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**Daniel Geleta**

Tropical and Infectious Diseases,  
Jimma University, Jimma,  
Ethiopia

**Delenesaw Yewhalaw**

Professor of Medical  
Entomology, Jimma University,  
Jimma, Ethiopia

## Molecular xenomonitoring for surveillance of mosquito-borne diseases

**Daniel Geleta and Delenesaw Yewhalaw**

### Abstract

Mosquito-borne diseases spread through the bite of infected mosquito and existed with human creating direct and indirect unhealthiness to over 40% of the world populations. For each disease caused by mosquito, appropriate diagnostic methods are recently available and recognized as a key component in clinical intervention and community level disease mentoring activities.

One of the diagnostic methods, molecular xenomonitoring predicts the infected mosquitoes and serves as a proxy for human infection in surveillance. The proxy further helps to spot the impact of mass treatment under a condition where mass-drug administration is considered as a disease control & elimination strategies and to verify elimination of neglected mosquito-borne diseases.

Scientists are progressively exploring new ways to predict human infection with mosquito-borne diseases, such as molecular xenomonitoring. However, concurrent to its benefits, the application of this technique perplexes some researchers, and has limited its widespread use in several programs. Researchers hope that it will continue to thrive.

**Keywords:** Molecular xenomonitoring, surveillance, mosquito-borne diseases

### Introduction

Definitions, causes and epidemiology of mosquito-borne diseases Mosquito-borne diseases are diseases that transmit through the bite of infected mosquitoes to a person or an animal when they feed on blood. During their feed the mosquitoes ingest blood with any viruses or parasites that live in the blood. These ingested viruses and parasites will further be transmitted through saliva to the next person or animal that gets bitten by the mosquitoes<sup>[1, 2]</sup>. Mosquitoes and the pathogens they carry are existed much longer on the earth than the present generations of humans with troublesome, hazardous and abundance characteristics in most of the world's temperate and tropical regions. For these peculiar characteristics, mosquito-borne diseases are pretty important to public health, and their occurrence depends on the interaction of several factors like biological, ecological, social and economic factors<sup>[3]</sup>. The interactions of the factors then lease the number of people affected by mosquito-borne diseases prevailing a rapidly growing global health problem that threatens more than 40% of the populations globally. According to reports from several countries, the direct or indirect impacts of mosquitoes were accounted more than any other pest. Recently they suffer nearly 700 million people each year and cause more than a million deaths worldwide<sup>[1, 4]</sup>.

Mosquito-borne diseases didn't only affect the human population; they also spread various diseases and parasites to friendly animals. These groups of diseases include dog heartworm, West Nile virus and Eastern equine encephalitis. Mosquito species can be divided into subgroups in respective of different aspects. Center for disease control (CDC) classify them according to the habitats where the larvae generally develop, whereas, other scholars divide them based on the pathogen group they give raise to. The later sub-groups of mosquito-borne diseases hereby include protozoa (malaria, filariasis) and viral diseases (Dengue, Encephalitis, West Nile virus, Chikungunya, Yellow fever and zika)<sup>[5]</sup>. Many of these diseases, including dengue, zika, chikungunya, and yellow fever virus are spread by Africa's *Aedes aegypti* mosquito that initially originated in Africa and spread to tropical and subtropical regions around the world between the 15<sup>th</sup> and 19<sup>th</sup> centuries. In recent years, population growth, the displacement of people from rural areas to cities, increased international travel, and climate change have all increased the spread of *Aedes aegypti* mosquito. According to the World Health Organization and other public health experts, dengue fever is the most common viral disease in the world caused by mosquitoes and is the fastest spreading disease in the world in the last 50 years. Some scholars call it the silent killer and the universal threat<sup>[1, 6-8]</sup>.

**Corresponding Author:**

**Daniel Geleta**

Tropical and Infectious Diseases,  
Jimma University, Jimma,  
Ethiopia

## Diagnosis and novel control strategies of mosquito-borne diseases

For various mosquito-borne diseases, various diagnostic methods are available, as shown in Table 1, which depend on the need for an accurate diagnosis for appropriate clinical intervention as well as community prevention and control. The best way to prevent and control mosquito-borne diseases is to ward off mosquitoes by repellents, cover exposed skin, keep mosquitoes at outdoors, draining pooled waters, control mosquitoes and pay attention to mosquito-borne diseases in different countries [1, 7-9]. In the fighting process of mosquitoes and mosquito-borne diseases today, a novel bio-control approach, particularly maternally inherited endosymbiotic *Wolbachia* is dominating the stage. *Wolbachia*, a genus of intracellular bacteria, will be trans-infected into mosquitoes to limit or interfere with pathogen transmission capacity of the vector. *Wolbachia* is a highly common bacterium that occurs naturally in 60% of insect species and is safe for humans and the environment when used for molecular modification of mosquitoes. In so far experience, a trans-infected *Wolbachia* strains in *Aedes aegypti* mosquitoes directly inhibit pathogen replication and also affect mosquito reproduction to allow *Wolbachia* to spread through mosquito populations. An independent risk analysis indicated that the spread of mosquitoes infected with *Wolbachia* pose a negligible or no risk to humans and the environment because of the reduced

pathogen in the mosquito or the mosquito become non-pathogen to human [5, 10, 11]. Similarly, surveillance and surveillance-driven mosquito-borne disease control methods are a similar control strategy across the entire landscape, so both control programs are conducted for a lifetime. This process served as important entomological parameters for repeatedly collecting and assessing the occurrence of mosquito species (the presence or absence of a species supporting the growth of a particular pathogen) and density. Occurrences are especially useful measure in the case of invasive species, which, by their occurrences, may be sufficient to guarantee the initiation of a vector control response. On the other hand, population density in a given area and its abundance relative to other species in a given area determine the level of risk and which species should be the prime targets for vector control [12, 13].

Generally, prevention and control strategies for mosquito-borne diseases are complex and difficult platform. Moreover, their recent connection with biotechnological like *Wolbachia*, mosquito repellents, genetic modification, fungi, protozoan control, control of aquatic stages using elephant mosquito and fish predators has worsen the situation. Within the worsen situations, the prevention and control strategies of mosquito-borne diseases have continued successfully, but have not achieved its expected maximum age of progress [14].

**Table 1:** Summary of some transmitters, members, causative agents and diagnostic methods of mosquito-borne diseases.

Mosquito species	Diseases caused	Type of pathogen	Suggested diagnostic methods
<i>Aedes</i>	Chikungunya	RNA virus	Serological tests, including ELISA to confirm IgM and IgG anti-chikungunya antibodies [10]
	Dengue	Flavivirus with four distinct serotypes: DEN 1-4	Viral isolation and serotype identification, nucleic acid detection, and antigen detection (within 6 days), IgM Antibody Capture if 7 days or above. Plaque reduction neutralization test for special groups like pregnancy [9]
	Lymphatic filariasis Rift Valley fever	Parasite	Membrane filtration, Ultrasonography, Immunochromatographic test or DNA probe's
		Virus	Serological assays (OM, ItiG), Genomic amplification assays (Reverse-Transcriptase-Polymerase Chain Reaction (RT-PCR)) [6]
	Yellow Fever in	Arbovirus of Flavivirus genus	Differ based on the stage of infection. In early stages, PCR may be suitable to detect the virus in
	Zika	Flavivirus	Blood or urine. In later stages, ELISA is used to identify anti-YFV IgM antibodies, or the PRNT test is used to detect neutralizing antibodies directed toward yellow fever virus [9].
<i>Anopheles</i>	Malaria	Protozoa of the genus	Nucleic acid testing (NAT) is used for the detection of viral RNA or DNA (within 7 days Duration) and Serological methods for IgM detection after 7 days [10]
		Plasmodium parasite (malariae, ovale, vivax and falciparum)	Giemsa stained blood smears.
<i>Culex</i>	Japanese encephalitis West Nile fever	Virus	Fluorescent microscopy and acridine-orange staining or rapid diagnostic tests (RDTs) (parasite identification) Serological methods (past exposure) [11]
		Flavivirus	Detection of JEV-specific IgM antibody in cerebrospinal fluid (CSF) or serum [17] ELISA to determine IgG antibody seroconversion, IgM antibody capture ELISA, cerebrospinal fluid IgM [9]

## Surveillance activities in vector-borne diseases

The efforts of public health agency to control and monitor mosquitoes and to maintain population health are critical in preventing mosquito-borne diseases and protecting public health. To attain this, the ongoing systematic collection, analysis, interpretation, and dissemination of mosquito-borne

diseases data contributes a paramount role in assessments of threats, risks and trends for prompt and appropriate public health action against these disease. However, the type of surveillance activity we conduct could be indicator-based (traditional & structured way of reporting diseases to public health officials) or event-based (looks at reports, stories,

rumors, and other information about health events that could be a serious risk to public health and hence non-standardized or subjective) depending on the specific diseases under attention [18–20].

Nowadays, laboratory tests are a key component of many surveillance systems and are essential in order to establish patterns of progression of infectious diseases. They provide gears for epidemiologists and entomologists to move beyond risk factor epidemiology and gain insight into the overall disease system. To this end, molecular-level laboratory-based surveillance helps to understand the transmission system, including the incidence, prevalence, and transmission probability, duration of carriage, effective dose, and probability of effective contact. Among these many molecular-level techniques that exist today, molecular xenomonitoring has awarded the efforts of researchers and scientists against monitoring and control of mosquito-borne diseases [21, 22].

### **Molecular xenomonitoring and its importance in mosquito-borne diseases**

Molecular Xenomonitoring (MX) is a disease surveillance technique that involves the collection and testing of hematophagous insects including mosquitoes to detect the DNA or RNA of a pathogen or parasite of human or animal health importance. Literally, MX represents the process of collecting and testing of hematophagous insects for the presence of a pathogen's genetic material. For over 20 years, researchers in the field of neglected mosquito-borne disease have recognized that MX provided auspicious advantages to more rudimentary entomological techniques such as the microscopic observation of dissected insects. 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 [23].

Molecular xenomonitoring (MX) helps to monitor the parasite infection in the mosquito population, and sensitively determine the presence of parasite in the human population. Entomologists and Epidemiologists have premeditated MX as disease surveillance for long time in the past and recognized it as a non-invasive proxy for infection in the human or animal population. The scientific community also recognized that MX can allow infected mosquitoes to serve as a proxy for human infection in vector-borne disease surveillance. According to reports from previous studies, MX has been successfully used for the surveillance of neglected mosquito-borne diseases and is now a fundamental component of the World Health Organization's guidelines for stopping mass drug administration and verifying elimination neglected mosquito-borne diseases particularly the *lymphatic filariasis*. However, in some contexts MX serves as a 'supplement' to human surveillance and will not be considered as a 'replacement' because of limited understanding about the correlation between pathogen-infection rates in human and mosquito populations [22–26].

In some countries like India the MX used as alternative method for transmission assessment survey (TAS) to identify the impact of mass treatment and to decide on stopping mass treatment. During the survey, vector infection levels by MX were used to verify whether there were any infected mosquitoes. The technique has been applied in many studies and provisionally established mosquito infection threshold

level (0.25%) below which transmission could be considered interrupted. In regards to this, the post-validation MX survey in Togo has revealed the absence of infection in the *Anopheles gambiae* vectors of *lymphatic filariasis* (LF), confirming the results of TAS. Similarly, it showed continuation of LF transmission after Mass-drug administration (MDA) in Islands and helped to determine the landscape of human pathogens in western united states [24, 26, 27].

Based on findings from literature, therefore, MX could be a sensitive tool to confirm the interruption of LF transmission in areas considered at higher risk of recrudescence. This has been confirmed in countries like Bangladesh and Malaysia where entomological and laboratory capacity to perform MX is available. With similar pattern African countries including Egypt progress assessment toward elimination of LF has been conducted and concluded as MX is a powerful tool for monitoring the impact of MDA on filariasis endemicity and transmission [28, 29].

MX helped to bridge malaria landscape ecology and epidemiology. As to this MX of multi-drug resistant (MDR) falciparum and vivax malaria parasites in *Anopheles* vectors is proposed to monitor infection pockets in transmission control areas of forest and forest fringe-related malaria. MX (nucleic acid detection and differentiation of malaria parasites presence in mosquitoes) of MDR malaria parasites in *Anopheles* vectors can provide the proof of potential transmission and spread of MDR malaria parasites in any given malaria ecotope and ecotone [23].

Generally, the application of MX techniques has dramatically improved the measurement of disease and putative risk factors, increasing our ability to detect and track outbreaks, identify risk factors and detect new infectious agents. However, integration of these techniques into epidemiologic studies also poses new challenges in the design, conduct, analysis and dissemination [23, 28].

### **Confronting factors of molecular xenomonitoring**

Despite the attributed benefits in surveillance advancement, adopting MX for widespread use in programs presents a number of challenges that limit the implementation process. The development of molecular MX protocols, especially the integration of pre- and post-inspection monitoring, lack of technical expertise and ethical issues are some of the common challenges encountered during the implementation of MX. The variables that affect pathogen detection, such as the time between mosquito blood collection and mosquito treatment, the stability of pathogenic nucleic acids in the gut of the mosquito, and the burden of the pathogen in the mosquito are yet to be determined. The screening of field-collected mosquitoes for pathogens has been intensified in Europe, but findings usually remained unclear regarding whether the pathogens had just been ingested during blood-feeding or had really been able to infect their insect host and continue their developmental cycle in order to be transmitted during the next blood meal [24, 30, 31].

### **Fates of molecular xenomonitoring**

While it brings many advantages of a real-time estimate of disease, the application of MX Perplexes some researchers. However, its non-invasiveness and sensitivity for assessing the presence of some neglected mosquito-borne diseases gleamingly attracted the world health organization, hereby

included in operational guidelines of diseases monitoring and control programs. It also attracts the attention of academic institutions, therefore, believed to flourish in regards to financial support, political will, and related skill [5, 21].

### Recommendation

Molecular xenomonitoring necessitates highly sophisticated technology and expertise. Extraction of genetic material from insect samples, followed by amplification and detection of pathogen DNA or RNA to confirm the presence of pathogen within the sample, is one of the techniques used. In addition to sophisticated technology and skill, the methodology may require an existing cost to implement. However, for the methodology to have an effective impact on the control and elimination of some debilitating diseases, academic institutions and volunteer organizations should adopt it globally. Particularly, areas with mosquito-borne neglected tropical diseases must adapt the methodology in order to create a useful global public health tool.

### Conclusion

Mosquito-borne diseases are blood suckers that spread through the feeding habits of mosquito. They are not limited to human being only but also are responsible to affect other organisms. They are commonly of viruses or parasites group having a specific causative agent. Though the diagnosis of some of these diseases can be established clinically, laboratory tests are the fundamental requisites regardless of prevention, control or treatment purposes.

Scientists progressively explore the novel prevention and control strategies for mosquito-borne diseases. However, the suggested strategies are not in good progresses due to several factors including poor laboratory facilities, human skill and disparate distribution and spread of the mosquito-borne diseases across the regions of the world demanding variable approaches for each disease at respective region. Accordingly, molecular xenomonitoring was recently recognized as a method to detect the DNA or RNA of a pathogen in mosquito that help for surveillance. In the process of surveillance, molecular xenomonitoring confirmed to show the presence of pathogens in mosquito, the infectivity of mosquito, and transmission capacity of the mosquito or transmission assessment. Finally, the method helps to monitoring drug stoppage especially for neglected mosquito-borne diseases where mass-drug administration is an indication for elimination strategy.

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