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Public Health Open Access
ISSN: 2578-5001

Detection of *Wuchereria bancrofti* through Molecular Xenomonitoring of Putative Mosquito Vectors in the Philippines

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Research Article

Volume 8 Issue 1

Received Date: February 12, 2024

Published Date: March 14, 2024

DOI: 10.23880/phoa-16000279

Abstract

The advancement of molecular methods resulted in the application of molecular xenomonitoring (MX) as a complementary tool to detect the presence of circulating LF parasites in mosquito vectors. In the Philippines, a number of endemic areas are still undergoing evaluations by the World Health Organization (WHO) and Department of Health – Philippines (DOH). This study investigated the presence of filarial parasites in Oriental Mindoro (Region 4B) and Sultan Kudarat (Region 12), Philippines through MX. All collected and identified mosquito vectors were subjected to a real-time PCR assay targeting the *Wuchereria bancrofti* long-dispersed repeat *Wb-LDR* gene. This study detected the presence of *W. bancrofti* parasites in two species of mosquito vectors: (1) *Aedes poicilius* collected in Barangay Sta. Clara, Kalamansig, Sultan Kudarat (n=10 pools; 10.00%); and (2) *Culex quinquefasciatus* from Barangay Poblacion II, Victoria, Oriental Mindoro (n=344 pools; 0.29%) and Barangay Sta. Maria, Kalamansig, Sultan Kudarat (n=96 pools; 1.04%). These results support transmission assessment survey findings of continuous presence of LF parasites in the two endemic provinces of Oriental Mindoro and Sultan Kudarat. This study also suggests that further refinement of MX may produce broader applicability in the control and elimination of mosquito-borne diseases.

Keywords: Lymphatic Filariasis; Molecular Xenomonitoring; Philippines; *Aedes poicilius*; *Culex quinquefasciatus*; *Wuchereria bancrofti*

Abbreviations: MX: Molecular Xenomonitoring; WHO: World Health Organization; DOH: Department of Health – Philippines; LF: Lymphatic Filariasis; GPELF: Global Programme to Eliminate Lymphatic Filariasis; MDA: Mass Drug Administration; DEC: Diethylcarbamazine; MMDP: Morbidity Management And Disability Prevention; TAS: Transmission Assessment Survey; NFEP: National Filariasis Elimination Program; PSA: Philippine Statistics Authority; LDR: Long DNA Repeat; mct: Microcentrifuge Tube.

Introduction

Lymphatic filariasis (LF) is a major public health burden in which approximately 863 million people in 47 countries are still suffering from this neglected tropical disease [1]. LF is a parasitic disease caused by three species of thread-like nematodes: *Wuchereria bancrofti, Brugia malayi,* and *Brugia timori.* Bancroftian filariasis is responsible for 90% of infections, while the remaining 10% is attributed to brugian filariasis. These parasites spread from person to person

through mosquito bites. Mosquitoes belonging to the genera *Aedes, Anopheles, Culex, Mansonia,* and *Ochlerotatus* are incriminated as filaria vectors [2,3]. LF infection is acquired via repeated bites of infected mosquitoes for a duration of months or years, yet chronic conditions appear several years later such as lymphoedema, abnormal enlargement of body parts, and hydrocoele in men. Infected individuals experience severe pain and permanent physical disability, as well as become victims of social stigma and poverty [1].

In 2000, the World Health Organization (WHO) launched the Global Programme to Eliminate Lymphatic Filariasis (GPELF) as a response to end the escalating LF cases worldwide. GPELF has two key strategies: (1) to interrupt parasite transmission by providing mass drug administration (MDA) in endemic areas; and (2) to prevent disease progression and alleviate suffering of infected individuals through morbidity management and disability prevention (MMDP) [1,3]. Annual MDA, using a single dose of albendazole in combination with either diethylcarbamazine (DEC) or ivermectin for a span of at least five years, was the primary strategy anticipated to reduce density of circulating filaria parasites below the transmission threshold. Monitoring the success of MDA requires implementation of a surveillance program to confirm the interruption or reestablishment of disease transmission during the post-intervention period [2,4].

The transmission assessment survey (TAS) was recommended by WHO as a standard methodology for the confirmation of LF elimination in endemic areas. With roughly 24 months apart in implementation, there are three TAS, known as TAS-1, TAS-2, and TAS-3, to be conducted. TAS evaluates if the prevalence of microfilaria in the blood or circulating filarial antigen in infected human populations attained a certain level in which transmission can no longer be sustained, which, in turn, signals when MDA can be stopped. However, there are evidences suggesting inconsistencies in the current TAS guidelines, particularly test sensitivity, parasite level changes in human populations, and size of evaluation units. TAS alone is insufficient in carrying out MDA-related decisions [5-7].

A non-invasive surveillance tool, known as xenomonitoring, was developed to complement TAS. Xenomonitoring indirectly tracks the progress of LF elimination programs by utilizing infections in wild-caught mosquito vectors to diagnose if disease transmission is presently occurring in humans [2,7,8]. Dissection is the inexpensive, gold standard method for filaria parasite detection in mosquitoes, however, efficiency and sensitivity decline when prevalence is below 1%. In large-scale epidemiological surveys, mosquito dissection becomes more costly, labor-intensive, and time-consuming [2]. These limitations led to the development of molecular xenomonitoring (MX) which uses PCR techniques to detect filarial DNA in mosquito vectors [6,8]. MX has been extensively utilized for years due to its superior testing throughput, high sensitivity and specificity of detection, and species-level parasite identification [5]. Despite these advantages, LF MX has not been globally integrated as a routine monitoring tool for post-MDA surveillance [6,8]. The implementation of MX as an additional programmatic strategy will strengthen and standardize the current protocol of LF elimination programs [5].

In the Philippines, LF is caused by *W. bancrofti* and *B. malayi* parasites endemic in 46 out of 81 provinces in the country [9]. The National Filariasis Elimination Program (NFEP), established by the Department of Health, aims to eliminate LF as a public health problem in the Philippines by utilizing a comprehensive approach and providing universal access to quality health services. About 76% of cases are also evident in poorest municipalities in the country [10]. NFEP initially encountered setbacks particularly in formulating effective MDA coverage plans and in sustaining MMDP activities. By 2015, there were significant improvements in logistic chain management, MDA implementation, and monitoring and surveillance. Regrettably, there are remaining provinces which need to accomplish and pass the evaluations conducted by WHO. Endemic areas declared LF-free are also at risk of potential disease resurgence [9].

Oriental Mindoro was declared LF-free last 2013 after successful results from TAS-1. However, the province failed TAS-2 in 2015 which prompted recommendation from WHO to conduct TAS strengthening by implementing two more rounds of MDA (2017-2018). Sultan Kudarat has been implementing MDA from 2011 to 2017, but high burden municipalities in the province are still not able to reach the threshold level (<1%). Sultan Kudarat will continue with MDA until the threshold is achieved, followed by the initiation of TAS-1.

As evidence to support TAS, this study was conducted to detect the presence of filarial parasites in mosquitoes from two endemic provinces, Oriental Mindoro (Region 4B) and Sultan Kudarat (Region 12), in the Philippines through molecular xenomonitoring.

Materials and Methods

Study Area and Household Selection

This study was conducted in 30 barangays from 12 municipalities and one city of Oriental Mindoro (Region 4B) and nine barangays located in three municipalities of Sultan Kudarat (Region 12), Philippines. The selection of the study area was based on previously conducted TAS in humans in the

said provinces. Identified sentinel or spot-check barangays were prioritized in accordance with the LF data provided by the regional entomologists.

Collection Date	Province	Municipality	Barangay	No. of households
April to May 2018	Oriental Mindoro	Puerto Galera	Villaflor	11
		Baco	Water	6
		City of Calapan	Santa Rita (Bungahan)	7
			Ilaya (Pob.)	16
			Wawa	5
			Bayanan II	11
		Naujan	Mulawin	6
			San Jose	2
			San Agustin I	6
			Gamao	4
			Mabini	2
			Pinagsabangan II	6
		Victoria	Bambanin	12
			Poblacion II	3
		Socorro	Malugay	3
			Mabuhay II	7
			Fortuna	12
		Pola	Tiguihan	3
		Pinamalayan	Wawa	16
		Gloria	Santa Maria	8
			Manguyang	7
			Banutan	3
		Bongabong	Morente	7
			Anilao	13
			San Jose	4
		Roxas	Happy Valley	5
			San Rafael	7
		Mansalay	Poblacion	18
		Bulalacao (San Pedro)	Milagrosa (Guiob)	9
			Balatasan	9
			Total	228
July to August 2018	Sultan Kudarat	Lebak	Pasandalan	8
			Salaman	13
			Bolebak	6
		Kalamansig	Sta. Clara	14
			Sta. Maria	7
		Palimbang	Badiangon	8
			Dumolol	4
			Kisek	5
			Namat Masla	7
			Total	72

Table 1: Selected sampling sites for Oriental Mindoro and Sultan Kudarat, Philippines.

Oriental Mindoro is part of the MIMAROPA Region, which comprises the islands of Mindoro, Marinduque, Romblon, and Palawan. Situated 45 kilometers south of Batangas, Oriental Mindoro occupies the eastern section of Mindoro Island and has a total land area of 4,238.38 sq. km. The western side of the province is mountainous or rugged, whereas the eastern side consists of hills and flood plains [11]. According to Philippine Statistics Authority (PSA), the total population of Oriental Mindoro is 908,339 as of May 2020 [12]. Oriental Mindoro is considered as largely rural. Approximately 70% of the total population is engaged in agriculture and fishing, while 30% are living in urban centers. Major industries include rice, vegetables, coconut, high value commercial crops, fisheries, and livestock production [11].

Sultan Kudarat is part of the SOCCSKSARGEN Region, which comprises the provinces of South Cotabato, Cotabato City, Cotabato Province, Sultan Kudarat, Sarangani, and General Santos City. Sultan Kudarat occupies the southwestern part of Mindanao and has a total land area of 5,135.3 sq. km. On the eastern side, there are two major mountain ranges in the province which are Alip Mountain Range in the town of Columbio, and Daguma Mountain Range found in the towns of Bagumbayan, Isulan, and Esperanza. There are also coastal towns (Kalamansig, Lebak, and Palimbang) on the western side which are directly facing Australia, Brunei, Indonesia, and Malaysia [13]. According to Philippine Statistics Authority (PSA), the total population of Sultan Kudarat is 854,052 as of May 2020 [14]. Sultan Kudarat is predominantly agricultural in which majority of the crops produced are rice, corn, banana, coconut, coffee, durian, mango, and African palm. Fishing is the source of income for the three coastal towns in the province [13].

For this study, selected households per barangay were considered as mosquito trapping sites. A 1:1 ratio of BGS trap to household sampling site was followed. Table 1 shows the list of selected barangays from each province and the corresponding number of selected households for this study (Table 1).

Households were prioritized according to: (1) having known LF case/s, either previously or currently; (2) availability of 24-hr electricity for the BGS traps to operate; and (3) accessibility and safety of the involved field staff. Households that satisfied the first criterion were recommended by barangay health workers who assisted our team. Randomization was conducted to select the remaining number of households per barangay. At least one BGS trap per sitio (a smaller unit or area within the barangay) was allocated to ensure wider coverage of collection.

Adult Mosquito Collection and Identification

One-time collection of adult mosquitoes was carried out in each study site from April to August 2018. For Oriental Mindoro, the head of each household was asked if they agree with the installation of the trap in their household and usage of their electricity as power source of the trap. An agreement letter was signed as proof of their permission for the use of their electricity. However, for Sultan Kudarat, a 12-volt rechargeable battery (BioQuip, California, USA) was utilized as power source of each trap due to inaccessibility of electricity. Batteries were replaced daily to ensure that the traps were functional and operational for the duration of the collection period.

Each BGS trap was assembled and installed outside of the selected household (≤20 m apart) to account for the biting behavior (exophagy) of target *Ae. poicilius*. The trap was placed in an undisturbed area away from animals, children, and ants which might feed on the collected mosquitoes [15]. The trap, baited with BG-Lure (a blend of lactic acid, ammonia, and caproic acid) (BioGents, Regensburg, Germany), was left for three consecutive days. Mosquitoes were collected daily in a catch bag labeled according to municipality, barangay, and household number. GPS coordinates were also recorded for future reference and pictures were taken to document the activity.

Daily collection of mosquitoes in each trap was accomplished by barangay health workers. These mosquito collections were brought to the assigned field team for further processing. Upon receiving of the samples, all collected mosquitoes were killed by freezing (temperature of at least -4^0C to -20^0C) for an hour or until mosquitoes were completely dead. Mosquito samples were immediately identified after each collection using Nikon SMZ 445 stereomicroscope (Nikon Corp., Tokyo, Japan) following published illustrated keys of mosquitoes [16-19].

Collected *Ae. poicilius* mosquitoes were stored using the dry/ desiccation method. Pools of *Ae. poicilius* (n≤15 mosquitoes) were prepared and placed in a properly labeled microcentrifuge tube (mct) with silica gel and filter paper. Each pool was distinguished by: (1) household/ trap site; (2) date of collection; and (3) sex of the mosquitoes. Then, mcts were placed inside a cryogenic box or Ziploc bag. Other collected mosquitoes were stored in a three-part plastic petri dish with silica gel and paper towel. Each part was designated and labeled for each day and date of collection. All petri dishes were wrapped with masking tape to secure the mosquitoes from ants. These post-collection tasks were repeatedly done for three consecutive days.

Proper storage and transport of mosquitoes were followed. Validation of species-level identification and subsequent molecular assay for detection of the presence of filarial parasites in primary and secondary mosquito vectors were conducted at RITM Medical Entomology Laboratory. Other mosquito species collected were identified and kept for possible future use.

Extraction of DNA From Mosquito Vectors

Mosquito samples validated as primary and secondary filaria vectors were prepared for nucleic acid extraction. Only the head and thorax from each mosquito were pooled (n≤15) and properly coded based on area of collection and species identification. Filarial DNA was extracted from the pooled mosquito vectors based on the commercially-available DNeasy Blood and Tissue kit (QIAGEN Catalog No. 69506, Dusseldorf, Germany) protocol with few modifications. These modifications include: (1) addition of 180 µL Buffer ATL to each pooled mct with mosquito heads and thoraxes; (2) mechanical homogenization using sterile, single-use blue polypropylene pestle and handheld motorized pellet pestle; and (3) elution with 100 µL Buffer AE. Pure, high quality extracted DNA can be immediately used for PCR assays or stored at -20 to -70ºC.

Real-Time PCR Detection of *Wuchereria bancrofti*

The real-time PCR assay described by Rao, et al. (2006) was validated and utilized in this study [20]. This assay was specifically designed to target the "long DNA repeat" (LDR) of *W. bancrofti*. A standard 25 µL PCR reaction mixture was prepared from the following reagents: (1) TaqMan qPCR Master Mix (Applied Biosystems Catalog No. 4304437, Massachusetts, USA), (2) *Wb-LDR1* forward (5' – ATT TTG ATC ATC TGG GAA CGT TAA TA – 3') and *Wb-LDR2* reverse (5' – CGA CTG TCT AAT CCA TTC AGA GTG A – 3') primers, (3) *Wb-LDR* Taqman probe (6FAM – ATC TGC CCA TAG AAA TAA CTA CGG TGG ATC TCT G – TAMRA), and (4) nuclease-free water. The thermal cycling parameters used for the amplification of *Wb-LDR* gene involved: (1) initialization at 50^0C for two minutes, (2) initial denaturation at 95^0C for 10 minutes, and (3) 40 cycles of denaturation at 95^0C for 15 seconds and annealing at 60^0C for one minute. Mosquito samples were amplified using CFX96 Touch Deep Well (Bio-Rad, California, USA) and Applied Biosystems 7500 Fast (Thermo Fisher Scientific, Massachusetts, USA) real-time thermal cyclers. Both positive control and no template control (NTC) were added in each PCR run.

Data Analysis

GPS coordinates of all selected households in each province were obtained using cellular phones. For real-time PCR detection, the validity of each completed PCR run was verified by determining the presence of amplification in the positive control/s and absence of amplification in the NTC. Baseline threshold was also adjusted accordingly. Positive mosquito samples were identified by the presence of amplification curve and Cq value of ≤35.99. Any sample with Cq value of ≥36 was confirmed by repeating the PCR run. After the repeat run, if the Cq value is ≤35.99, the sample is considered positive; however, if the Cq value is ≥36, the sample is considered negative.

Results

Collection of Filaria Mosquito Vectors

Adult mosquito collection was successfully performed from April to August 2018 in all 30 barangays in Oriental Mindoro (Region 4B) and nine barangays in Sultan Kudarat (Region 12), Philippines through the support from barangay health workers and other local officials (barangay captains, kagawad, midwives, sanitary inspectors, etc.). Only 227 out of 228 BGS traps were installed in Oriental Mindoro because one BGS trap was defective during that time. All 72 BGS traps were successfully installed in Sultan Kudarat.

Province	Municipality	No. of female *Ae. poicilius*
Oriental Mindoro	Puerto Galera	1
	Naujan	4
	Victoria	4
	Socorro	12
	Total	21
Sultan Kudarat	Lebak	5
	Kalamansig	30
	Palimbang	1
	Total	36

Table 2: Municipalities in Oriental Mindoro and Sultan Kudarat with collected female *Ae. poicilius* mosquitoes.

A total of 15,238 mosquitoes were collected in both provinces. In Oriental Mindoro, 6,118 out of 11, 595 mosquitoes were female, but only 21 *Ae. poicilius* mosquitoes, the primary vector, were captured. In Sultan Kudarat, on the other hand, there were 1,893 females out of 3,643 mosquitoes, but only 36 female *Ae. poicilius* mosquitoes were obtained. Female *Ae. poicilius* mosquitoes were present in the following municipalities (Table 2).

Aside from the primary vector *Ae. poicilius*, secondary vectors of LF were also identified in the two provinces. Secondary vectors found in Oriental Mindoro include *Culex quinquefasciatus* and *Mansonia uniformis*, while in Sultan Kudarat, *Cx. quinquefasciatus*, *Anopheles flavirostris*, and *Ma. uniformis* were observed (Table 3).

Total no. of female mosquitoes collected		
Filaria mosquito vectors	**Oriental Mindoro**	**Sultan Kudarat**
Ae. poicilius	21	36
An. flavirostris	0	1
Cx. quinquefasciatus	5,119	1,422
Ma. uniformis	6	1
Other mosquito species	972	433
Total	6,118	1,893

Table 3: Number of female mosquitoes collected in Oriental Mindoro and Sultan Kudarat.

Detection of Filarial Parasites in Mosquito Vectors

All identified female *Ae. poicilius, An. flavirostris, Cx. quinquefasciatus*, and *Ma. uniformis* mosquitoes were extracted from July 2019 to September 2020. All DNA extracts were stored at -30ºC for subsequent real-time PCR assay. Molecular testing of all extracted samples was conducted last December 2020. A total of 470 mosquito pools were generated from the collected filaria vectors. Table 4 presents the total number of mosquito pools in the two provinces (Table 4).

Mosquito vectors	Total number of pools n ≤ 15 mosquitoes		Number of filaria (+) pools	
	Oriental Mindoro	**Sultan Kudarat**	**Oriental Mindoro**	**Sultan Kudarat**
Ae. poicilius	17	10	0	1
An. flavirostris	0	1	0	0
Cx. quinquefasciatus	344	96	1	1
Ma. uniformis	1	1	0	0

Table 4: Number of mosquito pools produced for each vector species.

Results revealed the presence of filarial parasites in *Ae. poicilius* and *Cx. quinquefasciatus* mosquitoes, but not detected in any of the *An. flavirostris* and *Ma. uniformis* mosquitoes. In Oriental Mindoro, one positive pool of *Cx. quinquefasciatus* (n=344 pools; 0.29%) was collected in Brgy. Poblacion II, Victoria. Three households near the Bucayao Silonay River were selected for sampling in this barangay. Conversely, in Sultan Kudarat, one positive pool of *Ae. poicilius* (n=10 pools; 10.00%) was found in Brgy. Sta. Clara, and another positive pool of *Cx. quinquefasciatus* (n=96 pools; 1.04%) was obtained in Brgy. Sta. Maria. Both of these positive pools are from Kalamansig. BGS traps were placed in 14 households in Sta. Clara, while only seven households in Sta. Maria (Table 5).

Province	Municipality	Barangay	Household No.	GPS coordinates	
				Lat (N/S)	Long (E/W)
Oriental Mindoro	Victoria	Poblacion II	001	13°6'17" N	121°10'22" E
			002	13°6'14" N	121°9'47" E
			003	13°6'16" N	121°9'49" E
Sultan Kudarat	Kalamansig	Sta. Clara	001	6°29'30" N	124°3'49" E
			002	6°29'44" N	124°3'49" E
			003	6°32'47" N	124°3'15" E
			004	6°32'20" N	124°3'49" E
			005	6°32'31" N	124°3'23" E
			006	6°29'30" N	124°4'15" E
			007	6°29'30" N	124°4'16" E
			008	6°29'26" N	124°4'12" E
			009	6°29'25" N	124°3'13" E
			010	6°29'30" N	124°3'49" E
			011	6°31'26" N	124°2'58" E
			012	6°31'24" N	124°2'56" E
			013	6°32'47" N	124°3'15" E
			014	6°31'58" N	124°2'14" E
		Sta. Maria	001	6°53'30" N	124°18'22" E
			002	6°34'53" N	124°3'9" E
			003	6°53'30" N	124°18'22" E
			004	6°34'49" N	124°3'11" E
			005	6°34'51" N	124°3'12" E
			006	6°39'12" N	124°3'18" E
			007	6°39'26" N	124°3'55" E

Table 5: GPS coordinates of households with collected primary and secondary filaria vectors.

Discussion

The significant vectors of *W. bancrofti* parasites are (1) culicines in most urban and semi-urban areas; (2) anophelines in more rural areas of Africa; and (3) aedines in the Pacific Islands [21]. In the Philippines, there are five incriminated mosquito vectors of LF. *W. bancrofti* is transmitted by *Ae. poicilius, Cx. quinquefasciatus,* and *An. flavirostris* mosquitoes. Contrarily, *Ma. uniformis* and *Ma. bonneae* mosquitoes are responsible for the transmission of another filarial parasite, *B. malayi* [9].

This study collected four filaria mosquito vectors including aedines, anophelines, culicines, and mansonioides. *W. bancrofti* was detected in *Ae. poicilius* in Brgy. Sta. Clara, Kalamansig, Sultan Kudarat even though there were only 14 households. *Cx. quinquefasciatus* was collected in all sampling sites for both provinces, but *W. bancrofti* was detected in just two barangays – Brgy. Poblacion II, Victoria, Oriental Mindoro and Brgy. Sta. Maria, Kalamansig, Sultan Kudarat.

Collected mosquito vectors were pooled prior to real-time PCR detection. This hindered the identification of specific households positive for filarial parasites. Nevertheless, a specific real-time PCR assay is capable of detecting LF parasites in pools of 50 to 100 mosquitoes even if ingestion of microfilaria-positive blood occurred for more than two weeks [22]. Detection of *W. bancrofti* in primary and secondary mosquito vectors in Oriental Mindoro and Sultan Kudarat could be indicative of ongoing LF transmission. As indirect indicators of LF infection, PCR-positive mosquitoes in the sampling sites revealed that LF-infected individuals are or were recently nearby [23]. The existence of filarial parasites in mosquitoes denotes microfilaria reservoir in

the local human population [24] since humans are the only recognized definitive host of *W. bancrofti* [25]. Furthermore, the use of heads and thoraxes of mosquitoes potentially improved the chances of detecting third-stage infective larvae (L3), although for this study, the real-time PCR protocol used only detected filarial DNA. Further studies should be conducted on the correlation of mosquito vectors and LF prevalence surveys in humans to solidify evidence of ongoing disease transmission. Molecular protocol specific for filarial L3 detection should be utilized to incriminate other probable mosquito vectors collected within the study sites.

BGS traps were placed near previously or currently known LF case/s and presumed mosquito breeding sites. Both mosquito breeding sites and flight range are important factors to be recognized when conducting vector surveillance. *Aedes* mosquitoes usually breed in clean, stagnant water-holding natural and/or artificial containers [21]. The primary filaria vector *Ae. poicilius* is known to oviposit in water-filled leaf axils of abaca (*Musa textilis*), banana (Saba variety, *Musa sapientum*), screwpine (*Pandanus*), and taro (*Colocasia esculenta*) plants which are found near human habitations [9,26]. Most of the barangays with collected *Ae. poicilius* were noted to have at least one of these plants which serve as breeding sites of these mosquitoes. On the contrary, *Cx. quinquefasciatus*, a secondary filaria vector in the Philippines, is ubiquitous in nature because they can breed in a wide range of bodies of water, particularly in polluted and stagnant waters. These include canals, cesspits, drains, marshy swamps, septic tanks, and empty containers or tree holes filled with rainwater [21]. *Cx. quinquefasciatus* increases in numbers during the rainy season, and in areas with open sewers, poor sanitation, and untreated waste waters [27]. The sampling sites in both provinces displayed the above-mentioned breeding sites which support the abundance of *Cx. quinquefasciatus* in the mosquito collections.

Typically, the maximum mosquito flight distance spans from 50 meters to 50 kilometers depending on the species. The most common type of mosquito dispersal is associated with shorter daily flights in relation to the mosquitoes' behavioral response for survival. This non-oriented dispersal is intentional and integral for their mating, host-seeking, biting, resting, and egg-laying behavior [28]. A study of Bockarie reported two contradicting scenarios: (1) filaria-positive mosquitoes were detected in households with microfilaria-negative residents; and (2) filaria-negative mosquitoes were detected in households with microfilaria-positive residents [24]. This may be connected to the flight capacity, availability of suitable oviposition sites, and host preference of mosquito vectors.

Ae. poicilius is acknowledged as an anthropophilic, night-biting species suggesting preference for human hosts over animals. Previous findings concluded approximately 70% human blood index for this species. People who have outdoor activities at night are more at risk of acquiring LF because *Ae. poicilius* also shows activeness during the first few hours of the night. Furthermore, *Ae. poicilius* has prominent rate of survival in nature and favorably permissive for development of third-stage infective larvae [29,30]. *Cx. quinquefasciatus* is also identified as night-biter which favors both avian and mammalian hosts [27]. *Cx. quinquefasciatus* was formerly characterized as a weak LF mosquito vector in the Philippines because the microfilaria experimentally fed to these mosquitoes showed comparatively poorer development than in *Ae. poicilius* [26]. *W. bancrofti,* the predominant filarial parasite in the Philippines, exclusively infects humans which indicates lack of other epidemiologically significant non-human host. Additionally, *W. bancrofti* exhibits nocturnally periodic form synchronous with its competent night-biting mosquito vectors *Ae. poicilius* and *Cx. quinquefasciatus* [31,32].

With reference to the GPELF launched by WHO, endemic countries were committed to eliminate LF as a public health problem by 2020. MDA was the main strategy which focused on limiting the number of circulating filarial parasites in endemic areas. Monitoring LF infection in human populations through TAS and in mosquito vectors using MX were undertaken after stopping MDA [2]. In TAS, blood from infected individuals is tested for the presence of microfilaria or anti-filarial IgG4 using microscopic or serologic techniques, respectively. Challenges were observed during the implementation of TAS. Individuals became reluctant to submit to regular blood collections over time. Assessment of infections in humans is deemed as a "lagging indicator" because of the prepatent period which could extend for months or years. The prepatent period, defined as the period from infection to manifestation of the parasite in the host's body, should be substantially considered [33]. Due to these concerns, assessment of infections in mosquito vectors provides a more favorable approach. The primary basis of MX is the capability of mosquito vectors to competently take up filarial parasites via ingestion of infected human blood. MX is non-invasive and only involves mosquitoes [6] which makes it ideal to use as indirect guide of the progress of LF control and elimination programs [2,8]. MX, being highly sensitive and specific, is an effective complement to human-based TAS in quantifying persistent low levels of LF infection in endemic areas which require further treatment with MDA and follow-up intervention [2,4,8]. A previous study in American Samoa mapped LF after MDA and reported that DNA of *W. bancrofti* was still detected in pools of mosquitoes using a highly sensitive real-time PCR assay. In Sri Lanka, a comprehensive LF assessment showed that pools of *Cx. quinquefasciatus* were positive for *W. bancrofti* six years after MDA was stopped [4]. Household-based MX surveys are beneficial in conjunction with TAS for programmatic decision-making of stopping MDA

and post-MDA surveillance as evident in another study in Sri Lanka in which MX identified more areas with low levels of transmission than TAS. This is relevant in endemic areas which passed TAS but the number of LF-positive individuals is almost at the critical threshold value [8].

Lau, et al. (2016) mentioned several disadvantages and flaws of MX. MX demands entomological expertise in mosquito collection and identification, proficiency in molecular techniques, and well-equipped molecular diagnostic laboratory. There are also lapses in sampling strategies, interpretation of MX into operational policies, and unclear guidelines regarding critical thresholds to indicate ongoing transmissions [23].

As of 2021, 44 out of 46 endemic provinces in the country were already declared as LF-free. Sultan Kudarat and Zamboanga del Norte were the remaining provinces with ongoing LF transmission. The NFEP is keen and ambitious in preventing resurgence of the disease in LF-free areas by tracking down and immediately administering treatment to infected individuals, as well as implementing vector control strategies to impede transmission [9]. The role of mosquito surveillance in evaluating ongoing disease transmission should essentially be considered. Detection of filarial parasites in mosquito vectors functions as marker of efficacy of MDA in LF elimination or prognosis of pending LF resurgence.

Succeeding research related to this study should improve the adult mosquito collection and real-time PCR detection of filarial parasites. Longer collection time with more sampling days and/ or repeated collection for several months may be conducted to obtain more diverse collection of mosquitoes. Dorkenoo, et al. (2018) proposed increasing the frequency of monthly mosquito collections and additional sampling sites to achieve an extensive sample size [34]. Different adult collection techniques may be evaluated to establish the most suitable trap for each targeted vector species. For the real-time PCR detection, collected mosquito vectors may be tested individually instead of pooling to easily identify the household/s positive for filarial parasites. Adoption of a duplex real-time PCR protocol targeting L3 of both *W. bancrofti* and *B. malayi* yields more comprehensive and extensive data for LF elimination. Vector abundance and disease transmission dynamics are constantly changing which may bring in new vector species [8,34]. Testing non-vector mosquito species may provide further evidence for vector incrimination (with L3 detection adopted).

Conclusion

This is the first study in the Philippines utilizing molecular xenomonitoring to detect the presence of *W. bancrofti* filarial DNA in mosquitoes. Four mosquito vector species were collected from the provinces of Oriental Mindoro (Region 4B) and Sultan Kudarat (Region 12) namely, *Ae. poicilius, Cx. quinquefasciatus, An. flavirostris,* and *Ma. uniformis. W. bancrofti* parasites were only detected in *Ae. poicilius* (Brgy. Sta. Clara, Kalamansig, Sultan Kudarat) and *Cx. quinquefasciatus* (Brgy. Poblacion II, Victoria, Oriental Mindoro and Brgy. Sta. Maria, Kalamansig, Sultan Kudarat) mosquitoes. This result supports TAS findings of ongoing LF transmission in the two endemic provinces. Further research regarding diverse aspects of this disease are needed to accurately characterize LF transmission in the Philippines.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgement

The authors would like to thank the following: (1) Ms. Katherine Gass, Ms. Julie Kenyon, and colleagues from Task Force for Global Health and Neglected Tropical Diseases Support Center (NTD-SC) for their valuable involvement and advice throughout this study; (2) Mr. Nils Pilotte, Ms. Jessica Grant, Ms. Susan Haynes, and researchers from Smith College who provided the protocols and positive control of *Wuchereria bancrofti* parasites; (3) Dr. Leda Hernandez from Department of Health - Philippines, as well as Regional Entomologists Mr. Richard Delos Reyes (Region 4B) and Mr. Royfrextopher Boholst (Region 12) for providing status and insights of LF in the sampling sites; (4) local government units of Oriental Mindoro and Sultan Kudarat for their consistent participation and support; and (5) residents of selected sampling sites for allowing us to conduct adult mosquito collection in their household premises. We also acknowledge the contributions of the field and laboratory staff namely, Mr. Rommuel Gutierrez, Mr. Roland Parreno, and Ms. Jean Angela Ariles, of the Department of Medical Entomology, Research Institute for Tropical Medicine.

Funding

This study was funded by the Department of Health – Philippines through their Disease Prevention and Control Bureau (Sub-allotment Advice Numbers 2017-06-400 and 2017-12-2208).

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