficacy of the Filariasis CELISA antibody assay;
2) Its usefulness as a potential diagnostic tool for the inclusion into the LF programme; and,
3) Its role in future surveillance work.

This was achieved by validating the Filariasis CELISA for field applicability, assessing its efficacy for identifying areas of residual endemnicity, and investigating the spatial relationships between exposed and infected individuals. In addition, during the progression of the thesis, data became available concerning MDA compliance in Samoa. MDA compliance is also crucial for successful elimination of LF since systematic non-compliers remain as potential reservoirs of infection.

The Filariasis CELISA was easily applicable for field work using whole blood dried onto filter paper. Filter paper sampling had a sensitivity of 92% and a specificity of 77%, when compared to plasma samples. Five thousand four hundred and ninety-eight filter paper samples were assayed from four LF endemic South Pacific countries (Tuvalu, Tonga, Vanuatu, and Samoa). Antibody prevalence rates correlated with cessation of LF transmission in Tonga and Vanuatu, both of which have entered surveillance mode, and ongoing transmission in Samoa and Tuvalu. Most importantly, use of CFA prevalence in children alone, the current World Health Organization (WHO) recommendation, missed vital residual areas of endemic foci in Samoa, as observed by high antibody prevalence in children and Mf positive individuals. This observation required further investigation with an in-depth epidemiological study.

In Samoa, five villages were chosen for prevalence surveys, including Siufaga, which was originally believed to be LF-free. Results showed that the reservoir of infection was the older males and that there was a correlation between transmission (Mf/CFA positivity) and exposure in children. Crucially, ongoing transmission was occurring in Siufaga, as demonstrated by an overall CFA prevalence exceeding 1% and high antibody prevalence in children. CFA testing of children alone would not have identified Siufaga as an area of residual endemnicity.

Accurate identification of residual foci of transmission is challenging in areas where ***Aedes polynesiensis*** is endemic, such as Samoa, since no

geographical clustering of infection has been demonstrated. Results from the aforementioned epidemiological study were spatially linked to household of residence (community based analyses) and/or primary school (school based analyses) of attendance. “Community based” analyses revealed significant spatial clusters of households with infected individuals and a relationship to antibody positive children when they were included in the spatial analysis. Similar results were observed for “school based” analyses. These promising findings are the first evidence of spatial clustering of LF in a day-biting ***Ae. polynesiensis*** endemic area. In addition, these results are the first evidence of dual clustering of Mf/CFA individuals with exposed children.

In Samoa, MDA non-compliance of infected individuals may contribute to persistent transmission. Exploring why these individuals are non-compliant is of paramount importance to the LF programme. Individuals testing positive for LF and children aged 7 – 10 years were asked to participate in a questionnaire designed to ascertain: 1) level of LF knowledge, (2) compliance, and (3) a number of risk factors. For the infected individuals, there was a significant association between MDA compliance and knowledge of LF and, for the children, this association also extended to use of mosquito protection. This exploratory study highlights the need for restructuring current educational campaigns, and their deliverance, to appropriately target children and the systematically non-compliant infected individuals. In addition, the study highlights the necessity to instigate qualitative studies to explore cultural and religious beliefs; a strong driver of compliance.

The overall findings fill critical gaps in knowledge for the elimination of LF namely:

1) Incorporation of antibody serology should be a priority because:
    a. Certain areas of residual transmission will not be detected using Mf or CFA diagnostic testing alone; and,
    b. Surveillance requires a diagnostic test capable of detecting resurgence early so that action can be timely.
2) In Samoa:
    a. Identification of spatial clustering has a significant impact on the LF programme in terms of targeted treatment, re-introduction of vector control campaigns and aiding health personnel to locate potential Mf positive cases;
    b. Previously declared “LF-free” villages may have residual transmission; and,
    c. New health education campaigns are a necessity for targeting non-compliant individuals.

The addition of antibody serology into the repertoire of LF diagnostic tools holds huge promise for identifying areas of residual endemnicity and in future surveillance and control of LF.

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LIST OF ABBREVIATIONS

Ab	antibody
ADCC	antibody dependent cellular cytotoxicity
ADL	adenolymphangitis
ADLA	acute dermatolymphangioadenitis
AFL	acute filarial lymphangitis
APCs	antigen presenting cells
Bm	***Brugia malayi***
Bm14	***Brugia malayi*** 14
BmR1	***Brugia malayi*** recombinant antigen 1
X^2	Chi-squared
C	control
CDC	Centers for Disease Control and Prevention
CELISA	Cellabs enzyme linked immunosorbent assay
CFA	circulating filarial antigen
CI	confidence intervals
COMBI	Communication for Behavioural Impact
CTS	child transmission survey
DEC	diethylcarbamazine citrate
DNA	deoxyribonucleic acid
^oC	degrees Celsius
df	degrees of freedom
DOT	directly observed therapy
EDTA	ethylenediaminetetra-acetic acid
ELISA	enzyme linked immunosorbent assay
g	gravitational force
GIS	geographic information system
GMP	good manufacturing practice
GPELF	Global Programme for the Elimination of Lymphatic Filariasis
GPS	global positioning system
GSK	GlaxoSmithKline
HH	household
HIV	human immunodeficiency virus

H_2O_2	hydrogen peroxide
ICT	immunochromatographic test
IEC	information, education and communication
IFNg	interferon gamma
Ig	immunoglobulin
IL	interleukin
iL3	infective larvae stage three
IU	implementation unit
JCU	James Cook University
KAP	Knowledge, Attitudes and Practices
kDa	kilodalton
kg	kilogram
km	kilometre
LF	lymphatic filariasis
LPS	lipopolysaccharide
LQAS	lot quality assurance sampling
m	metres
MDA	mass drug administration
µg	microgram
mg	milligram
µL	microlitre
mL	millilitre
Mf	microfilariae
n	number of participants
N/A	not applicable
ND	not done
NSW	New South Wales
NK	natural killer
nm	nanometres
NMEA	National Marine Electronics Association
NO	nitric oxide
NPV	negative predictive value
NTD	neglected tropical disease
OCP	onchocerciasis control programme

OD	optical density
Og4C3	***Onchocerca gibsoni*** 4C3
OPD	***o***-phenylenediamine
OR	odds ratio
PacCARE	PacELF Coordination and Review Group
PacELF	Pacific Programme for the Elimination of Lymphatic Filariasis
PAR	participatory action research
PBS	phosphate buffered saline
PC	phosphorylcholine
PCR	polymerase chain reaction
PICT	Pacific Islands Countries and Territories
PNG	Papua New Guinea
PPV	positive predictive value
QC	quality control
Rad	radius
RMS	root mean square
RNA	ribonucleic acid
RR	relative risk
RTPCR	reverse transcriptase polymerase chain reaction
SD	standard deviation
SPC	Secretariat to the Pacific Community
X	times
T	test
Th	T helper cell
TMB	3,3',5,5'-tetramethylbenzidine
TNFα	tumour necrosis factor alpha
TV	television
VIC	Victoria
vs	versus
Wb	***Wuchereria bancrofti***
WHA	World Health Assembly
WHO	World Health Organization