GIS Mapping of Lymphatic filariasis endemic areas in Gampaha district, Sri Lanka; based on the epidemiological and entomological screening

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The health issues related to vector borne diseases appear always to be related to space and time.
Development of a site directed GIS map for lymphatic filariasis (Lf) dispersed areas in Gampaha district, Sri Lanka as a guide to target control activities.
Methodology

pre-identified 9 sites in Gampaha district, screening of Lf

and

Epidemiological
night blood screening

Entomological
pool-screening
PCR-ELISA
Epidemiological Investigation

60 μl of Finger pick blood was drawn from each individual

Thick blood films were prepared

Stained with 5% Giemsa stain

Observed under the microscope

- All participants were examined by a medical officer for clinical manifestations of lymphatic filariasis.

- Questionnaire:
  - Awareness
  - Practice
  - MDA
Indoor-resting mosquito collection (6 a.m. to 11 a.m., manually)

Transportation to laboratory

Knocking down of mosquitoes (-20°C, 10 min)

Separation of female *Culex* mosquitoes

Sorting into 15 mosq/batch

Pools processing for DNA extraction (75%)

DNA extraction of pooled mosquitoes

PCR for extracted DNA

PCR-ELISA

Measurement of Absorbance

+ve samples     -ve samples

Figure 1: Laboratory evaluation of transmission levels of vector mosquitoes

Entomological Investigation
Epidemiological Investigation

Figure 02: Percentage and number of participants screened for Lf with respect to study sites
## Results

**Figure 3:** % of Adult vs. study sites

**Figure 4:** % of Children vs. study sites

**Figure 5:** Number of participants screened for Lf in each category

**Figure 6:** Site level representation of mf positivity

**Figure 7:** % of mf prevalence in +ve sites

Out of 9 sites:  
- Peliyagoda, 3.4%
- Meegahawaththa
- Pethiyagoda
- Weliamuna
- Hekiththa
- Alwis town
- Horape
- Kurukulawa
- Batuwaththa

mf -ve, 7, 78%
mf +ve, 2, 22%
Hekiththa, 0.5%

Total participants 1073

<table>
<thead>
<tr>
<th>Category</th>
<th>No. of participants screened for Lf</th>
</tr>
</thead>
<tbody>
<tr>
<td>Children (&lt;18 yrs)</td>
<td>286</td>
</tr>
<tr>
<td>Adults (&gt;18 yrs)</td>
<td>787</td>
</tr>
</tbody>
</table>

Legend:  
- Peliyagoda
- Meegahawaththa
- Pethiyagoda
- Weliamuna
- Hekiththa
- Alwis town
- Horape
- Kurukulawa
- Batuwaththa

<table>
<thead>
<tr>
<th>Study Sites</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peliyagoda</td>
<td>17%</td>
</tr>
<tr>
<td>Meegahawaththa</td>
<td>8%</td>
</tr>
<tr>
<td>Pethiyagoda</td>
<td>15%</td>
</tr>
<tr>
<td>Weliamuna</td>
<td>13%</td>
</tr>
<tr>
<td>Hekiththa</td>
<td>13%</td>
</tr>
<tr>
<td>Alwis town</td>
<td>13%</td>
</tr>
<tr>
<td>Horape</td>
<td>10%</td>
</tr>
<tr>
<td>Kurukulawa</td>
<td>11%</td>
</tr>
<tr>
<td>Batuwaththa</td>
<td>4%</td>
</tr>
</tbody>
</table>
Entomological Investigation

- **No. of mosquitoes collected;**
  varied from 0 - 45 per household

1. Conventional dissection and microscopic examination;

- **Rate of infestation** → 44.44%
- **% of positive mosquitoes** → 8.54%
- **L1 density** → 1 per +ve Mosq.
2. PCR-ELISA assay

**Figure 8:** PCR results with respect to percentage of mosquito pools

**Figure 9:** Prevalence of L1 – L2 larvae from Dissection and PCR-ELISA
According to geographical data, the highest number of cases was found at altitudes between 2.5 – 3.5 m and highly populated areas where transmission appears to be taken place.
Results

- Of 1073 individuals; 78% (837) - Aware of MDA, 65% (544) received MDA, 50% (272) had taken at least once, 34% (92) - 5-year MDA.

The rest did not give a clear answer to this question.

Questionnaires indicated limited community awareness can be a reason for the fairly static infection rate prevalent in Peliyagoda sentinel site.

Figure 10: Questionnaire analysis data
The maps derived indicate the substantial extent as well as the marked variability in the geographical distribution of Lf in Gampaha, demonstrating site related trends.
Conclusion

According to the results of this study:

• mf rate of Lf in this study population is greater than the currently reported in the country (0.18%).
• Awareness of MDA was less compared to other countries.
• Confirmed that active transmission of *W. bancrofti* is currently taking place in the Gampaha district.

Therefore, an intensive MDA programme is recommended in selected highly infected areas to contain the spread of infection and also control programs to interrupt transmission need to be continued in this district.
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- GlaxoSmithKline Pharmaceuticals, Sri Lanka
- International Atomic Energy Authority (IAEA) for the equipment received through technical co-operation programme.
Thank you
Entomological Investigation

- **Mosquito collections;** 30 households/site
- **Collected mosquitoes;**
  - Knocked down in the laboratory
    - (-20°C, 10 min)
  - Separated - species and sex
  - Female *Culex* mosquitoes
    - pooled by site of collection

1. **Conventional dissection and microscopic examination**
   - (25% of collected mosquito);
   - Head, thorax and abdomen
   - dissected separately in a drop of 0.15% saline
     (1.5 g of NaCl in 1 L of H₂O)
During dissections developing worms were classified as:

- $L_1$ sausage stage,
- $L_2$ motile short and
- $L_3$ motile, infective and with caudal papillae larvae

The number of larvae were counted to determine:
- the infection rate
- the no. of developing worms per mosquito
2. PCR Amplification and ELISA assay:

- DNA extraction

- PCR amplification
  (NV-1 and NV-2 primers specific for the Ssp I repeat)
  Reaction volume - 50 μl
  Distilled water without internal control – (-ve control)
  Pre-prepared *W. bancrofti* DNA – (+ve control)

Primer NV-2 was biotinylated to facilitate binding of the product to a 1 μg/ml streptavidin-coated microtiter plate.
PCR - ELISA assay:

Dilution of PCR product

180 μl, hybridization buffer

40 μl, PCR product

Add 100 μl/well

Fl-wild type specific probe

Alkaline phosphatase substrate

Streptavidin (1 μg/ml)

Anti-FL-AP, fab fragment

B-PCR product
In ELISA assay,

- amplified positive control DNA (0.1 μg of extracted *W. bancrofti* DNA) was used as positive controls.
- Negative controls included water and DNA extracted from a pool of 15 parasite-negative lab-reared mosquitoes.

- A positive sample was defined as 5 times the uncorrected optical density (OD) of a sample containing no template DNA.

- PCR_ELISA point estimates were computed and compared using Poolscreen 2.0 software (The University of Alabama, Birmingham).
2. Polymerase Chain Reaction (PCR) assay

Extraction of DNA

Mosquitoes pools

O/N 98 °C

Crushed Homogenized

TE buffer
(10 mM Tris-HCl pH 8, 1 mM EDTA pH 8)

Supernatant

Column purification

14,000 rpm, 10 min

100 °C, 10 min
**PCR Amplification**

Preparation of PCR master mix for mosquito DNA templates

- 1X PCR buffer (5x) 10.0 μl
- 1.5 mM MgCl₂ (25 mM) 3.0 μl
- 1.25 mM dNTP (25 mM) 2.5 μl
- 10 pMol NV1 (10 pM/μl) 1.0 μl
- 10 pMol NV2 (10 pM/ml) 1.0 μl
- 2 U Taq (5 U/μl) 0.4 μl
- H₂O 27.1 μl
- DNA 5.0 μl
- Total volume 50.0 μl

**PCR program**

- 5 min at 95 °C
- 1 min at 94 °C
- 1 min at 55 °C
- 1 min at 72 °C
- 10 min at 72 °C

35 cycles

**Qualitative analysis of PCR products**

- Ladder (+ve -ve)
- 188 bp samples

**Methodology cont...**
Dilution of PCR product
(40 μl, PCR product + 180 μl, hybridization buffer)

100 μl/well - added to streptavidin-coated plate

Hybridized with fluorescein-labeled wild-type specific probes - 55°C, 30 min

Incubated - alkaline phosphatase-labelled anti-fluorescein Fab fragment

Add substrate – 1 hr

Absorbance measured - 405 nm