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

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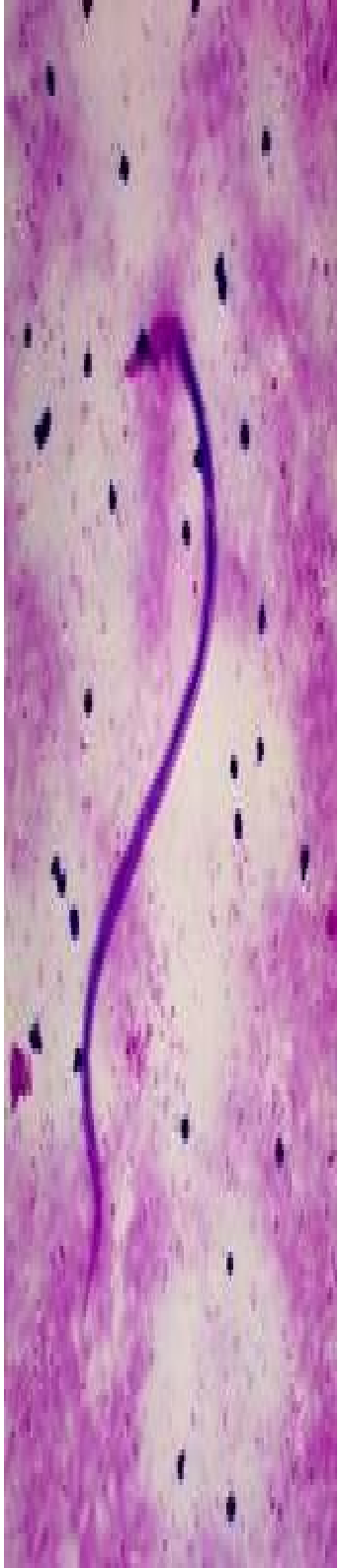
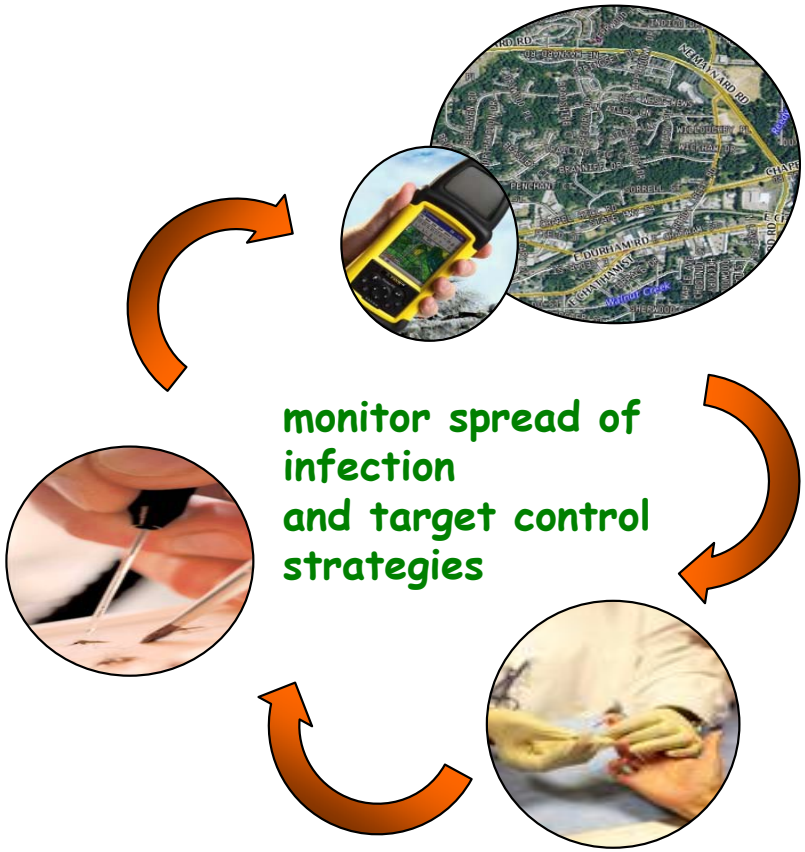
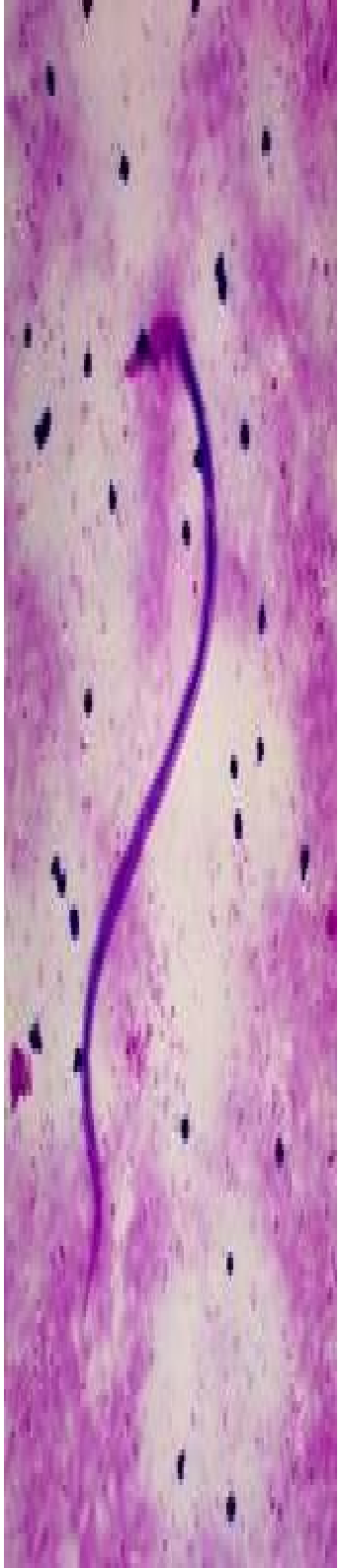


Background

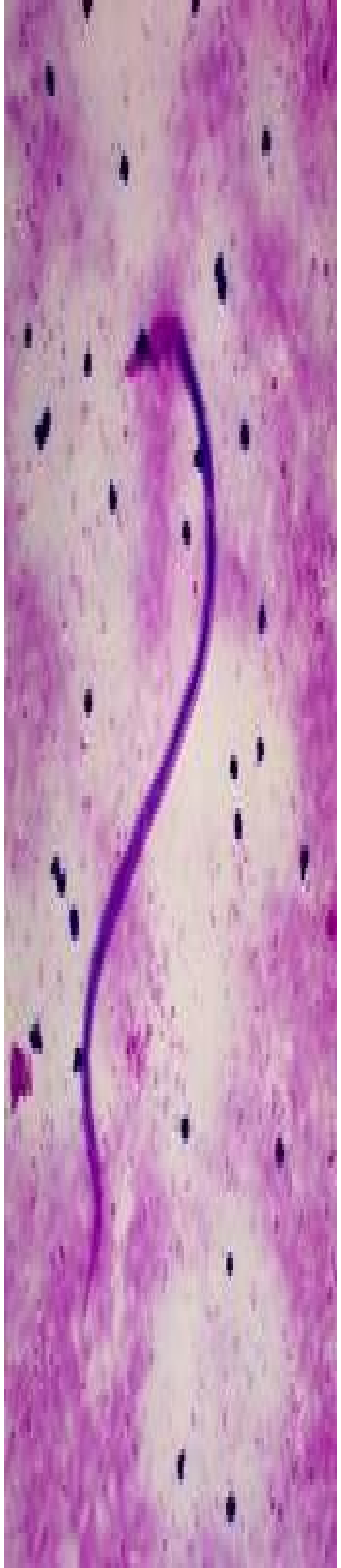
The health issues related to vector borne diseases appear always to be related to space
and time.





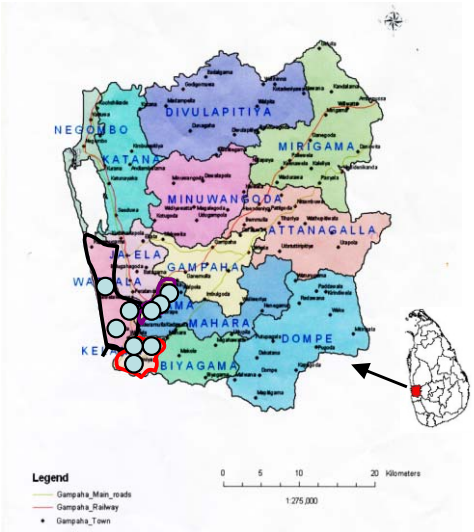
Objective

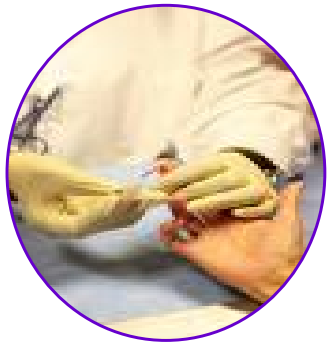
Development of a site directed GIS map for lymphatic filariasis (Lf) dispersed areas in Gampaha district, Sri Lanka as a guide to target control activities.



Methodology

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





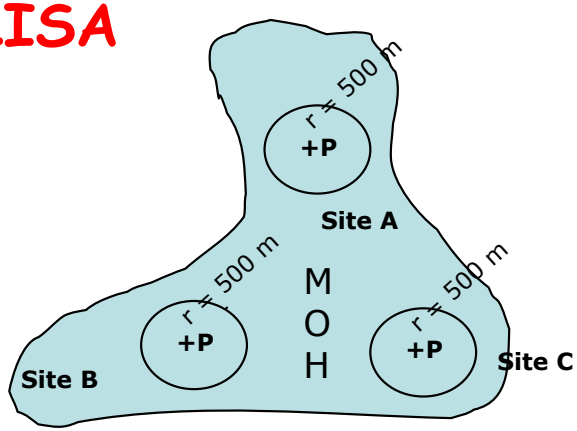
and

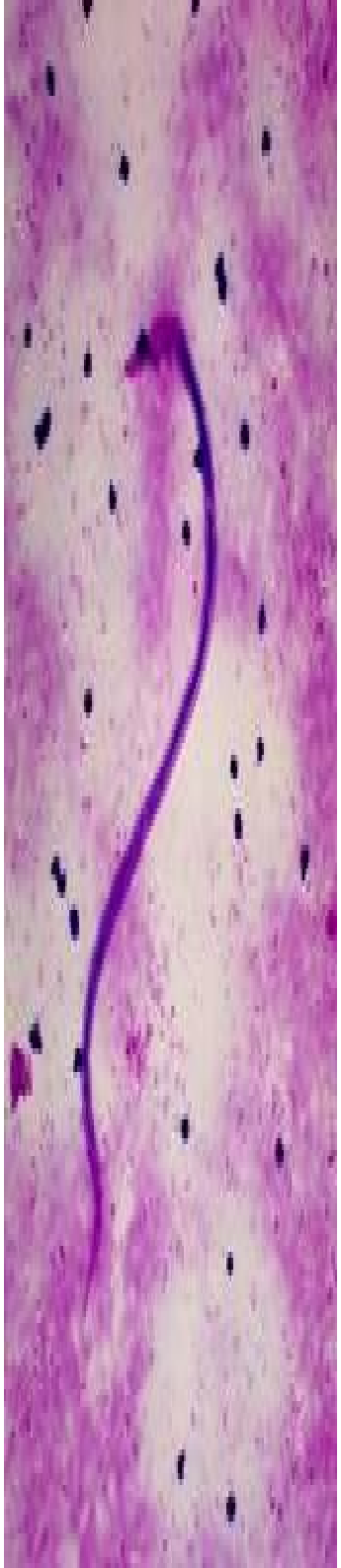
Epidemiological

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

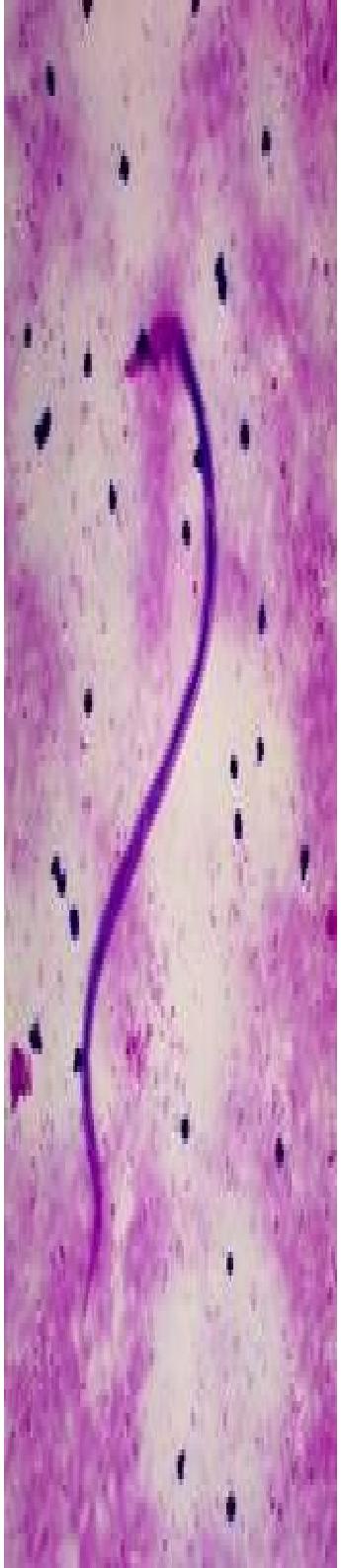
⇩

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 11a.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%)

Pools processing for dissection (25%)

↓

DNA extraction of pooled mosquitoes ← collecting dissected body parts of the mosquitoes

↓

PCR for extracted DNA

↓

PCR-ELISA

↓

Measurement of Absorbance

↙ ↘

+ve samples -ve samples

Entomological Investigation

Figure 1: Laboratory evaluation of transmission levels of vector mosquitoes

Results

Epidemiological Investigation

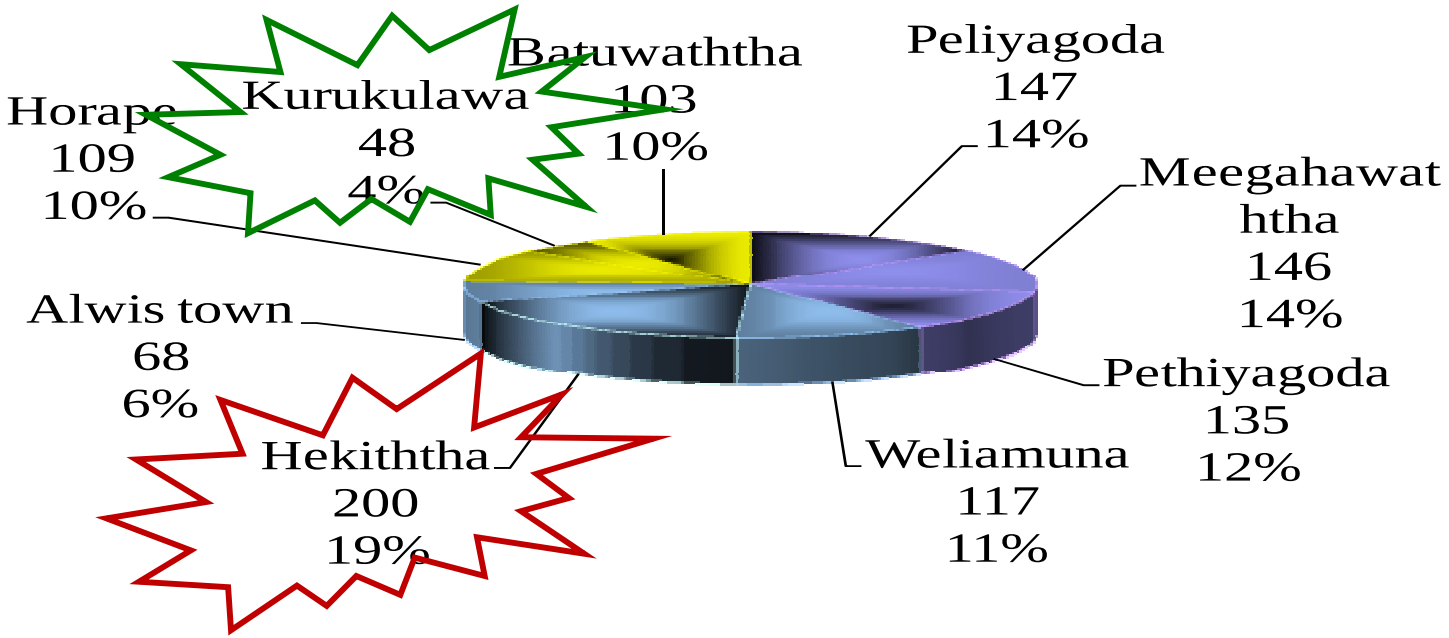
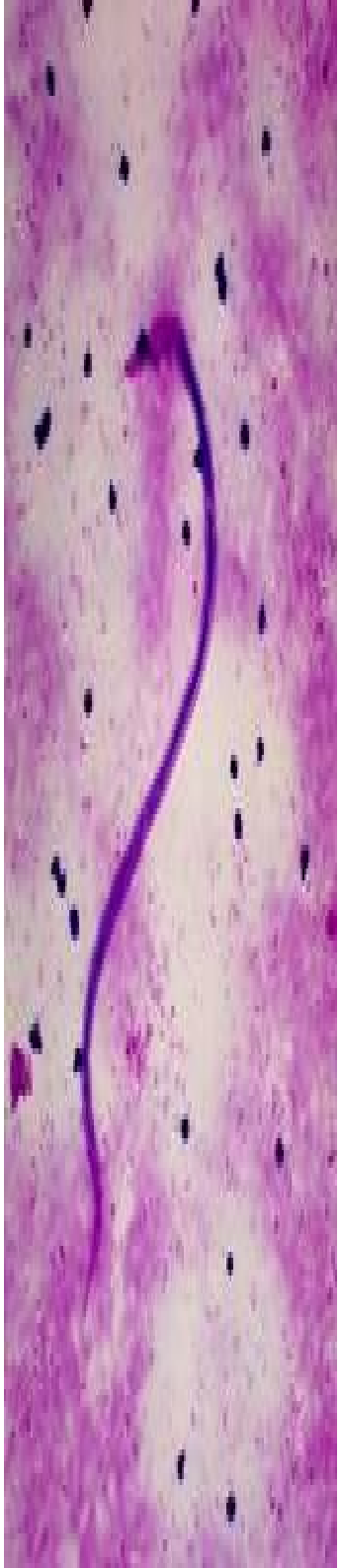


Figure 02 : Percentage and number of participants screened for Lf with respect to study sites



Results

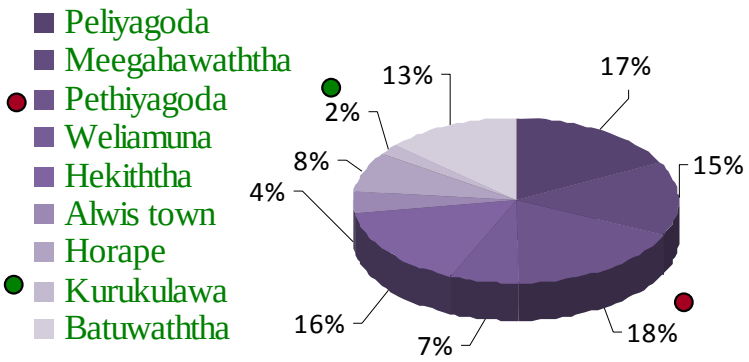


Figure 3: % of Adult vs. study sites

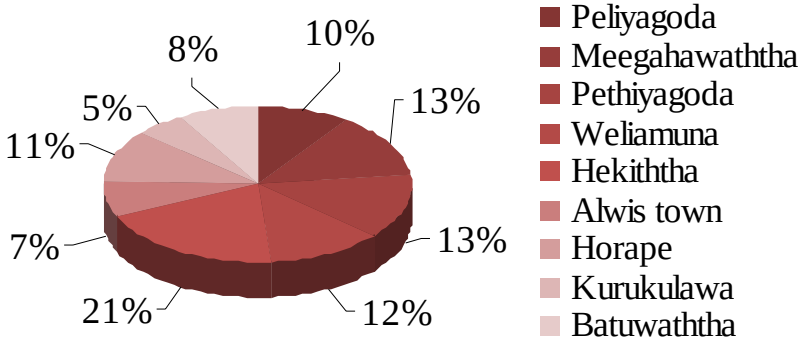
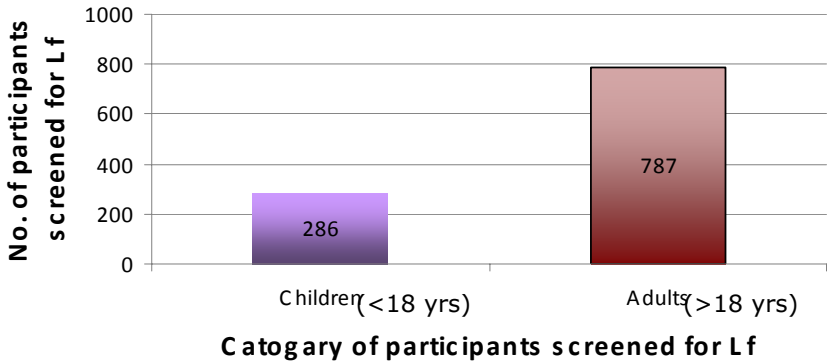


Figure 4: % of Children vs. study sites



Total participants 1073

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

Out of 9 sites

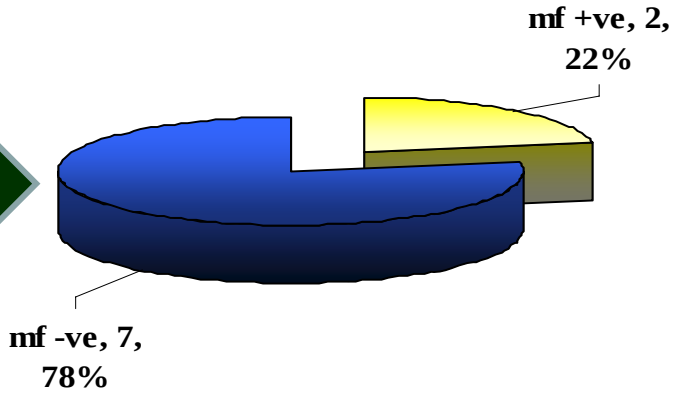


Figure 6: Site level representation of mf positivity

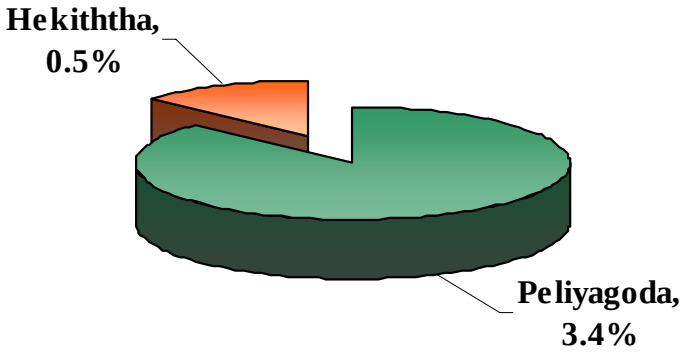
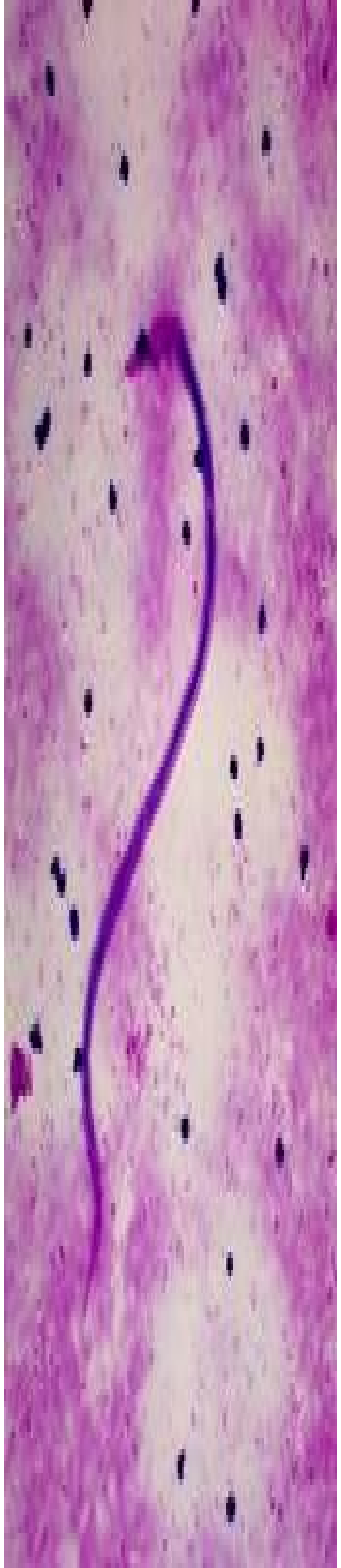


Figure 7: % of mf prevalence in +ve sites



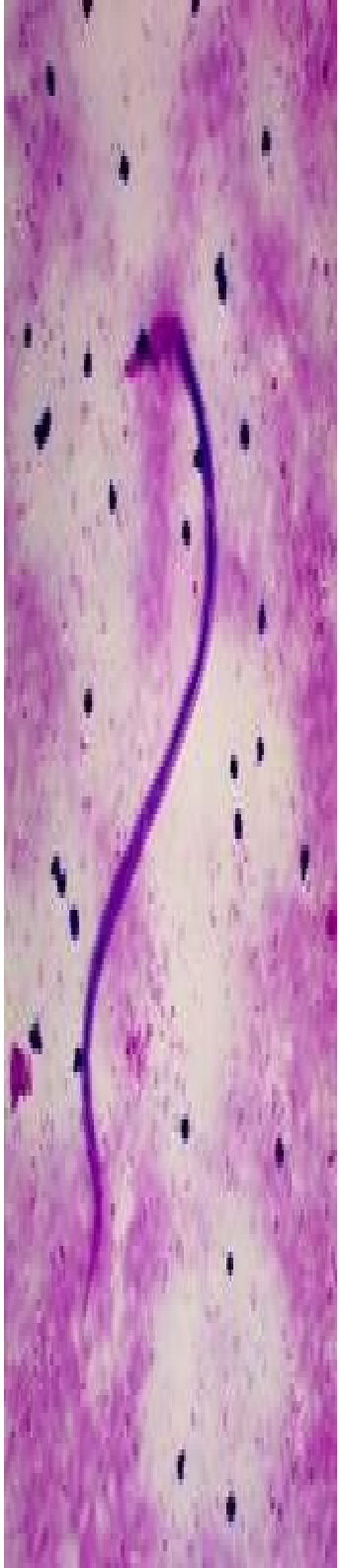
Results

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.**



Results

2. PCR-ELISA assay

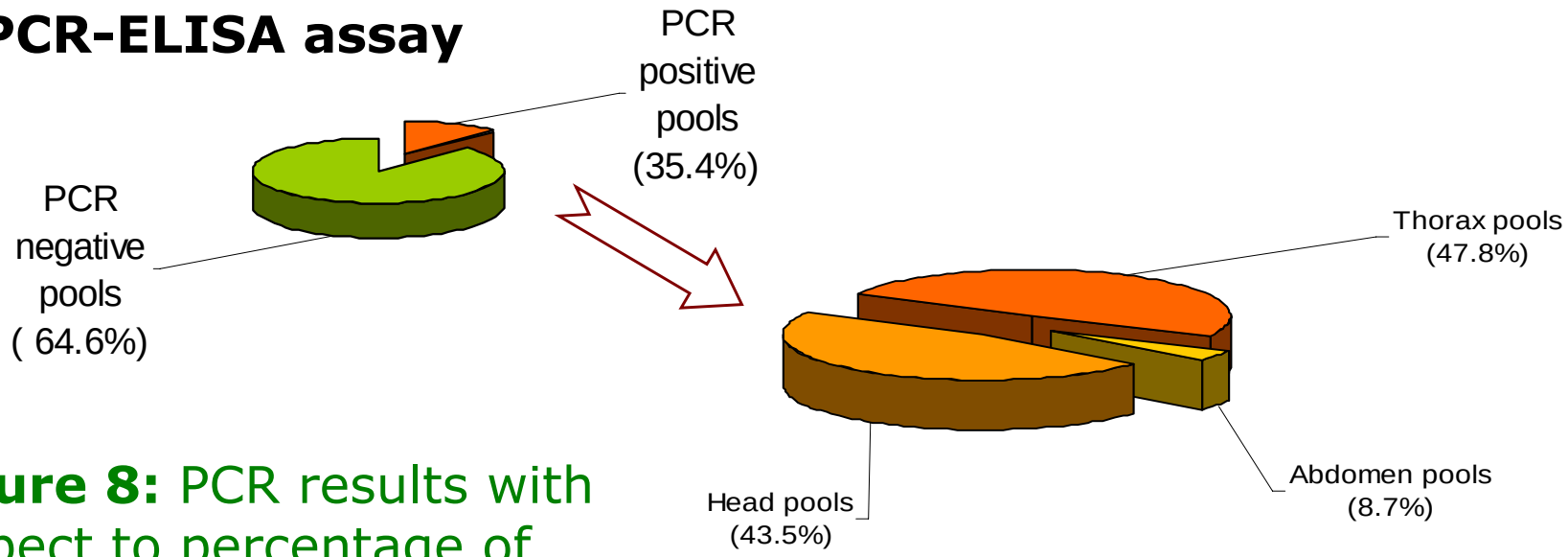


Figure 8: PCR results with respect to percentage of mosquito pools

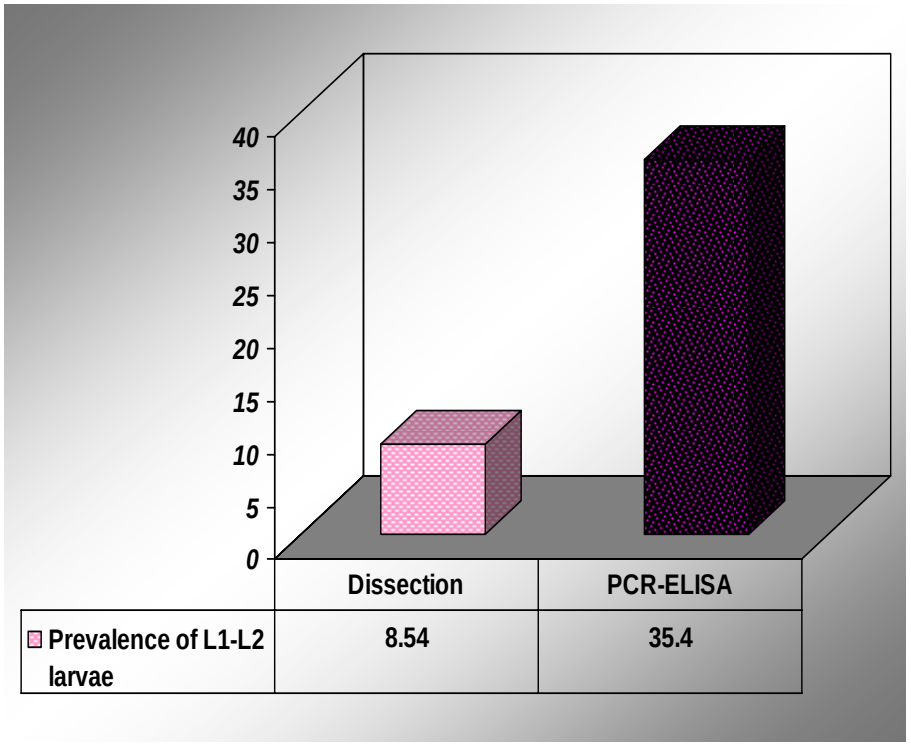
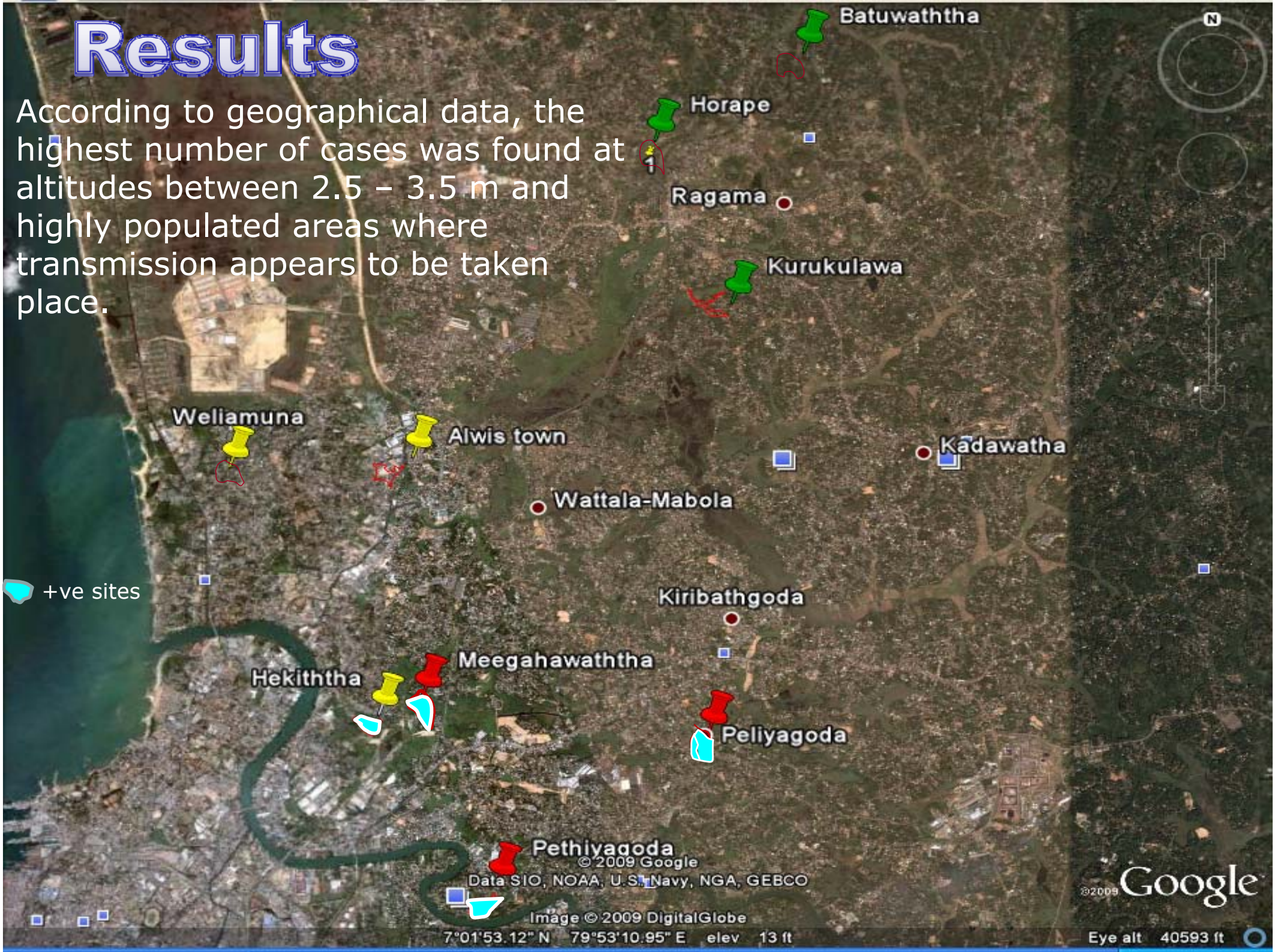
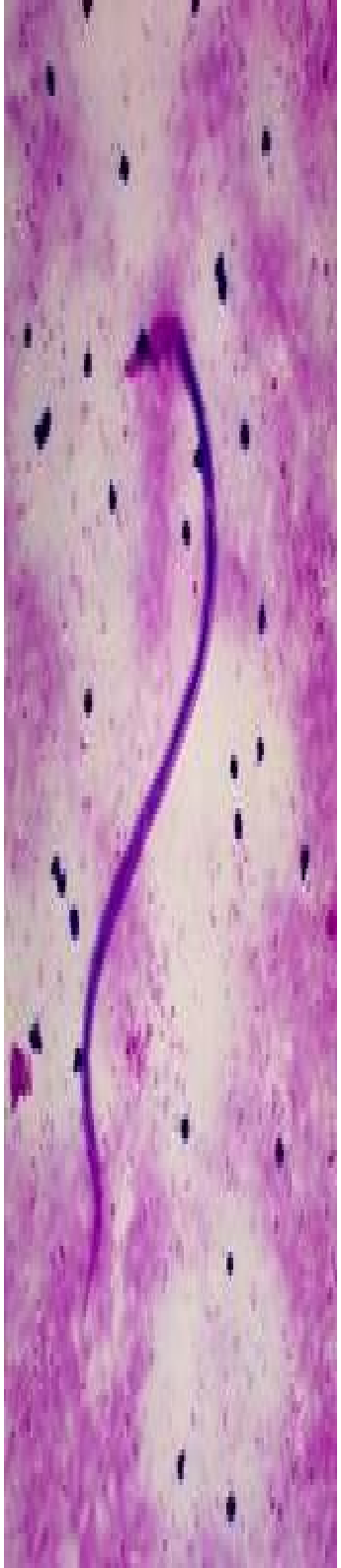


Figure 9: Prevalence of L1 – L2 larvae from Dissection and PCR-ELISA

Results

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,

8.57%

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

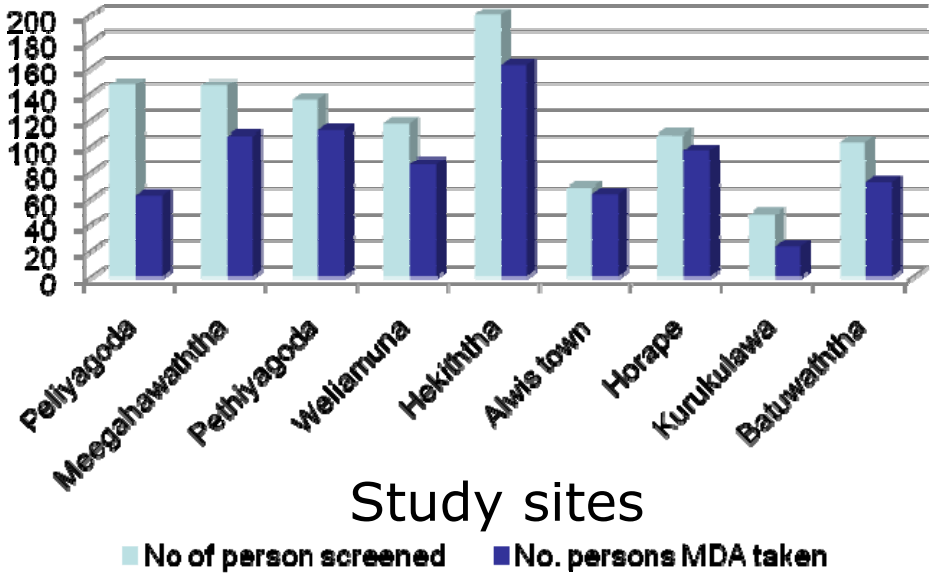
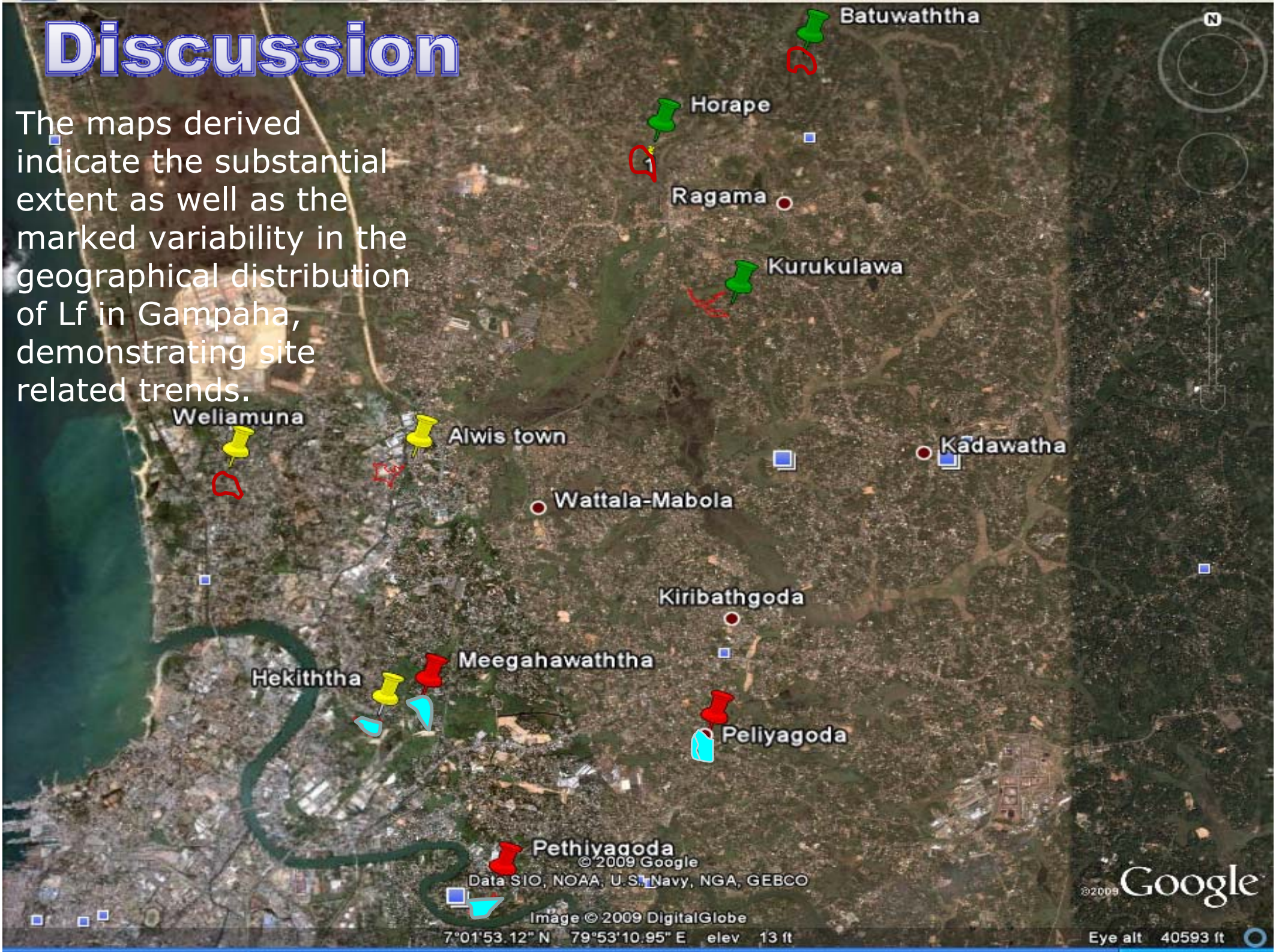


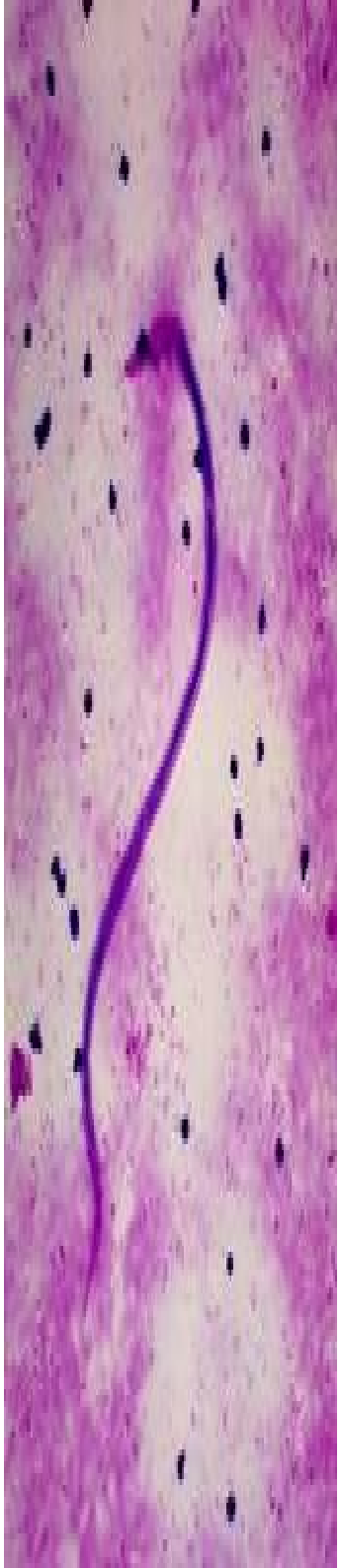
Figure 10: Questionnaire analysis data

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

Discussion

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.

Acknowledgement

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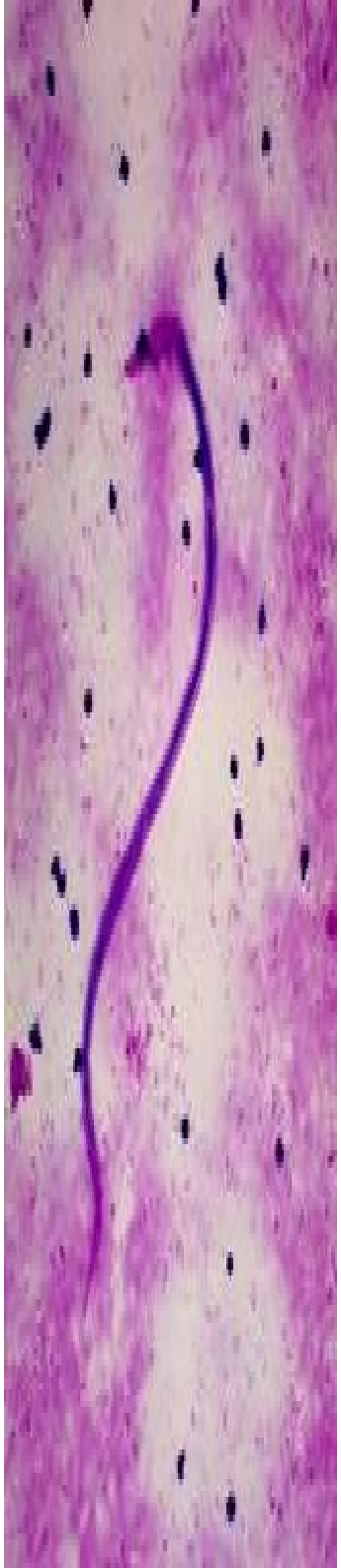
- International Atomic Energy Authority (IAEA) for the equipment received through technical co-operation programme.


Thank you



Entomological Investigation

- **Mosquito collections; 30** households/site
- **Collected mosquitoes**;

  Knocked down in the laboratory
  – (-20^{0}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 H_2O)

- **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)

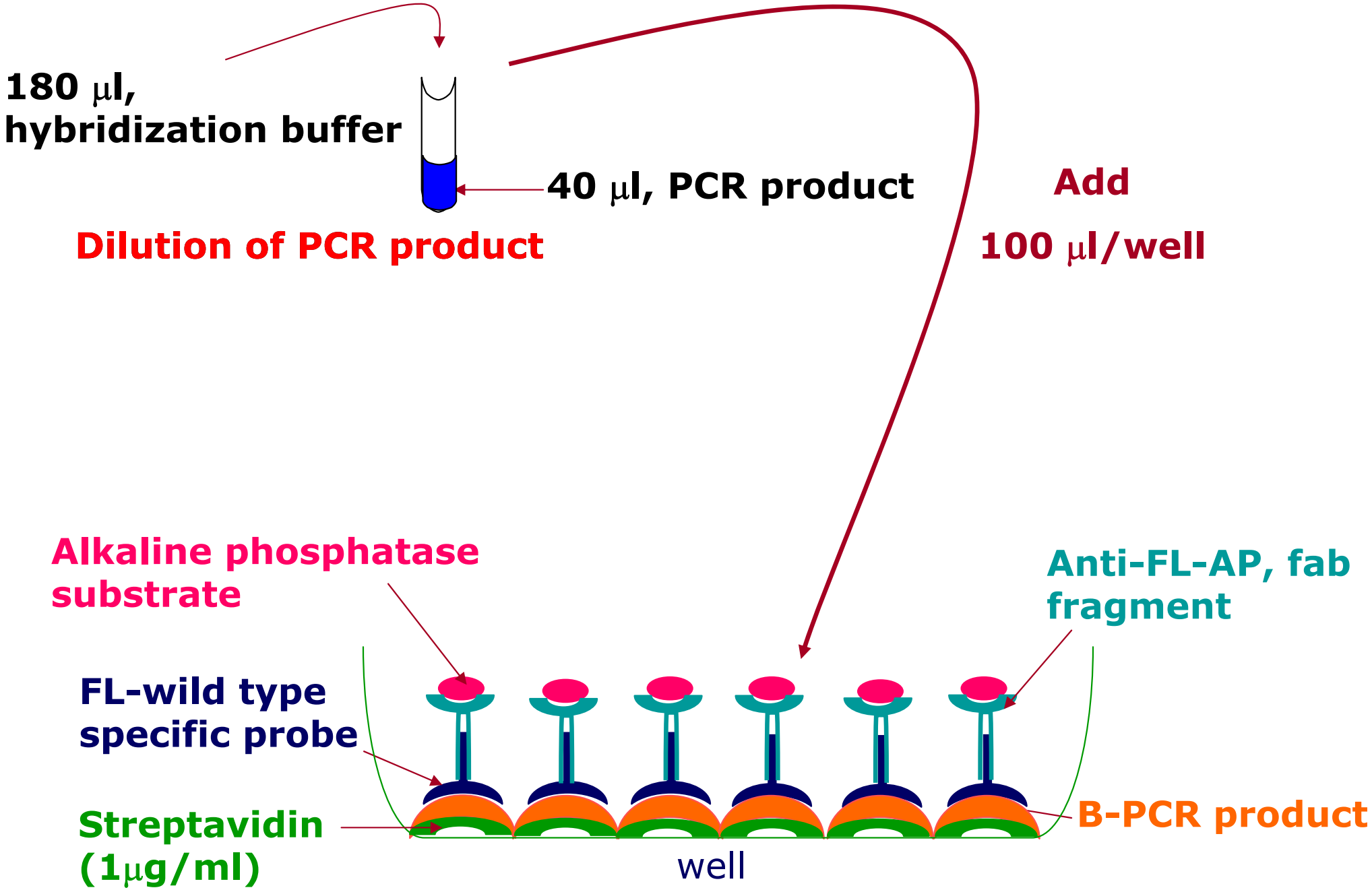
Reaction volume - 50 µl

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:



- 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.

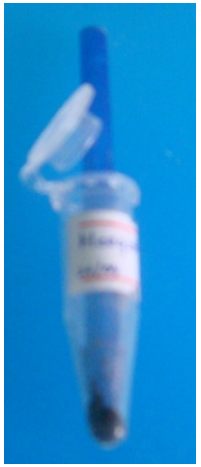
- PCR_ELISA point estimates were computed and compared using Poolscreen 2.0 software (The University of Alabama, Birmingham).

2. Polymerase Chain Reaction (PCR) assay

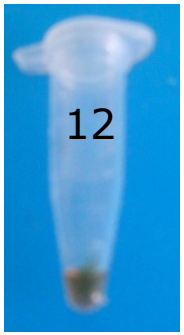
Extraction of DNA

Mosquitoes pools

O/N 98 ^{0}C



Crushed

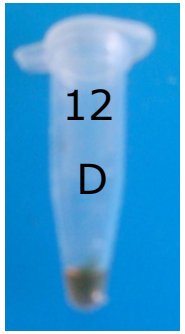


Homogenized TE buffer

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

100 ^{0}C, 10 min

14, 000 rpm, 10 min



Supernatant

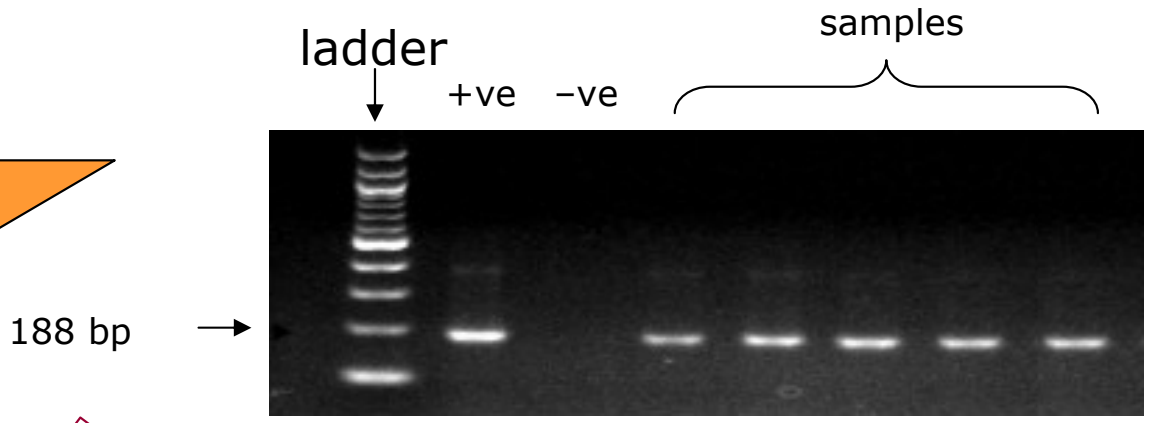
Column purification

METHODOLOGY cont…

PCR Amplification

Preparation of PCR master mix for mosquito DNA templates

	1X
1X PCR buffer (5x)	10.0 µl
1.5 mM $MgCl_2$ (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 ml
2 U Taq (5 U/µl)	0.4 µl
H_2O	27.1 µl
DNA	5.0 µl
Total volume	**50.0 µl**



Qualitative analysis of PCR products

5 min at 95 ^{0}C,
1 min at 94 ^{0}C,
1 min at 55 ^{0}C,
1 min at 72 ^{0}C,
10 min at 72 ^{0}C

35 cycles

PCR program

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