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

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in partial fulfilment of the requirements for the
degree of Doctor of Philosophy
in the School of Public Health, Tropical Medicine
and Rehabilitation Sciences,
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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 Information derived from the published or unpublished work of others has been acknowledged in the text and a list of references is given.

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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”.

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PRESENTATIONS

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Future Presentations:

PUBLICATIONS


**Future Publications**


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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<tr>
<td>Ab</td>
<td>antibody</td>
</tr>
<tr>
<td>ADCC</td>
<td>antibody dependent cellular cytotoxicity</td>
</tr>
<tr>
<td>ADL</td>
<td>adenolymphangitis</td>
</tr>
<tr>
<td>ADLA</td>
<td>acute dermatolymphangioadenitis</td>
</tr>
<tr>
<td>AFL</td>
<td>acute filarial lymphangitis</td>
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<tr>
<td>APCs</td>
<td>antigen presenting cells</td>
</tr>
<tr>
<td>Bm</td>
<td><em>Brugia malayi</em></td>
</tr>
<tr>
<td>Bm14</td>
<td><em>Brugia malayi</em> 14</td>
</tr>
<tr>
<td>BmR1</td>
<td><em>Brugia malayi</em> recombinant antigen 1</td>
</tr>
<tr>
<td>$X^2$</td>
<td>Chi-squared</td>
</tr>
<tr>
<td>C</td>
<td>control</td>
</tr>
<tr>
<td>CDC</td>
<td>Centers for Disease Control and Prevention</td>
</tr>
<tr>
<td>CELISA</td>
<td>Cellabs enzyme linked immunosorbent assay</td>
</tr>
<tr>
<td>CFA</td>
<td>circulating filarial antigen</td>
</tr>
<tr>
<td>CI</td>
<td>confidence intervals</td>
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<tr>
<td>COMBI</td>
<td>Communication for Behavioural Impact</td>
</tr>
<tr>
<td>CTS</td>
<td>child transmission survey</td>
</tr>
<tr>
<td>DEC</td>
<td>diethylcarbamazine citrate</td>
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<tr>
<td>DNA</td>
<td>deoxyribonucleic acid</td>
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<tr>
<td>°C</td>
<td>degrees Celsius</td>
</tr>
<tr>
<td>df</td>
<td>degrees of freedom</td>
</tr>
<tr>
<td>DOT</td>
<td>directly observed therapy</td>
</tr>
<tr>
<td>EDTA</td>
<td>ethylenediaminetetra-acetic acid</td>
</tr>
<tr>
<td>ELISA</td>
<td>enzyme linked immunosorbent assay</td>
</tr>
<tr>
<td>g</td>
<td>gravitational force</td>
</tr>
<tr>
<td>GIS</td>
<td>geographic information system</td>
</tr>
<tr>
<td>GMP</td>
<td>good manufacturing practice</td>
</tr>
<tr>
<td>GPELF</td>
<td>Global Programme for the Elimination of Lymphatic Filariasis</td>
</tr>
<tr>
<td>GPS</td>
<td>global positioning system</td>
</tr>
<tr>
<td>GSK</td>
<td>GlaxoSmithKline</td>
</tr>
<tr>
<td>HH</td>
<td>household</td>
</tr>
<tr>
<td>HIV</td>
<td>human immunodeficiency virus</td>
</tr>
</tbody>
</table>
H₂O₂  hydrogen peroxide
ICT  immunochromatographic test
IEC  information, education and communication
IFNg  interferon gamma
lg  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
RT-PCR  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