Introduction Xenomonitoring: Sampling issues for Lymphatic Filariasis

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Within the LF community, the term 'xenomonitoring' has generally been taken in the broad sense of testing mosquitoes to assess the prevalence of microfilaremia. 'MX' has been used to indicate that the analysis of the mosquitoes is by PCR, really a necessity where low prevalence requires large numbers of mosquitoes. The broad term also encompasses analyses for mosquitoes which are infected (all filarial stages) and those which are infectious (L3's only), used for the assessment of transmission potential. This paper uses the broad term although the focus is on the sampling of mosquitoes used for PCR analysis. The only vectors considered here are *Aedes, Anopheles,* and *Culex.*

After summarizing the pertinent conclusions on xenomonitoring from the most recent international meeting held in Copenhagen in 2006[1], this review

- describes the approaches currently recommended by WHO for monitoring in people[2] and their corollaries for xenomonitoring;
- describes approaches to sampling for mosquitoes and provides estimates of the numbers of mosquitoes required;
- reviews comparative costs for human transmission assessment surveys (TAS) and their counterparts for mosquitoes; and
- suggests areas for research and development.

1. Conclusions from 2006 WHO/DBL meeting: 'The Role of PCR Techniques for Assessing LF Transmission'

[1] Role of PCR for assessing LF transmission Copenhagen, November 2006.

[2] Monitoring and Epidemiological Assessment of Mass Drug administration in the Programme to Eliminate Lymphatic Filariasis WHO/HTM/NTD/2011.xx

Information on xenomonitoring for LF was reviewed in a 2006 meeting jointly sponsored by WHO and the Center for Health Research and Development, University of Copenhagen (DBL).[3] The conclusions most relevant to the present paper include:

- "Transmission of lymphatic filariasis is a function of *both* the prevalence of mosquitoes with infective-stage larvae (infectivity rate) and the human biting rate; therefore, transmission monitoring must also include estimates of human biting rates…

- MX (e.g. PCR) and mosquito sampling assessment should focus on individual villages (or cluster of villages when villages are small), rather than on implementation units…

- Traps should be distributed to cover all areas in study villages. A uniform number of pools/ mosquitoes should be tested from each trap when possible…

- The number of pools and mosquitoes for MX testing should follow power guidelines and sample size tables/graphs to target assessments of infection prevalence rates of 0.1, 0.25, 0.5, 1 and 2%.

- Provisional target thresholds for suspension of MDA are mosquito DNA rates by MX of <0.25% for *Culex*[4], <1% for *Anopheles*, and <0.1% for *Aedes*. No provisional target rate for *Mansonia* has been suggested at this time."

2. Xenomonitoring Prior to mass drug administration (MDA)

The current decision processes for deciding to start or stop MDA involve measuring some prevalence indicator in a population and deciding whether or not a defined starting or stopping threshold has been achieved.[5] These processes have drawbacks, but have provided practical guidance

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[3] Role of PCR for assessing LF transmission. Copenhagen, November 2006.

[4] More recently,Pedersen has suggested this be raised to an L3 infection prevalence of 0.085% or overall larval infection prevalence of 0.65%. These estimates were derived from dissection. Comparison of estimates from dissection and PCR were not consistent enough to suggest thresholds from PCR analysis. Pedersen, E. *et al.* (2009). The role of monitoring mosquito infection in the Global Programme to Eliminate Lymphatic Filariasis. *Trends in Parasitology*25, 319-327.

[5] WHO. op.cit.

for program managers to date, although the transmission assessment survey (TAS), used for the decision to stop, is proving expensive and questions remain about the size of the geographic area it should encompass. Larger areas carry higher risks of missing transmission zones and of failing an entire area when, in fact, only a portion has continuing transmission. Smaller areas increase the number of surveys required along with costs.

A fundamental difficulty in using filarial prevalence in mosquitoes for making these decisions is that no similar thresholds are generally accepted, in addition to the fact that collection methods at present appear suitable only for obtaining *Culex* in the numbers required for analysis. There will be, as with the TAS surveys, different thresholds for different mosquito species. There may also be the need for different thresholds for areas with different transmission potentials, although this has not been a variable used in establishing human prevalence thresholds. A meeting in late 2011 is being convened by the Atlanta-based Lymphatic Filariasis Support Center (LFSC) to review and discuss recent xenomonitoring work and will help to clarify the status of thresholds, the practicality of collecting adequate numbers of *Aedes* and *Anopheles* and the priorities for additional work. At present, however, it is premature to recommend xenomonitoring as a decision making tool for starting or stopping MDA.

3. <u>Post-MDA Surveillance</u>

Despite the recommendation to conduct surveillance in people after stopping treatment,[6] it has been difficult to identify approaches which are affordable and can be maintained. The most definitive current method for ensuring that a given area remains below the threshold prevalence level is the TAS.[7] As the time elapsed since the previous MDA increases, so does the confidence that a TAS which 'passes' the area surveyed indicates no recrudescence, especially if it also provides evidence of a decline in prevalence from the previous TAS. The current guidelines suggest waiting 2 or 3 years between surveys. This time interval takes into consideration the importance of identifying recrudescence at an early stage as well as the difficulty national staff may have in maintaining support until a TAS can be performed. If the first post-MDA survey is satisfactory, a final TAS is suggested 2-3 years later.

Whatever surveillance method is used should ideally be applied <u>before</u> MDA is stopped so as to provide data on trends in the post-MDA period. The TAS certainly accomplishes this. Its major

[6] Ibid. Section 8
[7] Ibid. Section 8.2.1

drawback is its expense, especially if low school enrolment precludes schools being used as the primary sampling units and the surveys must be done in communities. Up to now, the TAS has been employed in individual implementation units (IU's) or in two or more IU's considered epidemiologically similar. (When being assessed either individually or in a group, they are described as evaluation units (EU's)). It has proved challenging to use the TAS to assess all areas within a country considered eligible to stop MDA, and there have been no initiatives to date to try to assess areas excluded from consideration by the initial mapping surveys despite their known fallibility.

There is hope that one or more systems of passive surveillance which would cover large areas at low cost can be developed as an alternative to the TAS. Most promising appear approaches which entail screening blood for LF using specimens taken routinely over time for other purposes. Populations include persons attending anti-natal clinics, those seeking care for suspected malaria, blood donors, military and police recruits and persons included in the USAID supported Demographic and Health Surveys (DHS). One such a system using blood taken for malaria diagnosis has been implemented in Togo and other options are under consideration for assessment elsewhere in Africa.

Additional options for post-MDA surveillance need to be explored. It is in this context that xenomonitoring may have its most immediate application. While the use of thresholds may eventually become relevant, the only indicator needed post-MDA is a change in prevalence with the caveat that data are currently lacking about the size of change (especially if it is an increase) which is epidemiologically significant. Large changes should not raise difficulties, but in the case of small increases or decreases, continued monitoring will be needed, at least until a better indication of the significance of small changes is available. The size of the geographic area to be sampled for xenomonitoring poses the same epidemiological questions as for the TAS. The logistics of servicing traps makes sampling larger areas more difficult than for the TAS, however. The following sections address issues related to sampling and costs which mainly pertain to post-MDA surveillance in *Culex*.

4. Sample methods

A representative sample of either humans, or the mosquitoes which feed on them, can be most confidently chosen by using a method which randomly selects sites randomly with the human population proportional to size. It is convenient to use households (HH) as sample sites when collecting

Anopheles and *Culex* which are indoor crepuscular and nighttime feeders. From a sampling perspective, it does not matter whether the collection is made within or outside of the HH selected. For *Aedes,* an outdoor, daytime feeder, it is not so clear how to choose a representative sample, although HH based sampling can serve as a 'default' until some alternative approach proves better.

The Annex[8] describes a two-stage cluster sampling method in which HH are selected randomly with probability proportional to size. This is a more rigorous and more replicable approach than the recommendation from Copenhagen: "Traps should be distributed to cover all areas in study villages…"[9]

Samples of recently fed *Culex* mosquitoes using gravid traps were obtained from sites selected using this method in studies in Pondicherry (VCRC) India,[10] and Colombo, Sri Lanka[11]. These were supported by the Bill and Melinda Gates Foundation and coordinated by the LFSC. The method proved feasible, although some of the entomological teams experienced initial difficulties in using census data to identify HH and in moving the traps frequently between HH, as required by the sampling protocol.

At the time of writing this paper, only the results from Colombo had become available. It showed that the sampling method employed provides replicable results. [12] An average prevalence of 0.3% of all filarial stages was found with maximum likelihood estimates from 3 separate samples ranging from a low of 0.26% to a high of 0.32%. The 95% confidence intervals in samples of approximately 5,000 mosquitoes included a low of 0.13% prevalence and a high of 0.535%. Two of the samples were taken from the same set of traps and yielded a combined total of 7,635 mosquitoes. The maximum likelihood estimate was 0.30% with the 95% confidence ranging from 0.178% to 0.484% simply demonstrating the effect of a larger sample size in narrowing these limits. In the same resident human population, there were 2 individuals who were both ICT and Mf night blood smear positive among 1178 adults (0.17%). There were no ICT positives among 679 first and second graders. Both mosquito and human sampling suggest that LF prevalence is below sustainable levels of transmission.

Repeat studies in Pondicherry and Colombo are planned to measure changes of prevalence and to compare prevalence in mosquitoes with that assessed in school entrants.

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[8] Annex: Draft Generic protocol of mosquito sampling of Households

[9] Copenhagen. op. cit.
[10] Subramanian S., et al
[11] Laney S. Sri Lanka Mosquito sampling for molecular xenomonitoring (MX) for surveillance of lymphatic filariasis. August 31, 2010.
[12] Rao,R. Personal communication

In this two-stage cluster design, the first stage selects 'clusters' from within the area to be sampled and the second selects HH within each cluster. Given any desired total sample of mosquitoes desired, options exist for choosing both the number of clusters (30 is a minimum and was used in the *Culex* studies referred to above) and the number of HH. If only 1 pool of mosquitoes is to be collected from each HH, then the total number of HH is determined by the total number of mosquitoes desired divided by the size of the mosquito pools which will be analyzed by PCR (a pool size of 25 was used in the *Culex* studies referred to above). To reduce the number of collection sites (HH), more than 1 pool may be collected from each. Although in Colombo, reducing the sites from 208, from which 1 pool was collected, to 101, from which 2 were collected, showed no significant difference in prevalence,[13] the impact of further reductions in the number of sites remains to be determined.

5. Sample size and target prevalence

The following descriptions provide examples of the numbers of mosquitoes of different species which will be needed to demonstrate increases or decreases in prevalence. Charles Katholi has provided a convenient method for estimating those numbers on his web page[14]. His tables provide sample sizes which have an 80% power of detecting the designated difference between the null and an alternative hypothesis with a 5% chance of finding a higher prevalence when, in fact, it does not exist (type I or alpha error).

The Colombo study[15] provides examples relating to detecting increases of prevalence. It found a prevalence of 0.30% for all filarial stages. This can be taken as the null hypothesis. A sample of 10,525 mosquitoes, analyzed in 421 pools of 25 would justify accepting the alternative hypothesis that the actual prevalence was 0.45% or more, given that 40 or more of these pools tested positive. To assess a doubling of prevalence from 0.3% to 0.6%, a sample of 3,250 mosquitoes (130) pools is needed. So sample size increases as the difference between the null and alternative hypotheses decreases and vice versa. And sample size also increases as the prevalence for the null hypothesis decreases. For example, assessment of L3 stages at the threshold of 0.085% suggested by Pedersen[16] would require 10,850

[13] Laney. op.cit.
[14] Available at http://www.soph.uab.edu/bst/ps_samplesize - the current password is **ByInvitation2011.**
[15] Laney. op.cit.
[16] Pedersen. op.cit.

mosquitoes (434 pools) to be able to accept the alternative hypothesis of a doubling of prevalence to 0.17%. Mosquito samples of this magnitude are feasible for *Culex.*

At the 2006 Copenhagen meeting[17], a threshold of 1% was suggested for *Anopheles.* In this instance, a doubling of prevalence from 1% to 2% would require collecting only 1,100 mosquitoes (44 pools), although the numbers rise to 3,600 (144 pools) and 12,600 (504 pools) as the difference shrinks to 1.5% and 1.25%. The problem for monitoring studies for *Anopheles* is one of feasibility of collection.

The suggested Copenhagen threshold for *Aedes* is 0.1%. To assess a doubling to 0.2%, 9,250 mosquitoes (370 pools) are needed, and 30,625 (1,225 pools) would be needed to assess a prevalence of 0.15%. Realistically, only increases in prevalence can be assessed and the principle challenge for *Aedes* will be in capturing these numbers of mosquitoes in an affordable manner. This is especially true since the known potential for *Aedes* recrudescence makes the detection of relatively small increases in prevalence of relevance.

6. Costs

At this stage in the LF program, estimates of costs comparing human with mosquito monitoring are preliminary. Experience is still being gained, and those doing either a TAS or xenomonitoring are generally still on learning curves, not yet reaching full efficiencies. In addition, there is rapid development of PCR techniques which promises to reduce their costs and to make them more available within developing countries. Nevertheless, data are presented here to provide a broad sense of whether the costs in people and mosquitoes are comparable.

Estimates from Togo and Burkina Faso presented at the WHO Technical Consultation on LF elimination in low endemic and Loa Loa co-endemic areas (Lusaka, Zambia, 4-5 June 2011) suggest the cost of a TAS to be approximately $10,000 plus the cost of the ICT cards. These cards now cost about $5 each or a cost of $8,500 for a TAS sample of 1,700 persons (and double that in *Aedes* transmitted areas). It is hoped the cost for a new generation of ICT cards will decrease to about $1. The new cards will reduce the overall TAS costs by about a third from some $18,500 to $11,700. These estimates come from school-based sampling in Ghana and Togo and a modified community-based approach in Mali.

[17] Copenhagen. op. cit.

These are all relatively high-cost environments. While community-based samples are expected to cost more than school-based samples, they are also expected to be the less common of the two.

Xenomonitoring costs will represent community based sampling and can be expected to vary according to the collection method used and the prevalence level assessed. Data available from the survey in Colombo[18] provides one estimate for collecting *Culex* in gravid traps. The initial surveys collected 5,000-7,000 mosquitoes per sample. Two person teams were able to service an average of 4 traps per day. If one takes the estimate from the example in section 6 above, 6,500 mosquitoes (260 pools) would allow the identification an increase of prevalence from 0.3% to 0.5% or more. PCR analysis of 260 pools at $6.50/pool would cost $1,690. There would be 65 team days needed at $40 per day for a total of $2,600. So the total for this xenomonitoring survey would be in the neighborhood of $4,290. Two person teams were also used by the VCRC. Although a figure of cost/team/day is not available, these highly experienced teams were able to service 7 traps per day in a semi-rural environment suggesting their costs would be less than those observed in Colombo.

Egypt has also conducted *Culex* xenomonitoring studies, using a sampling method in which collection sites were distributed throughout the villages concerned but with a large degree of personal choice involved in their specific placement. No positives were found in either the mosquitoes or in the resident human population confirming the absence of filariasis but shedding no light on the sensitivity of xenomonitoring compared with human monitoring in identifying infection. Egypt employed 3-person teams at an estimated cost of $110/team/day rather than the $40/team/day in Colombo. Assuming these teams also serviced 4 traps/team/day the personnel cost for the survey described above for Colombo would have been $7,150. The total cost would have been $8,840.

These preliminary data suggest that xenomonitoring for *Culex* at prevalence levels of about 0.3% is likely to be no more expensive than a TAS, and might well be less expensive. If one supposes that technological advances related to human diagnostics such as the CFA and antibody testing and related to mosquitoes (PCR) will substantially reduce costs in the medium and long term, the basic driver of costs will be for the field personnel doing the surveys. Variables which then come into play include sample sizes and geographic extent of the surveys (which relate to the number of surveys necessary).

[18] Laney. op.cit.

Xenomonitoring may present advantages in avoiding the need to sample people. Sampling school children requires obtaining permissions from the Ministry of Education, from the authorities of the individual schools and sometimes from the parents. Especially if the traps are placed outside of houses, permissions related to xenomonitoring have been easy to obtain. For now, both human and mosquito sampling remain approaches which need continued exploration and refinement.

7. Suggestions for future work

While some specific areas for needed work related to mosquito sampling are indicated below, they should be seen in the context of similar work which is needed to sample humans. With respect to humans, it is hoped that antibody detection can provide a more sensitive and earlier signal of infection or exposure than antigen detection. The question may then arise as to what the earliest age for sampling children might be and, if this is before school age, the difficulties of sampling them. It will be important to continue to monitor comparative costs as both human and mosquito sampling evolves.

7.1 Epidemiological studies

- Monitoring changes in prevalence. This work has just begun and needs to be continued. Absent better collection methods, this is now restricted to areas where *Culex* is the principal vector. Because more experience with monitoring is also needed for the TAS, both approaches should be applied together pre- and post-MDA in a variety of settings. Comparative costs should be monitored.
- Assessment of large geographic areas. As IU's, India uses districts which may contain a million or more residents. Neither the TAS nor xenomonitoring have been applied on such a large scale and, as previously mentioned, the larger the size of the area assessed, the higher the risk that the area will pass despite zones within it of sustainable transmission being missed or that an entire IU will be failed when transmission continues in only a circumscribed part. Research is needed to develop assessment strategies which are feasible in such large areas.
- Anthropophagic *Culex* as a surrogate for other vectors. Such studies should be relatively low-cost and should be supported.

- Assessment of transmission thresholds. Further refinement of the estimates of transmission threshold levels provided at the Copenhagen meeting[19] and further modified by Pedersen[20] is needed. This is not likely to be an easy task, however, as, aside from the difficulties of collection of *Aedes* and *Anopheles,* one can anticipate observing different thresholds in areas with differing transmission potentials. It may suffice, however, to identify a threshold which correlates with the prevalence threshold accepted for children of school entrant age as determined by the TAS. That having been said, it remains to be demonstrated that transmission thresholds provide better decision-making indices than those currently recommended based on human prevalence.

7.2 Collection methods

Without better collection methods, xenomonitoring faces limitations where *Anopheles* is the principle vector, and the added numbers of mosquitoes required for *Aedes* apart from the questions concerning how to obtain a representative sample, raise additional difficulties. But it can be hoped that the increased investments related to concerns about dengue and a host of other mosquito-borne diseases as well as the initiatives to eliminate malaria may lead to improvements in trapping.

7.3 Sampling methods

Sampling methodology, in contrast to collection from the sample site, does not appear to be a major issue for *Anopheles* or *Culex*, for which household-based location of traps, similar to the method described in the Annex, appears to work well enough. While alternatives can be considered, the major need is to apply this approach in different epidemiological situations and in areas of differing geographic size. Evidence is needed as to whether household sampling is also valid for *Aedes,* however. Up to the present, no realistic alternative to setting traps at sites chosen randomly with population proportional to size has been suggested, although 'purposive' sampling (for example simply choosing a limited number of outdoor sites such as markets or bingo games) would certainly simplify logistics and might, in selected

[19] Copenhagen. op.cit.
[20] Pedersen. op.cit.

localities, prove replicable, it is difficult to envisage doing this systematically over an entire implementation unit or group of units.

7.4 Laboratory support

The primary focus of this review has been on sampling. But the results of sampling are needed in a timely manner to guide decision making and, unless facilities are locally available, delays of months are being experienced in obtaining results. As noted above, this is likely to change as better methods are developed and the capacities within national programs expand. But the international community should be encouraged to provide support for bringing new technologies to commercial production at affordable prices and to support national capacities to utilize them as is being currently done so effectively by the Bill and Melinda Gates Foundation, USAID, and DIFID.

7 Conclusions

Although it is premature to recommend xenomonitoring to LF program managers for routine use as a decision making tool, it shows enough potential to warrant continued investments in research and development. For areas in which *Culex* is the principal vector, it may already serve as a useful complement to the TAS in post-MDA monitoring and using it with the TAS pre-stopping would add to its relevance post-MDA. For both the TAS and xenomonitoring, approaches for assessing areas larger than a single implementation unit are needed.

Generic protocol of mosquito sampling of Households Rev.1 Sept 2011

Introduction

Within an area being assessed, here called the Evaluation Unit, a two-staged sample will be taken. A selection will first be made of larger areas and, within each, households (HH's) (or compounds) will be selected. There should be at least 30 sites within an EU from which samples are collected. A minimum sample would consist of 30 of the larger areas with one HH selected from each. The larger areas may often be census enumeration areas (EA's) but could also be villages, local government areas, or other areas with clearly defined boundaries. In this example, the larger areas are EA's and there are 1,020 EA's within the EU.

While different numbers may be used in specific surveys, in this example 4,000 mosquitoes in pools of 20 are collected from the EU, a total of 200 pools. If a different household (HH) is used for each pool, this requires the selection of 200 HH's.

A decision is required on the number of separate EA's from which to select HH's. Although 30 is the minimum number, there are some advantages, especially when the item being sampled is not evenly distributed, to increasing this number (but at increased costs). In this example, 50 EA's are chosen taking 4 pools of mosquitoes from each for the total of 200 pools.

Select the EA's to be sampled from the EU

1. Determine the total number of separate EA's within the EU. Number them according to geographic proximity. In this case, assign each of the 1,020 EA's a number.
2. Determine a sampling interval by dividing the total number of EA's by the number of EA's to be sampled. In this example, the sampling interval will be 1,020/50 = 20.4 (retain one decimal place
in this calculation).
3. Select a random number with the same number of digits as in the sampling interval (3 digits in this case). Multiply the number by 0 .1. If this number exceeds the total number of the sampling interval, repeat the selection until the number is less than the interval. Round **up** to the nearest whole integer to select the first EA. Example 1: the random number is 124 = 12.4 = 13. The 13th EA is the first selected. Example 2: the random number is 436 = 43.6 = 44. This is too large. Choose another number.
4. Select the additional 49 EA's by repeatedly adding the sampling interval (20.4) to the random number selected (12.4) and round **up** the result each time to the nearest whole number. But for each addition use the decimal numbers, not the rounded numbers. So the second EA selected will be 12.4 + 20.4 = 32.8 or, rounding up, the 33th EA. And the third will be 32.8+20.4 = 53.2, or the 54th EA. If the sampling interval for the final EA exceeds the total number, continue counting from the beginning of the list to finish.

Choose the HH's within the selected EA's

5. Determine a sampling interval to select HH's within a given EA. First obtain the average number of HH's per EA in the EU. Suppose it is 137. Then determine the number of HH's in the 50 EA's selected = (137)(50) = 6,850. Divide this number by the number of HH sites at which collections will be made, in this example 200. The sampling interval in each EA is 6,850/200 = 34.25 = 34.3.
6. To select the HH's within a given EA number all HH's. Now repeat the steps in numbers 3 and 4 above with one exception: stop selecting whenever the sampling interval for the next HH exceeds the total number of HH's. This may result in more or fewer than 4 HH's selected in a given EA.