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REVIEWS

Review

The Current Status of Molecular Xenomonitoring for Lymphatic Filariasis and Onchocerciasis

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The capacity of vector insect surveillance to provide estimates of pathogen prevalence and transmission potential has long been recognized within the global communities tasked with eliminating lymphatic filariasis (LF), the underlying cause of elephantiasis and hydrocele, and onchocerciasis (river blindness). Initially restricted to the practice of dissection, the potential of vector monitoring has grown due to the advent of molecular methods capable of increasing the sensitivity and throughput of testing. However, despite such advancement, operational research gaps remain. If insufficiently addressed, these gaps will reduce the utility of molecular xenomonitoring (MX) for onchocerciasis as elimination efforts expand into Africa. Similarly, such shortcomings will limit the programmatic usefulness of MX for LF, resulting in this technique's significant underutilization.

Introduction to Molecular Xenomonitoring

Molecular xenomonitoring (**MX**) (see Glossary) is the collecting and testing of haematophagous insects for the presence of a pathogen's genetic material. For over 20 years, researchers in the field of filarial disease have recognized that MX, utilizing techniques such as the polymerase chain reaction (**PCR**), provides advantages to more rudimentary entomological techniques such as the microscopic observation of dissected insects [1–9]. These advantages stem largely from the superior throughput of testing, the enhanced sensitivity of detection, and the facilitated species-level identification of parasites which MX methodologies enable. However, historically, despite these advantages, adapting MX for widespread programmatic use has presented a series of challenges that have hampered implementation efforts. Through the development of reliable and efficient vector trapping strategies enabling the elimination of human landing catch (**HLC**)-based collections [10,11], the advent of PCR-based diagnostic procedures facilitating the pooling of hundreds of vector insects [12,13], and algorithmic tools capable of reliably estimating parasite prevalence rates based upon pooled sample testing [12,14,15], global onchocerciasis elimination efforts have succeeded in overcoming many of these complications. As a result, xenomonitoring is now a fundamental component of the World Health Organization's 'Guidelines for Stopping Mass Drug Administration and Verifying Elimination of Human Onchocerciasis' [16]. In sharp contrast, trapping-related challenges (particularly in *Anopheles*-vectored areas) [17–19] and limitations to the throughput of testing [20] have hampered MX in its programmatic use for the monitoring of lymphatic filariasis (**LF**). However, given the recognized potential of MX, efforts to demonstrate the feasibility and usefulness of its

Trends

Preliminary recommendations now exist for the programmatic use of molecular xenomonitoring for lymphatic filariasis in *Culex*-vectored regions.

Novel methodologies for molecular xenomonitoring, such as excreta/feces testing, have the potential to increase the feasibility of mosquito-based monitoring efforts for lymphatic filariasis.

The Esperanza Window Trap has enabled the collection of black flies for the molecular xenomonitoring of onchocerciasis in the Americas and Uganda without the use of human landing collectors.

The high-throughput screening of onchocerciasis vectors enables the practical use of molecular xenomonitoring as a component of onchocerciasis verification of elimination efforts.

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programmatic implementation are increasing [21–23]. Yet despite these efforts, MX for LF would greatly benefit from additional technical advances and procedural standardization, allowing for the maximization of its usefulness in a world with a shifting global landscape of infection.

In this review, we have aimed to pinpoint the specific strengths and weaknesses of MX as it pertains to both LF and onchocerciasis. Implementation efforts are discussed, and technological and operational research gaps are identified. Furthermore, prospects for the future are addressed.

Molecular Xenomonitoring and Entomological Surveillance for Lymphatic Filariasis Elimination

Following the 1986 discovery of the *Hha*I tandem repeat in the *Brugia malayi* parasite [24], diagnostic assays targeting noncoding repetitive sequences have become the standard for the nucleic acid-based detection of filarial pathogens (Figure 1) [2,20,25–29]. Yet, despite many studies demonstrating the capacity of such tools to detect parasite signal in DNA extracts obtained from both human and mosquito-derived samples, programmatic implementation of such assays for the MX of LF has been limited, and most testing efforts have centered upon the use of human-based serological techniques such as the immunochromatographic card test [30,31]. Consequently, by providing a standardized set of serology-based instructions for determining when mass drug administration (**MDA**) can be discontinued, the transmission assessment survey (**TAS**) has become an invaluable tool for global LF elimination efforts [32,33]. However, due to factors relating to test sensitivity and evaluation unit (**EU**) size, a growing body of evidence suggests that, while the TAS can inform MDA-related decision making processes, the current TAS guidelines by themselves may be insufficient for the purpose of confidently demonstrating transmission interruption [34,35]. Accordingly, as a noninvasive methodology for monitoring infection recrudescence in post-MDA settings, the potential of MX to complement the TAS is considerable [36]. To this end, largely due to the efficient collection of blood-fed *Culex* mosquitoes enabled by the CDC Gravid Trap, MX presents a viable TAS-complementing methodology in *Culex*-vectored regions, and MX-based efforts are expanding in such environments [37]. Using these traps, recent work in Sri Lanka has provided a sampling strategy with the demonstrated capacity to more sensitively detect *Wuchereria bancrofti* persistence at the level of the large EU [21]. Additional work in India, focused on defining household-based clustering strategies within EUs, has further refined sampling methods in regions where the predominant vector is *Culex* spp. [38]. Accordingly, for the first time, elimination programs now have a suggested methodology for the programmatic utilization of MX. While studies in additional *Culex*-vectored regions will be required to demonstrate the broad application of this approach, and additional work is needed to refine and support proposed mosquito infection and infectivity break points [39,40], these findings represent a critical step forward. Similarly, recent work in American Samoa equating serological data with filarial persistence in both *Aedes* mosquitoes and the captured mosquito population as a whole, has demonstrated the capacity to utilize MX as a means of predicting the presence of seropositive individuals at the level of the village unit [22]. Conducted in a region having successfully completed two TAS assessments, this work demonstrates the potential capability of MX to identify hotspots in post-MDA settings. As these studies and others continue to correlate parasite levels within the mosquito population with corresponding levels in the human population, protocols for the standardization of use will improve. Accordingly, the practicality of implementing MX as a programmatic tool will begin to expand. After many years largely relegated to use as a research tool for purposes of prevalence mapping [41–43], intervention-surveillance [34,44–48], and vector incrimination [49,50], such expansion of the possible applications for mosquito monitoring will augment global efforts as elimination programs continue to progress.

Glossary

ATP: annual transmission potential (ATP) is an estimate of the intensity of infection transmission. With respect to vector-borne diseases of humans, it measures the number of infectious staged larvae annually transmitted by the vector host to the human host.

EU: an evaluation unit (EU) is the study area in which a transmission assessment survey (TAS) is conducted. An EU should be systematically selected such that the information obtained through its testing is sufficient to allow for programmatic decision making.

EWT: the Esperanza Window Trap (EWT) is a simple, inexpensive trap for the collecting of onchocerciasis vectors in the Americas. This trap design has not yet been sufficiently validated for programmatic use in African onchocerciasis elimination efforts.

GPELF: the Global Programme to Eliminate Lymphatic Filariasis (GPELF) is a World Health Organization-launched effort with the goal of eliminating lymphatic filariasis as a public health problem.

HLC: human landing catch (HLC) is the practice of using humans as bait for the collection of haematophagous insects. Upon landing in search of a blood meal, insects are collected, generally though the use of an aspirator.

LF: lymphatic filariasis (LF) is a neglected tropical disease resulting from infection by one (or more) of the parasitic nematodes *Wuchereria bancrofti*, *Brugia malayi*, and *Brugia timori*.

MDA: mass drug administration (MDA) is the systematic distribution of chemotherapeutics to a population without knowledge of the individual infection status of each person within that population. MDA is intended to decrease infection or prevent the spread of infection within a population as a whole.

MX: molecular xenomonitoring (MX) is the molecular-based testing of haematophagous insects for the presence of pathogen genetic material. Typical MX methodologies involve extraction of genetic material from insect samples, followed by the amplification and detection of pathogen DNA or RNA to verify the presence of pathogen within the sample.