Review

The role of monitoring mosquito infection in the Global Programme to Eliminate Lymphatic Filariasis

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In addition to monitoring infection in the human host, there is also a need to assess larval infection in the vector mosquito population to evaluate the success of interventions for eliminating lymphatic filariasis transmission from endemic communities. Here, we review the current status of the available tools for quantifying vector infection and existing knowledge and evidence regarding potential infection thresholds for determining transmission interruption, to assess the potential for using vector infection monitoring as a tool for evaluating the success of filariasis treatment programmes.

Monitoring the intervention

The main aim of the current Global Programme to Eliminate Lymphatic Filariasis (GPELF) is to achieve worldwide elimination of this vector-borne parasitic disease by the year 2020. The main intervention strategy adopted to attain this goal is yearly mass drug administration (MDA) in endemic populations for at least 4–6 years [1]. As for any intervention programme, close monitoring of progress will be necessary to ensure that the programme is on track to achieve this ambitious goal and to determine when the goal of elimination is achieved and mass treatments can be stopped [2,3]. Recent work has shown that the decision to stop treatment does not require the complete absence of filarial parasites but rather the reduction of parasite numbers to such low quantities that transmission will cease [4–6].

The above considerations indicate that accurate quantitative indicators predictive of parasite transmission interruption will be essential for evaluating the success of the current elimination programme [2,3]. Different tools are available for measuring the prevalence of filarial infection in the human population, including several tests for the detection of microfilaria or worm antigen in the blood [7]. However, a change in parasite levels in the human population does not necessarily result in a proportional change in the incidence of new infections because of density-dependent constraints on parasite development in both the host and vectors [2,4,6]. This signifies that in addition to monitoring changes in human infections, it is also important to monitor the corresponding changes and levels of infection indicative of transmission endpoints in the vector population, defined here as the vector infection threshold (see Glossary).

Classically, infection detection and quantification in vector populations were carried out via dissection of individual mosquitoes, but this method is thought to become inefficient when the prevalence of larval infection in mosquitoes falls to very low levels, implying that potentially large numbers of mosquitoes would be required to reliably estimate such low infection prevalences. This has led to the

Glossary

Gravid trap (CDC):: a suction trap placed over a pan containing an oviposition medium attracting mosquitoes. The trap was constructed to attract *Culex* mosquitoes, and hay infusion is used as attractant. The trap collects mainly gravid mosquitoes.
Human landing catch:: collection of host-seeking mosquitoes landing on exposed body parts using an aspirator.
Indoor resting collection:: collection of mosquitoes resting on the walls and roofs using an aspirator. Blood-fed, semi-gravid and gravid mosquitoes are collected.
Light trap collection:: collection of mosquitoes using a US Centers for Disease Control (CDC) miniature light trap, which has a battery-driven fan sucking mosquitoes into a collection bag. The trap is hung close to a sleeping person and is more efficient if the person is covered with a bednet. Mostly host-seeking mosquitoes are collected.
Molecular xenomonitoring:: introduced without a specific definition and used in two ways, either as (i) a molecular PCR technique used in xenomonitoring or (ii) a PCR test for identifying parasite DNA in mosquitoes, irrespective of the sampling method or larval stages. The second use is less specific and can create confusion.
Parasite transmission endpoint:: includes (i) worm breakpoints in the human population and (ii) vector biting threshold, or (iii) larval infection breakpoints in the vector populations.
Transmission monitoring:: detection and quantification of infective larvae in mosquitoes. The sampling method will be aimed at collecting mosquitoes coming for a bloodmeal.
Vector infection threshold:: infection thresholds in the vector population below which parasite transmission will cease.
Xenomonitoring:: defined within an LF elimination programme as 'the use of wild-caught mosquitoes to detect microfilaraemia in a community' [10], was inspired by the term xenodiagnosis introduced by Brumpt (1914) [76] 'as diagnosis via a stranger' using Reduvid bugs to diagnose human infection with *Trypanosoma cruzi* (Chagas disease). The mosquito sampling method is important in xenomonitoring and will optimally be aimed at collecting mosquitoes that have recently taken a human bloodmeal to get the highest sensitivity.

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development of more efficient and sensitive high-throughput methods for vector infection measurement, the most promising of which seem to be PCR techniques for detecting filarial DNA in pools of mosquitoes [8,9]. However, despite calls to deploy these molecular methods to monitor the progress of the GPELF [10,11], such approaches have been limited largely to research settings. Here, we review the current status of the available tools for quantifying vector infection and existing knowledge and evidence regarding potential infection thresholds for determining transmission interruption, to assess the potential for using vector infection monitoring as a tool for evaluating the success of filariasis treatment programmes in achieving parasite elimination from endemic communities.

Current tools for quantifying infection in vector populations

Vector dissections

Vector dissection is the traditional standard tool for measuring vector infection in filariasis and the only method that enables counting of all stages of filarial parasites in the mosquito. This is possible because in vector mosquitoes, microfilariae (Mf) taken up with the bloodmeal will pass the midgut wall and develop to infective larvae in the thoracic muscle, from where they migrate to the mouthparts to be transmitted when the mosquito feeds. Thus, dissection of different segments of an infected mosquito will enable both detection and measurement of different larval stages of the parasite.

Direct examination of freshly dissected mosquitoes for the infective third-stage larvae (L3) is adequate, quick and simple, facilitated by the size of the L3s and their characteristic movements. Similarly, dissection of unstained live mosquitoes is also appropriate for detection of Mf in recently engorged mosquitoes. The much less mobile developing larvae, first and second stage (L1 and L2), can also be seen in freshly dissected mosquitoes by experienced dissectors, but staining makes this much easier. It is generally accepted that a skilled technician is capable of dissecting and examining 50–100 mosquitoes per day, a limited number. Large numbers of mosquitoes could be screened for L3s by mass dissection [12,13]; however, to use the method quantitatively, care must be taken to macerate the mosquitoes just enough to enable the L3s to wriggle out undamaged (Box 1).

Fixing and staining the mosquitoes before dissection [14] is very useful when more mosquitoes are collected than can be dissected and/or when the place of dissection is far away because the mosquitoes just have to be fixed and can be processed later. However, although useful for detecting developing and infective larvae, this method cannot be used to find Mf in the bloodmeal of engorged mosquitoes owing to fixation of the bloodmeal. However, if mosquitoes are stained after they have been dissected on a slide [15,16], they can be stored and read later, Mf in bloodmeals can be counted and quality checks can be performed, provided the slides are treated and stored carefully to avoid losing L3s (Box 1).

The method of collecting mosquitoes will also influence the relative frequency of larval stages found by mosquito dissections. For example, to measure the transmission from mosquitoes to humans, the number of L3s in mosquitoes coming to bite human hosts need to be counted (Box 1). These mosquitoes, therefore, are best collected by human landing catch or light trap catches. Collecting mosquitoes resting indoors after a bloodmeal will give too low a transmission estimate because the mosquitoes will have lost some L3s during the recent bloodmeal [17]. By contrast, collecting blood-fed mosquitoes by indoor resting catch or pyrethrum spray catch then dissecting and examinating the midgut for Mf and the abdomen and thorax for early L1 larvae represents the optimal method for measuring the uptake of this parasite stage from the human population. Depending on the Mf prevalence and intensity of the inhabitants in the house and the mosquito vector species, half or more of these blood-fed mosquitoes will have Mf in their midgut [18]. Mosquitoes caught in this way are, thus, most useful for xenomonitoring.

Box 1. Dissection methods and transmission indices

- Dissection for infection: wings and legs are removed and head, thorax and abdomen are separated under 20X magnification. Each is placed in one of three drops of saline on a microscope to keep record of the location of the filaria. The mouthparts are separated with fine needles (40–50X magnification), enabling L3s to escape, and the remainder of the head and the thorax and abdomen are teased apart and examined for filarial worms.
- Dissection for Mf: in recently engorged mosquitoes, the midgut can be removed and the Mf counted in the bloodmeal. It can be useful to lyse the red blood cells by dissecting the midgut in distilled water.
- Staining and careful examination under a compound microscope at 100X magnification might be necessary to separate the larval stages and identify the species, the latter by examining the infective larva.
- Staining done before dissection: killed mosquitoes are placed immediately in 80% ethanol, in which they can be kept indefinitely. The specimens are taken through descending dilutions of alcohol to distilled water, stained in Mayer's acid haemalun and then differentiated in distilled water and taken to pure glycerol, awaiting dissection [14].
- Staining done after dissection: the slides are allowed to dry and abdominal preparations are dehaemoglobinized by immersing in water, then dried and fixed in 75% methanol. The slides are stained in hot Mayer's acid haemalun and differentiated in water, after which they can be stored for six months. The rather big L3s can easily fall off the slide, and it is recommended to keep a record of the number of L3s observed during dissection and to take care to avoid losing L3s from the slide (e.g. by using completely grease-free slides, dissecting in boiled water, keeping the slides horizontal until dry, being careful when staining the slides, storing the slides horizontally and avoiding knocking the slide boxes about) [15,16].
- The monthly biting rate, or MBR, is the estimated number of vector mosquitoes coming to bite one person who is exposed during all dark hours every night for one month (i.e. MBR is the number of mosquitoes collected by human landing catch multiplied by the number of nights in a month, divided by the number of catching nights) [81].
- The monthly infective biting rate, or MIBR, is the estimated number of infective bites delivered by the mosquitoes from which MBR is calculated (i.e. MIBR is the MBR multiplied by the number of infective mosquitoes found, divided by the number of mosquitoes dissected).
- The monthly transmission potential, or MTP, is the estimated total number of infective larvae carried by the mosquitoes from which MBR is calculated (i.e. MTP is MBR multiplied by the number of infective larvae found, divided by the number of mosquitoes dissected) [81].

Box 2. Sample sizes for detecting vector infection thresholds

The main objective of monitoring for management decision making in a parasite intervention programme is not to estimate infection prevalence but to detect with some certainty whether a set threshold has been met or crossed [3]. More formally, the objective is normally to test the hypothesis that the true prevalence (p) in the population is less than or greater than some critical threshold value (p_t). The usual formulae to determine how many sampling units (of n individual insects in the case of dissections and N pools of size m insects per pool in the case of the poolscreen PCR-based tool) are then given by Eqn 1–3 [82,83].

$$n = \frac{\log(1-P)}{\log(1-p)} \quad (1)$$

in the case of the total number of mosquitoes that require to be dissected, and

$$N = \frac{\log(1-P)}{m\log(1-p)} \quad (2)$$

and

$$N = \frac{-\theta\log(1-P)}{p\log(1+m\theta)} \quad (3)$$

in the case of the total number of pools of mosquitoes of size m that are required to be screened, all infection free, to conclude with (1–P)·100% confidence that actual or true infection prevalence is less than or equal to the specified threshold p. Eqn 2 is used when one can assume that infection in the vector population is distributed according to the binomial distribution model. If infection is clustered, then Eqn 3, derived from the beta-binomial distribution model, can be used to estimate sample sizes, with the magnitude of infection heterogeneity in the jth pool given by the value of θ [82,83].

Table I gives the results from applying these equations to estimate both n and N for 30 mosquitoes ($m = 30$) each per pool for detecting the two-vector infection thresholds quantified in this paper (Figure 1b,1c) at confidence levels of 95% and 99%. The results clearly show that: (i) large numbers of mosquitoes will be required to be dissected and found negative for infection to detect the L3 threshold prevalence of 0.085%, compared to the overall infection prevalence of 0.65%; and (ii) that for detecting either of these thresholds, pool numbers (N) will increase if infection aggregation occurs.

Table I. Sample size calculations of the total numbers of mosquitoes and mosquito pools[a]

		Sample size (n, N of m =30)					
		95% confidence level			99% confidence level		
Vector infection thresholds	p_t (%)	n	N_{binom}[b]	$N_{beta\text{-}binom}$[c]	n	N_{binom}[b]	$N_{beta\text{-}binom}$[c]
L3 infection threshold	0.085	3523	118	135	5416	181	135
All stages infection threshold	0.65	460	16	18	707	24	28

[a]Results are shown for detecting the two vector infection thresholds (see text), n denotes number of individual mosquitoes to be dissected, and N denotes number of mosquito pools of size 30 to be analyzed using poolscreen PCR.
[b]Number of pools of mosquitoes estimated (Eqn 2), assuming that infection is binomially distributed in the vector population.
[c]Number of pools of mosquitoes estimated (Eqn 3), assuming that infection is distributed non-randomly in the vector population approximated by the beta-binomial distribution model. A moderate degree of infection heterogeneity was used in the estimation by setting the value of θ to 0.01.

This indicates that vector dissection is the most unequivocal direct measure of infection status in vectors allowing estimates of transmission to be made through the identification of L3 larvae. It is important to note that the accuracy and precision with which lymphatic filariasis (LF) infection in vectors can be estimated might be limited by the ability to process sufficient numbers of mosquitoes by dissection, particularly when vector infections are low. However, using mosquitoes for estimating Mf prevalence in the human population could require far fewer mosquitoes than it takes to quantify transmission levels (Box 2).

PCR-based poolscreen assays

The PCR assay is capable of detecting genomic DNA from any stage of the parasite present in the mosquito. The basics of the poolscreen assay are: mosquitoes are collected, sorted and pooled for DNA extraction; the purified parasite DNA is amplified in a PCR amplification using parasite-specific primers; and, finally, the presence or absence results are analyzed using various statistical algorithms to determine a point estimate of infection prevalence [19].

For several years after the initial design, variations of the DNA-extraction method and the PCR detection method were developed [20–26] (Box 3), and this led to a multicentre standardization trial in 2002 [8]. A key result from this was the recommended use of the Qiagen DNA column extraction method combined with the Qiagen HotStart Taq PCR amplification. More recent modifications include using test strips [27] and the development of real-time PCR [9,28], with the latter being the most important of these advances. The major advantages to real-time PCR are the increased sensitivity, the decreased possibility of contamination from post-PCR handling and the decreased handling time of post-PCR products, which enables faster throughput of samples, increasing the efficiency of the assay. The one main disadvantage is that an expensive specialized instrument is required to detect the PCR product in real time.

Poolscreen PCR for detecting filarial DNA in mosquitoes can be used to monitor the Mf prevalence in a community, termed here as molecular xenomonitoring. This has the considerable advantage of being much less intrusive to the community members than the standard night blood sampling used to detect Mf-positive individuals and avoids the bias of individuals that are not willing to participate in the blood collection surveys and who might also be systematic non-compliers for the drug treatment. One of the disadvantages is that, as with dissections, a large number of mosquitoes will need to be captured and screened, especially as the parasite prevalence is decreased through national Programmes to Eliminate Lymphatic Filariasis (PELF) efforts (Box 2). Egypt, French Polynesia, Thailand, Haiti and Papua New Guinea [29–34] are some of the countries that have successfully used PCR detection of mosquito infections in various field studies, but, unfortunately, the necessary equipment and expertise are not available in all countries and no national PELF programmes are currently using this tool for monitoring their activities.

Box 3. PCR detection of filarial DNA in mosquitoes

The molecular detection of filarial DNA can be achieved by using PCR to amplify the *Ssp*I DNA repeat sequence in *Wuchereria bancrofti* [84] and the *Hha*I repeat DNA sequence in *Brugia malayi* parasites. This technique was first developed in 1994 [20,85]. These diagnostic assays were designed to detect parasite DNA in either blood samples or pools of mosquitoes with the sensitivity level of detecting DNA from a single microfilaria. Both assays use a segment of their genomic DNA known as a 'repeat sequence' because it occurs many times in the DNA. Real-time PCR (qPCR) detection assays were developed in 2006 [9,28] for both of these parasites using primers and probes that amplify different regions of each of these repeat sequences. In *W. bancrofti* conventional PCR (cPCR), the *Ssp*I repeat sequence is a section of DNA that is part of a larger repeat sequence known as the long dispersed repeat, or LDR (Genbank Accession No. AY297458). The qPCR assay amplifies a region of the LDR downstream of the *Ssp*I region. In *Brugia malayi* detection, both cPCR and qPCR are based on amplifying different regions of the repeat DNA sequence known as the *Hha*I repeat (Genbank Accession No. M12691) (Table I).

The primary difference between cPCR and qPCR lies in the method of detection of the amplified target. In cPCR, the DNA sequence is amplified millions of times during the PCR reaction and then, upon completion of the reaction (post-PCR), a portion of the sample is run on an agarose gel, stained with ethidium bromide that binds DNA and detected by exposure to ultraviolet light. qPCR detection involves using a probe labelled with a fluorescent molecule that hybridizes to the amplified target sequence (between the two primers) during the PCR reaction. During each cycle of PCR, as more product is made, more probe hybridizes and fluoresces. The fluorescence is detected and the information is collected and recorded on the instrument at each cycle during PCR, making it possible to see the accumulation of PCR product in real time. qPCR is less subject to individual variations in staining, detecting and scoring of samples, resulting in a more sensitive analysis.

Table I. PCR primer and probe sequences used in detection of *W. bancrofti* and *B. malayi* DNA in mosquitoes

PCR assay	Forward primer sequence (5′–3′) Reverse primer sequence (5′–3′)	Probe sequence (5′–3′)
Wb-cPCR (SspI)	CGTGATGGCATCAAAGTAGCG	
	CCCTCACTTACCATAAGACAAC	
Bm-cPCR (HhaI)	GCGCATAAATTCATCAGC	
	GCTTTTGTAATTAAGTTTTGC	
Bm-qPCR (HhaI-MGB)	GCAATATACGACCAGCAC ACATTAGACAAGGAAATTGGTT	*FAM*-TTTTTAGTAGTTTTGGC-*MGBNFQ*
Wb-qPCR (LDR)	ATTTTGATCATCTGGGAACGTTAATA	*FAM*-ATCTGCCCATAGAAA
	TCACTCTGAATGGATTAGACAGTCG	TAACTACGGTGGATCTCTG-*Tamra*

Abbreviations: *Wb, Wuchereria bancrofti; Bm, Brugia malayi*; FAM, reporter dye; TAMRA, quencher dye; MGB, molecular grove binder moiety; MGBNFQ, molecular-groove binding non-fluorescence quencher hybridization probe (Applied Biosystems, Foster City, CA).

The recent development of both *Brugia* L3 and *Wuchereria* L3 molecular detection assays illustrates the feasibility of estimating the transmission potential by using RNA instead of DNA [35,36]. Detection of the L3-stage parasite in a pool of mosquitoes is based on the reverse transcriptase PCR amplification of a specific target mRNA transcript that is activated, or first expressed, in the infective stage of the parasite. This enables the determination of infectivity in the vectors, a component necessary for the estimation of transmission potential.

A recent study has shown that filarial DNA can be detected for two weeks or longer after the infected bloodmeal, even in non-vector mosquitoes in which the Mf do not leave the midgut [37]. If partly digested Mf and even fragments of Mf DNA give positive PCR results, it could indicate a problem in using the DNA-based tool in its present form (i.e. it will overestimate vector infection prevalence).

Vector infection thresholds: theory and empirical data

A key need for using vector infection data to assess the elimination of LF is not only determining whether larval infection thresholds exist in the vector population that could signify interruption of parasite transmission but also quantifying what the numerical values of these thresholds might be. There are two principal reasons for this interest. First, larval infection thresholds, rather than vector biting densities, clearly represent the parasite elimination target for drug intervention programmes in vector-borne infections such as filariasis [3]. Second, information regarding the numerical value of such vector infection thresholds is required if xenomonitoring tools, such as the all-stage parasite PCR-based poolscreen methods described above [8,26,28], are feasibly applicable on the basis of mosquito sampling sizes (Box 2) in filariasis monitoring programmes, compared with methods based on measurement of infection in humans [3].

Here, we use results from recent modelling work and analyses of vector infection data, as quantified via both carrying out mosquito dissections and the application of the pooled PCR method, to assess the existence (as well as compare the likely values) of larval infection thresholds that could signify parasite transmission endpoints in filarial vector populations. Given that extinction thresholds in any parasite system essentially represent system boundaries, the crossing of which can cause a parasite population either to persist in an endemic state or to become extinct [6], it is apparent that analysis of mathematical models of filariasis transmission can offer a useful initial tool for estimating the likely values of infection endpoints in vector populations. Figure 1a shows the results from a numerical stability analysis carried out on a deterministic model of filariasis transmission [6], which depict that the existence of an Mf prevalence breakpoint threshold in the human population (for culicine-transmitted filariasis) can indeed give rise to the occurrence of a corresponding L3 larval density threshold in the vector population. For the given plausible parameters of the transmission model [6], the results illustrated in Figure 1a indicate that the average L3 transmission threshold value in the culicine vector population could lie in the region of 0.0009 L3 per mosquito, corresponding to the breakpoint Mf prevalence value of 0.53% in the human host.

However, because proposed vector infection monitoring tools (as described above) are most likely to be based on

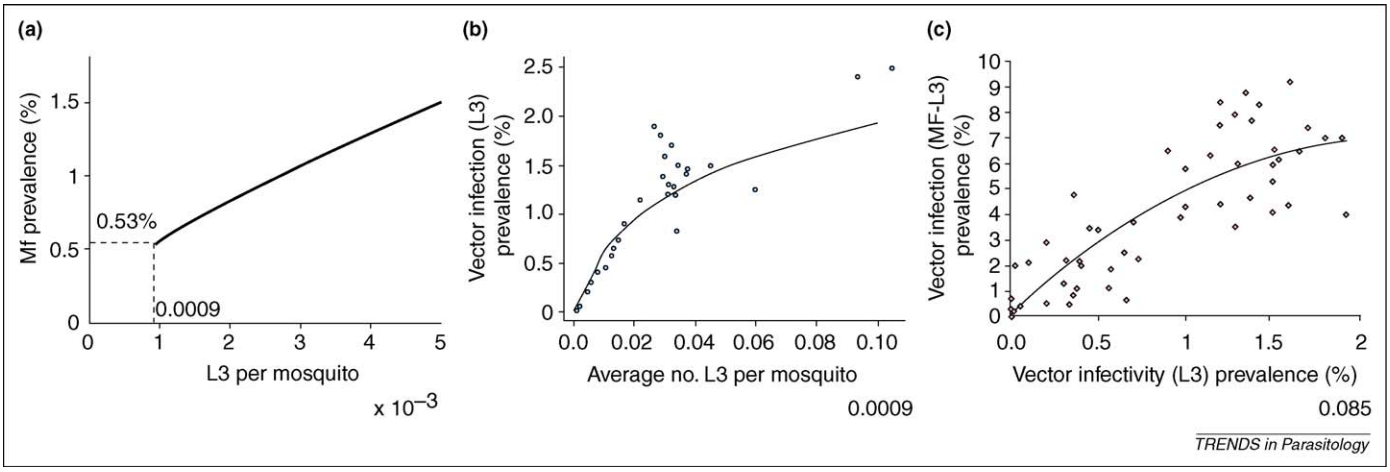

Figure 1. Quantifying vector infection thresholds in lymphatic filariasis. **(a)** Results from a numerical stability analysis of the coupled deterministic transmission model of lymphatic filariasis, indicating the existence of a L3 larval density transmission threshold (value of 0.0009 per mosquito given by the dashed line crossing the *x*-axis) in culicine mosquitoes corresponding to the existence of a Mf prevalence threshold in the human host (value of 0.53% given by the dashed line crossing the *y*-axis). Details of model and analysis methods are as given in Ref. [6]. **(b)** The relationship between average L3 per mosquito and prevalence of L3 infection obtained in wild-caught mosquitoes from filarial endemic communities. Each circle in the figure denotes paired data on these variables obtained from mosquito samples within each study community ($n = 29$; see Refs [15,16,38–71]). The solid curve shows the fit of the negative binomial model relating infection intensity to prevalence given by:**(1)**. $P(M,k) = 1 - (1 + M/k)^{-k}$
where P is the L3 infection prevalence, M is the L3 density and k is the aggregation parameter of the negative binomial distribution. The model was fitted to the data by maximum likelihood using binomial errors, giving an estimation of k of 0.007. Application of the model to an M value of 0.0009 (the L3 transmission threshold density) gives a corresponding value of 0.085% as a potential L3 threshold prevalence for determining filariasis elimination (shown in bold on the graph). **(c)** The relationship between overall all-stages vector infection prevalence and prevalence of L3 infection obtained in wild-caught mosquitoes from filarial endemic communities. Each solid circle in the figure denotes paired data on these variables obtained from mosquito samples within each study community ($n = 63$; see Refs [15,16,38–71]). The solid curve shows the best-fit quadratic logistic regression model of the form:**(2)**. $\log\left[\frac{p}{1-p}\right] = a + b_1X + b_2X^2$
where p denotes the prevalence of overall all stages infection, X is the prevalence of L3 infection, a represents the intercept and b_1 and b_2 represent the linear and quadratic terms, respectively, with estimated values of $a = 0.239$, $b_1 = 0.210$ and $b_2 = -0.008$. Application of this model to the L3 threshold prevalence value of 0.085% gives an empirical value of 0.65% as the corresponding all-stages larval infection prevalence threshold (figures in bold on the graph).

measurement of larval infection prevalence rather than density, the comparable infective L3 or infection (Mf-L3) prevalence figures need to be estimated to gainfully use the theoretical larval breakpoint result depicted in Figure 1, depending on whether proposed vector infection detection tools can measure L3 infection or overall all-stages larval infection in vector populations. Figures 1b and 1c show how we can use published data on mosquito dissections to derive these prevalences, based on: (i) estimating the relationship between average L3 density recorded among dissected mosquitoes versus the prevalence of L3 infection observed in these mosquitoes to obtain the threshold L3 infection prevalence (Figure 1b); and (ii) using this threshold value, in conjunction with estimation of the relationship between vector L3 infection prevalence and overall infection prevalence, to derive the vector overall or all-stages infection threshold (Figure 1c). These results are clearly crude (e.g. the relationships are estimated for both culicine and anopheline mosquitoes, combined, and the data are derived from a variety of mosquito collection methods, including a mix of resting and/or biting catches – for details, see Refs [15,16,38–71]). Nonetheless, they provide a first estimate of the magnitudes of the infective L3 and overall larval infection threshold prevalences that might be used as preliminary transmission endpoint targets in the global LF elimination programme. More specifically, the results in Figures 1b and 1c suggest that if L3 infection thresholds obtained through mosquito dissections are to be used as endpoint targets, the relevant endpoint value could be preliminarily set as 0.085%, whereas for overall larval infection, the corresponding value would be higher at 0.65%. These actual threshold values are important because they will play a major role in the choice of the infection indicator for monitoring [3]. For example, if dissections are to be used for estimating infection levels in the vector population, the higher value of the overall larval infection prevalence indicator – which would reduce the required sample size (Box 2) and the ease of dissections for estimating this prevalence – would mean that this indicator would be the choice for transmission monitoring when using this method.

Figure 2 plots the currently available data on the relationship between PCR-based poolscreen estimates of larval infection prevalence and prevalence of infection obtained by dissection in comparable mosquitoes. This indicates that it is presently not possible to estimate the PCR-based poolscreen larval infection threshold prevalence, as compared to the larval infection threshold value of 0.65% obtained by mosquito dissection (Figure 1c), largely as a result of the considerable between-study variability in the observed relationship between the two infection measurement methods. Clearly, more carefully standardized data providing parallel information on vector filarial infection obtained by dissection or PCR-based poolscreen of comparable mosquito samples (i.e. either resting or biting mosquito catches), using standardized dissection and PCR methods, are required to estimate this threshold, if parasite elimination endpoints based on quantifying infection prevalence in vector populations by the PCR-based poolscreen method are to be successfully applied in filariasis elimination monitoring programmes.

Mosquito sampling and estimation of vector infection

Using infection in mosquitoes to assess when transmission thresholds have been crossed – and, hence, to decide when to stop MDA – will necessarily mean sampling

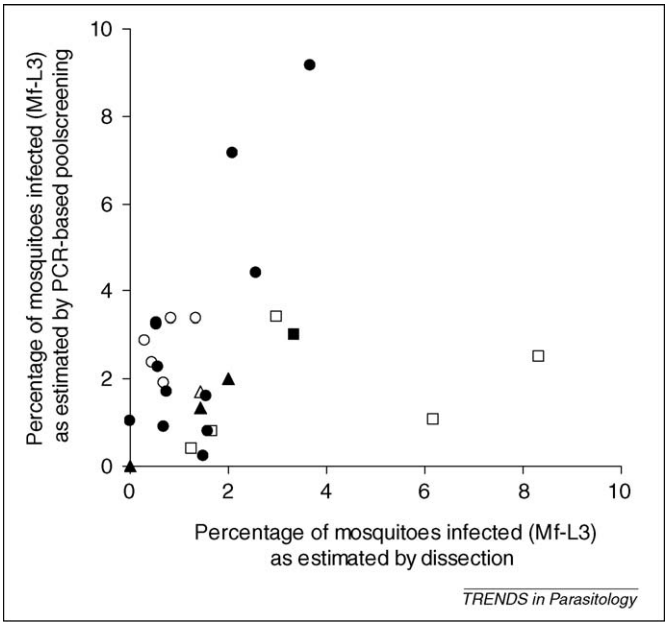

Figure 2. Comparison of infection prevalence in mosquitoes, as measured by dissection versus PCR-based poolscreening. The prevalence is given as the percentage of mosquitoes that are infected with any-stage parasites (Mf–L3). Symbols denote the paired prevalence data obtained using these methods on mosquito samples from different studies (closed triangle [77]; open triangle [34]; closed circle [33]; open circle [78]; closed square [79]; and open square [80]). The relative paucity, and between-study heterogeneity, in the data precluded empirical modelling of these data.

for mosquitoes with very low infection rates (see estimated threshold values above). This clearly implies the need to test for filariae in many mosquitoes, using a highly sensitive method. The number of mosquitoes needed if mosquitoes are tested for filariae by dissecting for infective larvae will be more than 3500 (Box 2, Table I), whereas if all filarial stages are included, only 460 mosquitoes would be required. The PCR-based poolscreen method should clearly constitute the method of choice because more mosquitoes can be tested during a shorter period and with a higher sensitivity compared with dissections, provided that funds and issues surrounding standardization of the method are resolved and a PCR laboratory is available.

However, the population structure of the mosquitoes sampled should also be considered carefully when the current PCR tools (which amplify DNA from any stage of parasites, whether dead or alive, in mosquitoes) are used to quantify larval infection. It is important to recognize that a group of mosquitoes collected just after a bloodmeal will have a much higher chance of being PCR positive for filaria than mosquitoes coming for a bloodmeal because a substantial proportion of the mosquitoes collected in human landing catches will have come for their first bloodmeal (in the coastal area of East Africa, this is normally approximately 35%) [15,16,72]. This suggests that the best use of PCR methods would be for xenomonitoring rather than for transmission monitoring using mosquitoes collected as soon as possible after bloodfeeding.

With regard to actual numbers of mosquitoes that are required to be sampled for detecting the infection thresholds in vector populations (Box 2), it is clear that a large number of mosquitoes (from at least 460 to above 3500; Box 2, Table I) will have to be collected and examined. This indicates that there is a requirement to ascertain the relative efficacy of the available methods for collecting mosquitoes in each ecological setting, so that the most efficient method is used for assessing the crossing of these vector transmission thresholds in endemic communities. A study in Egypt suggested that for culicine mosquitoes, gravid traps might be more practical for collecting large numbers of recently fed mosquitoes than resting collections [30]. However, the filarial infection rates might be lower; gravid traps collect mosquitoes coming to lay eggs, including those that have taken non-human bloodmeals, and *Culex* filariasis vector mosquitoes sometimes prefer birds to humans [73].

Thresholds and indicators

Monitoring multiple infection indicators pertaining to different stages of the parasite transmission cycle will no doubt greatly increase our confidence in correctly judging the progress of the GPELF, as well as deciding when parasite elimination has taken place owing to implemented interventions [2,3,74]. For vector-borne diseases, such as LF, it is important to note that multiple indicators signifying parasite elimination could encompass both Mf breakpoint values in the host population and either vector biting thresholds or larval infection breakpoints in the vector population [4–6]. Because these thresholds are dynamically interlinked, one can theoretically choose to monitor one of these indicators, based on best diagnostic accuracy, inherent variability, sensitivity to change in underlying parasite population, statistical power and sample size requirements, and cost and ease of tool deployment [2,75]. Thus, it is pertinent to understand that using multiple infection elimination target indicators is to overcome flaws in any one indicator. Although there is some information available regarding the efficiency of current methods to detect infection elimination thresholds in the human host [7], including values of infection prevalence breakpoints based on both microfilaraemia and antigenemia detection [5], the corresponding data on the usefulness of the present vector infection estimation methods for performing the same function has been scarce. This review represents a first attempt to systematically appraise both the advances made in biomarker development and the current state of knowledge of infection endpoints in vector populations, as well as the continuing gaps that should be resolved if these methods are to be successfully applied in monitoring and evaluating filariasis elimination.

It is undeniable that accurate, high-throughput vector infection measurements will be required if filarial infection thresholds in the vector population are to be used to evaluate the achievement of parasite elimination. The primary reason for this is that these thresholds are likely to be very low (~0.085% if L3 is to be used as the threshold measure or 0.65% if overall larval infection is to be used as the threshold measure), indicating that large mosquito samples would be required to be monitored for detecting the crossing of either threshold (Box 2). Note, also, that these infection thresholds will depend on the transmitting vector species, being lower for culicine vectors exhibiting the 'limitation' larval infection process and higher for anopheline vectors showing the 'facilitation' process [6].

However, although these considerations have led to the development of PCR-based poolscreen tools for estimating these prevalences, there are several questions that should be resolved before we can use these tools successfully to monitor the elimination of filariasis transmission. Current PCR tools, which detect parasite DNA from any stage in the mosquito, are best used as xenomonitoring tools (i.e. for detecting the presence of Mf infection in human communities), given the age structure of mosquitoes in both resting and biting catches. This might not be a major problem, given that thresholds based on overall infection prevalence are equally useful as L3 infection prevalence for determining parasite elimination, but it does indicate that it will be important to sample households randomly across a community so that the data obtained are reflective of the true overall infection prevalence in the vector population and not simply of infected individuals in a particular household [30]. The greater difficulty of using this method, however, lies in the fact that current mosquito collection techniques and tools might not enable sufficient numbers of mosquitoes to be caught for analysis, although if overall infection thresholds are used, adequate collection of *Culex quinquefasciatus* could be facilitated by using gravid traps. Finally, a difficulty in using PCR for estimating larval infection is that this might detect fragments of DNA in non-vector mosquitoes that could stem from killed larvae, suggesting that this might not necessarily indicate a live parasite infection [37].

The estimates of the larval infection threshold values for use as elimination targets are preliminary. More standardized data (stratified by vector species and endemic regions) are required both to estimate and to validate the threshold interrelationships between L3 intensity and L3 and overall infection prevalences before we can confidently set threshold values for monitoring. Standardized data need to be acquired on the relationship between overall infection prevalence obtained by mosquito dissection versus poolscreen PCR-based tools to estimate the infection prevalence thresholds to be used as targets when applying PCR-based tools. Given that filariasis elimination programmes have now been in progress for several years in many endemic regions, a research programme to collect mosquito samples from these sites in which infection levels have presumably declined to near threshold levels to dissect and validate the interrelationships and threshold estimates would clearly constitute another crucial priority in efforts to develop efficient monitoring tools in filariasis elimination.

Concluding remarks

Although tremendous progress has been made in developing alternatives to mosquito dissections for estimating vector infection prevalences in filariasis, the effective application of these new tools (including the PCR-based poolscreen method) requires careful considerations of mosquito collection methods and age structure in these collections, diagnostic characteristics of the tool and the actual target infection thresholds that need to be monitored to determine whether parasite elimination has occurred. Only an integrated research programme that brings together entomologists, molecular biologists, epidemiologists and modellers to address this problem in an interlinked manner, aligning tool development with threshold estimation using models and empirical data, will lead to progress in this area. Given that many areas in endemic countries have successfully implemented the proposed 4–6 years of annual mass drug treatments and, thus, are close to deciding when to stop these treatments, it is clear that undertaking this work has now become an urgent priority in filariasis research.

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Meetings of interest to parasitologists

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III International *Giardia* and *Cryptosporidium* Conference
Conference Centre in Orvieto (Terni, Italy)
Simone Caccio simone.caccio@iss.it

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Athens, Greece
www.epidemics.elsevier.com

9–12 March 2010
14th International Congress on Infectious Diseases
(Organised by the International Society for Infectious Diseases)
Miami, Florida
info@isid.org
www.isid.org

15–20 August 2010
XIIth International Congress of Parasitology (ICOPA)
''Understanding the global impact of parasites - from genomes to function and disease''
The Melbourne Exhibition and Convention Centre
http://www.icopaxii.org/index.php?option=com_frontpage &Itemid=1
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