

Development and validation of sampling strategies for xenomonitoring of infection in *Culex* vector by PCR as a surveillance tool for assessing post-MDA situation of lymphatic filariasis elimination programme (Funding: Gates Foundation)

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Introduction: PCR assay has been proved to be useful for detecting filarial infection in vector. The assay could be used for evaluating the success of filariasis elimination programme. However, its application in programme (in place of human sampling) requires sampling methods for mosquito collection. This study aims to develop sampling strategies for mosquito collection that can be used as a tool in the monitoring and evaluation of the LF elimination programme.

Objectives:

- To evaluate a mosquito collection sampling strategy that can be used to assess the usefulness of vector infection monitoring by PCR as a surveillance tool for assessing post-MDA situation
- To assess the usefulness of gravid traps for monitoring vector infection in relation to IDR collection by insecticide impregnated fabric traps

Methods: This study involves two steps: (i) mosquito collection using gravid and fabric traps and (ii) PCR assays using two extraction methods (TE for conventional PCR and Qiagen for quantitative PCR). Two mosquito surveys (targeting a minimum of 5000 gravid *Culex quinquefasciatus* females) each were done from (i) the entire PHC (all the 33 sites, Ammapettai, Thanjavur district) and (ii) from four 'hotspots' (consist of 17 clusters, active transmission sites) in the same PHC. Mosquitoes collected from each selected household were separated in to two pools of 25 gravid females each for cPCR and qPCR assays. In addition in one of the hotspot surveys, gravid females were collected in pools of 25 each for detecting L3 by RT-PCR and qPCR assays.

Results

Pool positivity: A total of 2049 pools containing 47,710 gravid females were collected. The pools collected from the two hotspot surveys (414 pools) and two PHC surveys (461 pools) were processed by qPCR. The qPCR assays showed infection in 99 (47.8%) and 83 (40.1%) of the 207

pools of each hotspot surveys, and 54 (23.4%) and 41 (17.8%) of the 231 pools from each of PHC survey.

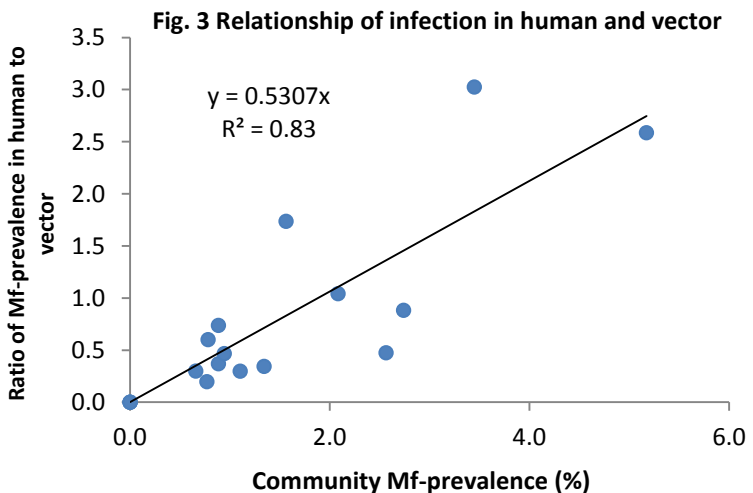
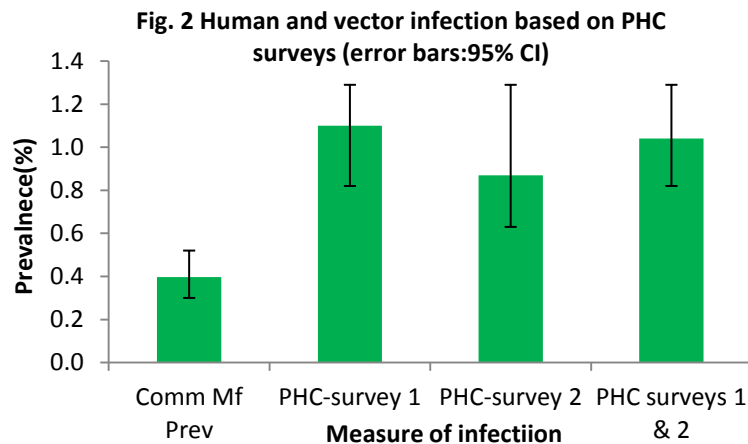
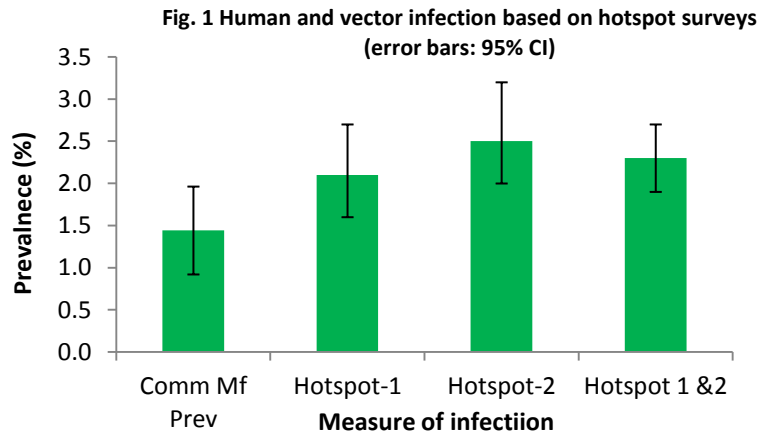
Vector infection

PHC cluster surveys: The vector infection rates varied from 0 to 4.9% in survey-1 and 0 to 6.2% in survey-2 in different sites. The overall infection rate (95% CI) based on surveys-1 and 2 are 1.1% (0.8-1.5%) and 0.85% (0.6-1.2%) respectively, showing that the estimates are consistent. The overall infection rate after combining all the pools of surveys-1 and 2 was 1.0% (0.8-1.2%).

Hotspot surveys: The vector infection rates varied from 0.5 to 5.4% in survey-1 and from 0 to 8.2% in survey-2 in different sites. The overall infection rate (95% CI) based on surveys-1 and 2 are 2.1% (1.6-2.7%) and 2.5% (2.0-3.2%) respectively, showing that the estimates are consistent. By combining all the pools of surveys-1 and 2 together, the overall infection rate was 2.3% (2.0-2.7%).

Human and vector infection:

The community Mf-prevalence (95% CI) was 1.4% (0.9-1.96%) in the hotspots and 0.4% (0.30-0.52%) in the PHC clusters. While the estimated vector infection rates based on surveys 1 and 2 are not significantly different from overall community Mf-



prevalence (Fig. 1; 95% CIs overlap) in the hotspots, they differed significantly from the overall community Mf-prevalence in the PHC clusters (Fig. 2; 95% CIs do not overlap).

The data from hotspot survey 1 was used to examine the relationship between human and vector infection. A significant positive relationship ($r=0.91$, $P<0.001$) was observed between the ratio of community Mf-prevalence to vector infection rate and community prevalence (Fig. 3). The 95% CI for the slope (0.5307; 95% CI: 0.404-0.66) of the regression line was used to obtain an estimate of vector infection rate corresponding to the threshold community Mf-prevalence of 1% and was found to be 1.9% (95% CI: 1.5 -2.5%). This suggests that a vector infection rate of 1.5% could be a threshold for stopping MDA.

Conclusions

Vector infection rates based on qPCR assay are consistent between surveys based on entire PHC or hotspots indicating that a sample of >5000 gravid females would suffice for assessing infection in mosquitoes.

The rates are significantly higher in the hotspots than those in the entire PHC suggesting that the hotspots surveys cannot be a complement to the entire PHC survey. If hotspots are to be used for post-MDA monitoring, the challenge is developing sampling strategies that could identify the hotspots.

Our preliminary results of the relation between human and vector infection suggest that xenomonitoring could be used as a surveillance tool for monitoring infection in human.

A vector infection rate of 1.5% could be a threshold equivalent to an Mf-prevalence of 1% at community level.