

# 5. Population Science, Modelling & Bioinformatics

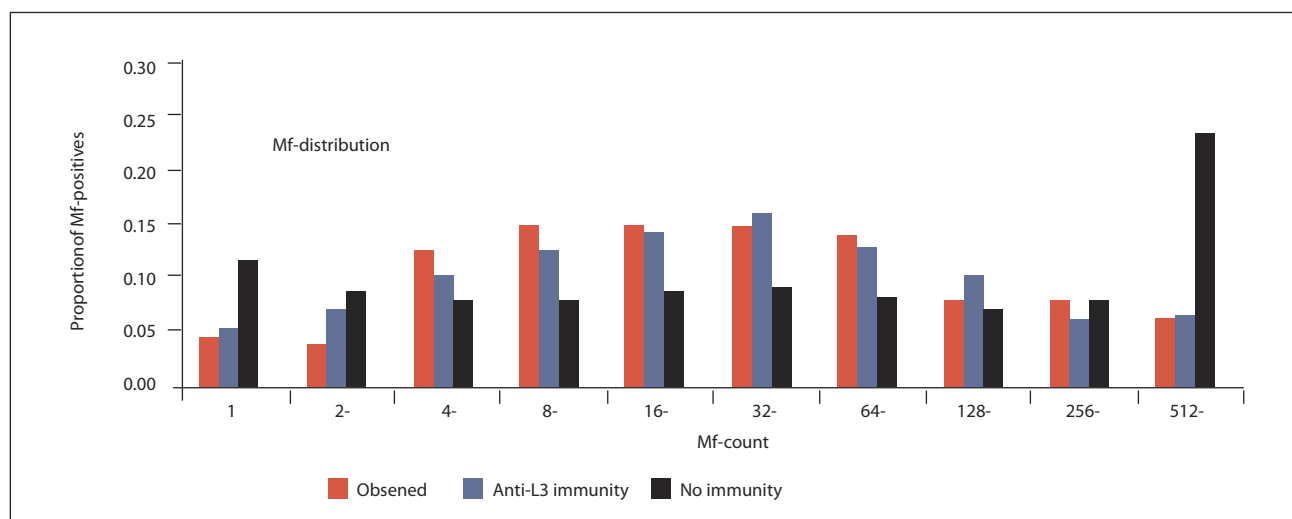
|                        |                          |
|------------------------|--------------------------|
| Dr. P.K. Das, Director | Mr. Raja Packirisamy, TA |
| Dr. P. Vanamail, TO    | Ms. T. Sankari, RA       |
| Mr. S. Subramanian, TO | Mrs. R. Sundarammal, LIO |
| Mrs. A. Srividya, TO   | Mr. S. Kandasamy, TA     |

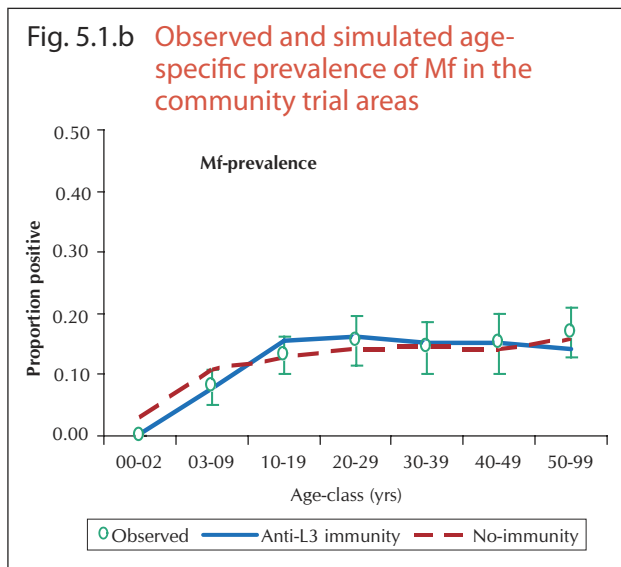
## 5.1. Developing model variants of Lymphatic Filariasis Simulation (LYMFASIM) to be used in planning and evaluation of LF programmes (P.K.Das and S.Subramanian; EM 0301 PMB; Duration: 1 year, Jan 2004 - Feb 2005)

The LYMFASIM model developed earlier could not mimic the epidemiological patterns observed in different parts of the globe. It was realised that the difference in epidemiological patterns are due not only to differences in transmission dynamics across countries, but also to differences in heterogeneity in exposure to mosquito bites. Therefore three different model variants were proposed and tested: (i) model without immunity ('exposure model'); (ii) model with anti-L3 immunity; and (iii) model with anti-fecundity immunity. An 'exposure

model' assumes that mosquito biting varies not only according to age and sex but also between persons. The two immunity models, in addition to exposure differences, assume that immunity is the main factor driving the dynamics of infection in humans. Anti-L3 immunity is triggered by exposure to L3-antigens and causes reduction in the successful maturing of L3 inoculated larvae in the human body. Anti-fecundity immunity is triggered by the presence of adult worms and causes reduction in Mf production through concomitant immunity. As far as possible, most of the parameters of different model variants were quantified using literature and local data and, for some biological parameters, estimates from the Pondicherry model variant were used. However, five (three parameters related to human exposure to

Fig. 5.1.a Observed and simulated distribution of Mf per 60 µl of blood in the community trial areas





mosquito biting, one each related to individual personal variability in Mf-counts in smear and variability in eliciting immune response) of the ten parameters (not directly observable) were quantified by systematic fitting of different model variants to the community trial data. An automatic fit-procedure was developed to quantify the parameters of different model variants. The results suggest that a model without immunity failed to fit the observed prevalence and distribution of Mf in the rural community ( $\chi^2 = 104.8$ , D.F.=11,  $P < 0.05$ ; Fig. 5.1), whereas a model with anti-L3 immunity was found to provide a reasonably satisfactory fit to the observations ( $\chi^2 = 12.1$ , D.F.=11,  $P = 0.36$ ; Fig. 5.1.1). The fitting of the anti-fecundity immunity model is in progress.

## 5.2. Density-dependent development of *Wuchereria bancrofti* in *Culex quinquefasciatus*: evidence in nature (S.Subramanian, P.K.Das, K.D.Ramaiah; IM 0401 PMB; Duration: 1 year, Jan 2004 – Dec 2004)

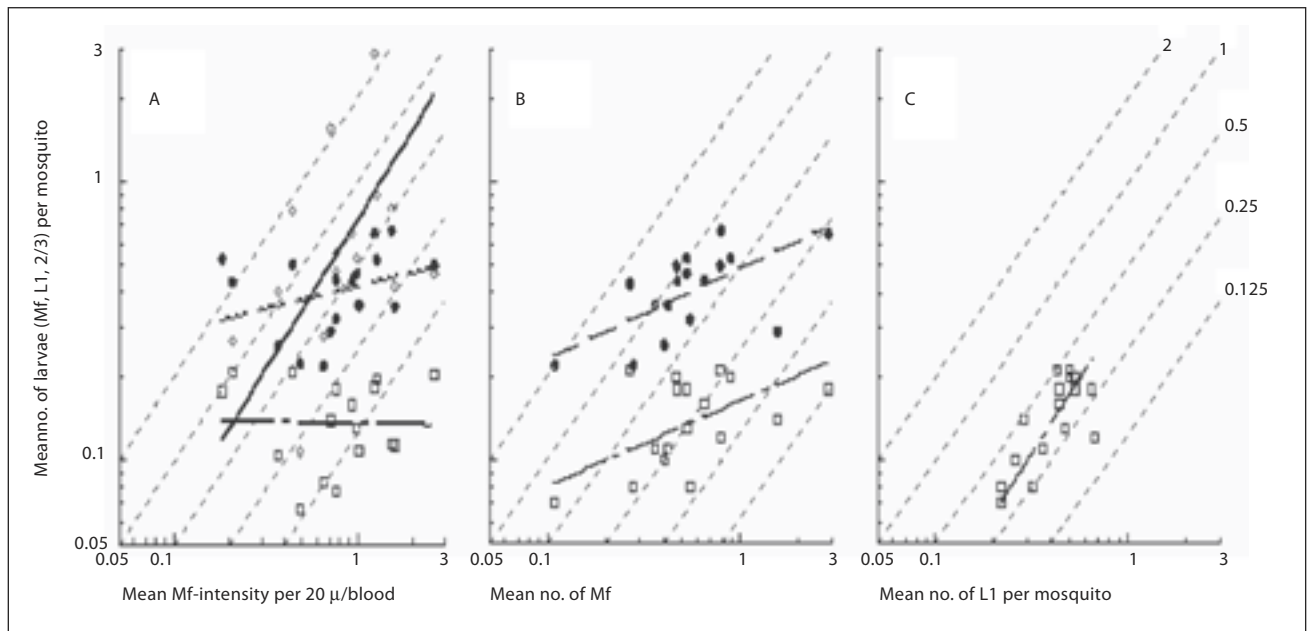
Experimental transmission studies showed density-dependence in the uptake and development of Mf in the vector mosquito *C. quinquefasciatus*. This means that there is a limitation to the number of larvae that can develop in the vector. This will have a negative effect on the chance of eliminating infection

through MDA with antifilarial drugs, because under 'limitation' mosquitoes can pick up and transmit the parasite even when the Mf load in a person is microscopically not detectable. Therefore it is important to know whether, and to what extent, evidence can be obtained under field conditions as well. The objective of this project was to provide evidence for density-dependence in nature and identify the stage at which parasite regulation is most likely to occur.

Two methods were applied to examine evidence for density-dependence: (a) by fitting a power model of the form ' $y = ax^b$ ' to describe the nature of relationships of mean counts of parasitic stages (Mf, L1, and L2/3) in mosquito with human Mf-density as well as among parasite stages (Mf, L1, L2/3) for different sites; and (b) by examining the relation between the degree of parasite aggregation (measured by 'k' parameter of the negative binomial distribution) and mean larval count in different sites. The infection status of 18738 resting female *C. quinquefasciatus* mosquitoes and 10455 persons from 17 sites in Pondicherry formed the database for this study.

The fitted power model revealed that the relationships of Mf-uptake with L1 (slope  $b = 0.32$ , 95% confidence interval: 0.13-0.52) and L2/3 ( $b = 0.31$ , 95% CI: 0.08-0.53) in vector significantly deviated from proportionality (Fig. 5.2.1B; in both cases 95% CI does not include 1.0). However, the relationships between Mf-uptake and mean human Mf-count (Fig. 5.2A;  $b = 1.07$ , 95% CI: 0.6-1.5) and densities of L1 and L2/3 (Fig. 5.2C;  $b = 1.09$ , 95% CI: 0.8-1.4) were proportional. These results suggest that there is a strong density regulation during parasite development from Mf to L1-stage larvae. This was further confirmed from the decrease in degree of aggregation with an increase in mean L1/2/3 count ( $k = 0.015 + 0.105 \times L1/2/3$ ). The constant relation between human Mf-density and L2/3 larvae per mosquito (Fig. 5.2A) implies that even at low human Mf densities, there can still be a considerable

Fig. 5.2. Relation between human blood Mf-density with larval density in the vector (A); between mean no. of Mf in the vector and mean no. of L1 or L2/3 larvae (B); and (C) between the mean no. of L1-larvae and L2/3 larvae



Observations: Mf ( $\diamond$ ); L1 ( $\circ$ ) and L2/3 ( $\square$ ). Lines are results of Deming's linear regression on log - log scale. Mf (Solid line), L1 (dashed line) and L2/3 (dot-dashed line)

level of transmission. The results imply that DEC-based LF-elimination programmes must consider supplementation of MDA with DEC-fortified salt/vector control.

### 5.3 Pharmacogenomic approach to unravel the variation in response to DEC (T. Sankari, S.L. Hoti & P.K. Das; IM 0303 PMB; Duration: 3 years, Nov 2003 - Oct 2006)

DEC acts thorough the enzymes of prostaglandin and luektriene cascade of the arachidonic acid pathway. Non-synonymous SNPs (nSNPs) detected in the genes coding for the enzymes of PGE2S, PGI2S, LTA4H and LTC4S are the determinants of variation in response to DEC. In order to assess the functional effects of non-synonymous SNPs, geometric location of nSNPs have to determined. We predicted the geometric locations of nSNPS resulting in amino acids substitutions. Amino acid residues of protein can be classified into three categories based on their geometric locations: (i) those located in the pocket or void (type P); (ii) those located on a convex region or a shallow depressed region

(type S); and (iii) those buried in the interior of proteins (type I).

There is an increased probability that if the nSNP is located in the well-formed surface pocket or void region that may affect the protein function leading to various disease phenotypes, the residues in the surface pockets or voids are involved in molecular interactions. nSNPs are far less likely to be in the shallow depressed region or convex region that may be involved in protein-protein and protein-membrane interactions. Finally, the nSNPs that are less likely to be buried in the interior of the protein, are essential for structural stability and folding.

As the protein structure is not available for all the four enzymes, (PTGES2, PTGIS, LTA4H, LTC4S) the protein sequences were aligned with the PFAM HMM database containing a library of protein family with default parameters. The pdb structures aligning with the domains of target proteins were used for CAST P server analysis, to locate the geometric position of amino acid residues. The nSNP amino acids in each gene,

Table 5.1. nSNPS and their geometric locations in four genes responsible for DEC action

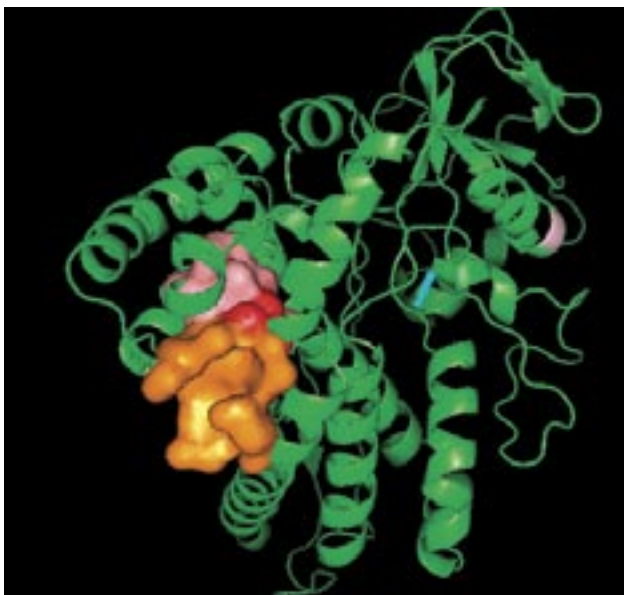
| Gene Name | Amino acid change | SwissProt position | Pdb position | Consensus with pfam | Geometric location |
|-----------|-------------------|--------------------|--------------|---------------------|--------------------|
| PTGIS     | V/G               | 69                 | 69           | A                   | P                  |
|           | S/R               | 118                | 118          | A                   | P                  |
|           | Q/H               | 134                | 132          | A                   | P                  |
|           | M/T               | 140                | 138          | NA                  | -                  |
|           | E/A               | 154                | 151          | NA                  | -                  |
|           | F/L               | 171                | 168          | A                   | P                  |
|           | K/T               | 227                | 224          | A                   | P                  |
|           | R/C               | 236                | 233          | NA                  | -                  |
|           | P/S               | 500                | 499          | NA                  | -                  |
| PTGES2    | R/H               | 107                | 107          | A                   | P                  |
| LTA4H     | T/S               | 600                | 599          | NA                  | -                  |
| LTC4S     | R/Q               | 142                | 140          | NA                  | -                  |

A-Aligned residues; NA - Non aligned residues

aligned with the PFAM HMM protein, were then searched for the position of the amino acids in the pocket or surface region. (Table1)

Six nSNP amino acids are found located in pockets, in two genes coding for the enzymes of DEC action, that may disrupt the molecular interactions of proteins resulting in variation in response to DEC. (Fig. 5.3)

Fig. 5.3. SNP in the position 118 (shown in red) is buried in the pocket region (shown in orange and pink) of the pdp homologue of PTGIS



#### 5.4. Cochrane review on the short and long term effects of DEC medicated salt on filariasis infection (A. Srividya, Julia Critchley, Paul Garner, Helen Gelband and P.K.Das; IM 0302 PMB – Duration: Jul 2003 – Dec 2004)

Global Filariasis Elimination programme had suggested mass treatment with DEC fortified salt as a tool for eliminating persistence foci of transmission in LF. With this in view, a Cochrane review was undertaken to assess the short and long term effects of DEC salt on patients or populations with filarial infection. Twenty-one studies were included (the details of the search strategies, eligibility criteria, etc., were given in Annual Report 2003).

Percentage reductions in Mf prevalence were large (43% to 100%) and consistent in most studies, provided that a high proportion of the community received DEC salt. Meta regression showed that the percentage reduction in the prevalence of filarial infection was associated with the duration of the trial and the concentration of DEC in the medicated salt. Large reductions in Mf density were also observed, though most studies only reported changes in Mf density for those who were Mf positive at baseline.

Only two studies compared DEC salt with other forms of DEC (such as annual dose, or standard 12-day treatment), but in both, DEC salt performed favourably in the reduction of filarial infection levels, compared with alternatives.

A few studies included long-term follow-up of more than one year (range: 2-19 years). There appeared to be a relationship between per capita

DEC consumption and the percentage reduction in Mf prevalence in these studies, but this was not statistically significant.

DEC salt appears promising, but is only effective if high levels of community coverage can be maintained. Further studies are required to assess the effects of continuous low dose DEC on adult worms and disease prevalence and severity.