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Research Article

Field Validation of Post-MDA LF Surveillance by using Molecular Xeno-monitoring: Preliminary Study in Belitung District, Indonesia

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Abstract

This study aimed to validate molecular xenomonitoring (MX) as a post-MDA surveillance tool for lymphatic filariasis in Belitung District, Indonesia. Lymphatic filariasis (LF) is a mosquito-borne parasitic disease caused by filarial worms such as *Brugia malayi*, which remains a significant public health concern in tropical regions, including Indonesia. Belitung District, historically endemic for LF, implemented the Mass Drug Administration (MDA) from 2005 to 2010 as part of Indonesia's national eradication program. Despite achieving WHO certification as filariasis-free in 2017, sporadic LF cases persisted, prompting the need for effective post-MDA surveillance tools. MX, which detects parasite DNA in mosquitoes, has emerged as a complementary strategy to assess transmission risks and validate elimination efforts. This study conducted field validation of MX in two LF-endemic villages in Belitung District: Cerucuk and Lassar. Mosquitoes were collected using resting catches, human bait, and traps (CDC Light Trap and BG Sentinel Trap) across various habitats. Collected mosquitoes were pooled and tested for *B. malayi* DNA using conventional PCR. A total of 1,270 mosquitoes were collected, and 144 pools were analyzed for parasite DNA. No *B. malayi* DNA was detected in any of the 144 mosquito pools tested. The dominant mosquito species collected included *Culex gelidus*, *Anopheles letifer*, and *Armigeres subalbatus* in Cerucuk, and *A. letifer*, *Culex vishnui*, and *Culex quinquefasciatus* in Lassar. The absence of parasite DNA suggests that LF transmission may have been interrupted in these areas, likely due to the success of previous MDA campaigns. The findings indicate that MX is a valuable tool for post-MDA surveillance, particularly in areas with persistent ecological risks. While the results suggest interrupted transmission, continued surveillance is essential to confirm these findings and prevent resurgence. MX can complement existing methods, contributing to more effective LF elimination strategies in Indonesia and other endemic regions.

Keywords: Belitung district, *Brugia malayi*, lymphatic filariasis, molecular xenomonitoring, post-MDA surveillance

1. INTRODUCTION

Lymphatic filariasis (LF) is a parasitic disease transmitted by mosquitzoes, posing a significant public health concern in many tropical and subtropical regions, including Indonesia [1]. This disease is caused by filarial worms such as *Wuchereria bancrofti*, *Brugia malayi*, and *Brugia timori*, which can lead to chronic complications like lymphedema and elephantiasis, significantly diminishing the quality of life of those affected [2]. In Indonesia, the Belitung District has historically been an endemic area for LF, with *B. malayi* identified as the primary causative parasite [3]. The transmission of LF in this region is driven by several mosquito species, including Mansonia, Anopheles, Culex, and Aedes, which act as vectors for the filarial parasite [4]. Belitung District has adopted Mass Drug Administration (MDA) as part of Indonesia's national eradication program to eliminate lympathic filariasis. The MDA initiative in Belitung involved the annual distribution of a two-drug regimen—diethylcarbamazine citrate (DEC) and albendazole—to the entire at-risk population for at least 5 consecutive years from 2005 to 2010 [5]. This strategy was designed to lower microfilarial prevalence and disrupt transmission cycles.

The transmission assessment survey (TAS) is a WHO-recommended protocol [5] used to determine when MDA can be stopped (TAS 1) and to assess whether infection levels remain below the target threshold (antigen (Ag) prevalence <2% in children aged 6 to 7 years] after MDA cessation (‘post-MDA surveillance’). The current guidelines for post-MDA surveillance suggest conducting TAS twice—first at 2–3 years (TAS 2) and again at 4–6 years (TAS 3) after stopping MDA [6]. Any evaluation unit that successfully passes all three TAS stages

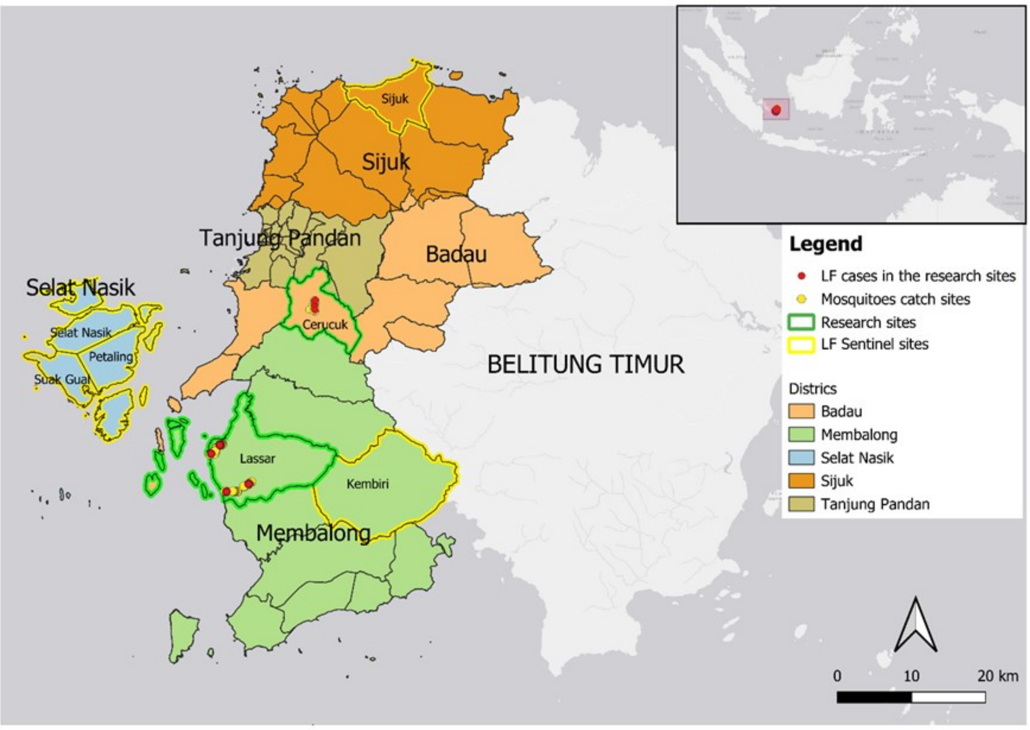

Figure 1. Distribution of study site and sampling site in Belitung regency.

will undergo post-validation surveillance for at least 5 years. Belitung completed all required steps for filariasis elimination and was officially recognized as filariasis-free by WHO in 2017. Despite these efforts, surveillance data from the District Health Office continued to record LF cases in 2017 [5] and 2021. Due to concerns about persistent transmission and the need for accelerated elimination, Belitung was selected as one of the pilot areas for implementing triple drug therapy (IDA). This new regimen, consisting of ivermectin, DEC, and albendazole, was introduced to enhance the efficacy of MDA and potentially reduce the number of rounds needed to achieve elimination. The IDA implementation in Belitung faced both positive reception and some resistance from communities, with initial rounds showing drug uptake of approximately 70%, varying between villages.

This persistence of LF cases post-MDA highlights the critical need for effective post-MDA monitoring tools. Molecular xenomonitoring (MX) is a technique that detects parasite DNA in mosquitoes to indirectly assess transmission risk, providing a community-level measure of infection without requiring invasive sampling in humans. MX has been recognized as a valuable complementary tool to TAS for monitoring the resurgence of infection during post-MDA surveillance and validation phases [7]-[9]. The MX process involves collecting a large number of mosquitoes using appropriate sampling techniques to ensure a representative sample, followed by PCR-based detection of parasite DNA in mosquito pools. Previous research has shown that MX is more sensitive and efficient in detecting parasite DNA than Mf testing in humans [10]. It is also useful in identifying residual or re-emerging infection foci following multiple rounds of MDA [11]-[13]. Several LF-endemic countries have utilized MX to confirm TAS findings, particularly by verifying the absence of transmission during post-MDA and validation phases [7]-[9][14]. Despite its advantages, implementing MX in operational settings requires careful evaluation of cost-effectiveness relative to TAS, as well as considerations regarding the availability of well-equipped laboratories and trained personel [10]. This technique offers an indirect yet sensitive method to assess ongoing transmission, providing valuable data for decision-making in LF elimination programs.

The objective of this study was to validate the application of MX in Belitung District as a tool for post-MDA surveillance, by assessing mosquito species composition and testing for the presence of *B. malayi* DNA. This study represents the first field validation of xenomonitoring activities in Indonesia, specifically focusing on the Belitung District. By evaluating its implementation and effectiveness, this research aims to contribute essential insights into the feasibility of xenomonitoring as a post-MDA surveillance strategy. Compared to traditional methods such as TAS or microfilaria surveys in humans, MX offers several advantages. It provides higher accuracy in detecting low-level or residual transmission, can be more cost-effective in settings where sampling large populations is logistically difficult, and avoids the ethical and operational challenges of human blood collection. However, MX also requires adequate laboratory infrastructure, molecular diagnostic facilities, and trained personel, which may limit its applicability in resource-constrained settings. Considering these factors, MX serves as a complementary tool rather than a replacement, supporting more appropriate and sustainable strategies for post-MDA surveillance in Indonesia and other endemic regions.

2. MATERIALS AND METHODS

2.1. Study Sites

This study was conducted in two villages within Belitung Regency (Figure 1), Indonesia: Cerucuk Village (Badau District) and Lassar Village (Membalong District), which are filariasis sentinel areas in Belitung Regency. A sentinel area is a village/urban area selected during the mapping survey before the implementation of MDA. These sites were selected based on LF case data recorded in 2017, 2021, and 2023, which indicated ongoing transmission risks in these areas. Cerucuk Village is characterized by a diverse ecological landscape that contributes to mosquito breeding and LF transmission: numerous swamps and small rivers; extensive oil palm and rubber plantations; and residential areas are typically built in proximity to mosquito breeding sites. Lassar Village presents a more rural and forested environment than Cerucuk: mixed vegetation and forested areas; swamps and brackish water zones; and cattle farming areas. Including these two distinct ecological landscapes allows for a comprehensive evaluation of xenomonitoring as a post-MDA surveillance tool, considering variations in mosquito habitats and human exposure risks across different settings in Belitung. Sampling was conducted in 2023 during the rainy season, a period typically associated with increased mosquito breeding and higher vector densities in tropical regions. These seasonal conditions are relevant for interpreting the abundance and species composition of the collected mosquitoes.

2.2. Sampling Sites

Mosquito collections were conducted in areas with known LF cases within Cerucuk and Lassar villages (Figure 1). The habitat selection was based on the habitat types found around filariasis cases in each region, ensuring that the sampling strategy directly targeted environments where transmission was most likely to occur. In each village, sampling efforts focused on three distinct habitat types to capture potential variations in vector populations and *B. malayi* infection rates. In Cerucuk Village, the following habitats were targeted for mosquito collection: village (residential areas), village + forest (edge habitats), and cattle + swamp (livestock and wetland zones). In Lassar Village, the following habitats were targeted for mosquito collection: village (residential areas), mix vegetation (diverse plant communities), and brackish swamp (coastal wetland).

2.3. Mosquito Collection

Mosquito collection began at houses with filariasis cases and then continued in a circular pattern, spreading to 100–250 houses. The catching methods applied were resting walls indoors and outdoors, carried out for 30 min in each house for five hours of catching (7 p.m. to 12 p.m.). The human bait method and mosquito traps (CDC light trap and BG-sentinel trap) were also used in certain areas to increase the chances of capturing more mosquitoes. The coordinates of the mosquito-catching locations were mapped using GPS. Mosquito collection began at houses with filariasis cases and then continued in a circular pattern, spreading to 100–250 houses. Mosquitoes were collected using four complementary methods to maximize species diversity. (1) Indoor and outdoor resting catches – Adult mosquitoes resting on house

Table 1. Summarized mosquito collections by species in Cerucuk and Lassar villages.

No	Species	1st survey (Cerucuk Village)				2nd survey (Lassar Village)			
		Habitat type			Total	Habitat type			Total
		Village	Village + Forest	Cattle + Swamp		Village + Mix Vegetation	Village	Brackish Swamp	
1	*Ma. Dives*	11	-	2	13	33	-	-	33
2	*Ma.annulata*	-	-	1	1	-	-	-	0
3	*Ma. Annulifera*	-	-	-	0	-	3	-	3
4	*Ma.uniformis*	-	-	-	0	17	18	4	39
5	*An. letifer*	7		15	22	230	15	4	249
6	*An. Peditaeniatus*	-	-	-	0	1	7	11	19
7	*An.subpictus*	-	-	1	1	-	-	2	2
8	*An. vagus*	-	-	9	9	-	-	-	0
9	*An. nitidus*	2	-	6	8	-	-	-	0
10	*An.nigerimus*	-	-	2	2	-	-	-	0
11	*An.limosus*	-	-	1	1	-	-	-	0
12	*Cx. quenquefasciatus*	8	-	-	8	1	61	76	138
13	*Cx.vishnui*	12	-	4	16	109	9	33	151
14	*Cx.gelidus*	76	-	23	99	5	1	2	8
15	*Cx.tritaeniorhynchus*	18		114	132	-	-	-	0
16	*Cx.hutchinsoni*	-	4	-	4	-	-	-	0
17	*Cx.bravivalpis*	-	-	2	2	-	-	-	0
18	*Cx.whitei*	-	-	8	8	-	-	-	0
19	*Cx.malayi*	-	-	1	1	-	-	-	0
20	*Cx mumulus*	-	-	2	2	-	-	-	0
21	*Cx edwardsi*	-	-	3	3	-	-	-	0
22	*Cx. Scanloni*	-	-	1	1	-	-	-	0
23	*Cx.polietis*	-	-	2	2	-	-	-	0
24	*Cx. Nigropunctatus*	-	-	13	13	-	-	-	0

Table 1. *Cont.*

No	Species	1st survey (Cerucuk Village)				2nd survey (Lassar Village)			
		Habitat type			Total	Habitat type			Total
		Village	Village + Forest	Cattle + Swamp		Village + Mix Vegetation	Village	Brackish Swamp	
25	*Ae. albopictus*	5	3	2	10	7	6	17	30
26	*Ae. aegypti*	9	4	-	13	-	-	-	0
27	*Ae. (Ver.) butleri*	-	-	-	0	-	-	9	9
28	*Ae. (Am) caecus*	-	-	-	0	-	-	24	24
29	*Aedes sp.*	-	-	-	0	-	-	2	2
30	*Ar. Subalbatus*	47	-	34	81	3	36	22	61
31	*Ar. kuchingensis*	29	-	20	49	-	-	-	0
32	*Ar. Kesseli*	-	-	1	1	-	-	-	0
					502				768

walls and outdoor structures were aspirated using manual mouth aspirators. Each resting collection lasted 30 min per house, both indoors and outdoors, across 6 h (18:00–24:00). (2) Human landing catches (HLC) – Conducted in selected households with prior LF cases, following local ethical approval. Two trained collectors, protected with aspirators and flashlights, captured mosquitoes that attempted to land on exposed lower legs. Collections were alternated hourly between collectors to reduce fatigue. (3) CDC light traps – Traps were installed inside houses near sleeping areas with an occupied bed net, set at dusk (18:00) and collected at dawn (06:00). (4) BG-Sentinel Traps – Deployed outdoors near houses and vegetation using BG-Lure attractants, operating overnight for approximately 12 h.

Each method has inherent sampling biases: resting catches tend to collect endophilic or exophilic resting species, human landing catches capture anthropophilic mosquitoes, while light traps and BG-Sentinel traps attract nocturnal or host-seeking mosquitoes. Combining these techniques was intended to minimize bias and obtain a representative sample of the local vector population. The coordinates of the mosquito-catching locations were mapped using GPS. It should be noted that mosquito populations are strongly influenced by weather factors such as rainfall, temperature, and humidity. As this study was conducted during the rainy season, mosquito density may have been higher than in dry months. Future studies incorporating longitudinal sampling across different seasons are needed to capture the influence of weather variability on vector abundance and infection rates.

2.4. Sample Processing

Mosquitoes were first identified to species level using standard morphological identification keys [15]. Identification was performed by trained entomologists to ensure accuracy. After identification, all mosquitoes collected were grouped in pools of 25 or fewer, according to the village and method of collection. The pooled mosquitoes were then sent to the laboratory for molecular identification of *B. malayi* infection in the vector species. Collected specimens were killed by freezing and then stored at −20 °C until processing. Each pool of mosquitoes was homogenized using sterile pestles in lysis buffer prior to DNA extraction. DNA was then extracted using the Quick-DNA™ Miniprep Plus Kit (Zymo Research) following the manufacturer's protocol. The method for detecting the presence of *B. malayi* DNA in mosquitoes was conventional PCR by employing 35 cycles of amplifications and the employed temperatures were 95 °C for 5 min, 95 °C for 60 s, 59.6 °C for 60 s, 72 °C for 60 s, 72 °C for 10 min, and finally held at 4 °C until further analysis.. The primers used were Hha1 F (5'-GCGCATAAATTCATCAGC-3') and Hha1 R (5'-GCGCAAAACTTAATTACAAAAGC-3') 8. DNA was extracted from pools using Quick-DNATM Miniprep Plus Kit from Zymo Research. Amplified PCR products were electrophoresis and visualized by SYBR® safe DNA gel staining (Invitrogen, Carlsbad, CA, USA). A 100 bp DNA ladder was used to measure the size of PCR products (targeted band 322 bp) .

3. RESULTS AND DISCUSSIONS

A total of 1,270 mosquitoes were collected from 114 houses in Cerucuk Village and 123 houses in Lassar Village. A breakdown of mosquito collections by species is presented in Table 1, showing that dominant mosquito species collected included *Culex gelidus*, *Anopheles letifer*, and *Armigeres subalbatus* in Cerucuk, and *A. letifer*, *Culex vishnui*, and *Culex quinquefasciatus* in Lassar. Resting catches yielded primarily Culex and Armigeres, human landing catches predominantly captured Anopheles, while BG-Sentinel traps were more effective for collecting Aedes. This highlights the importance of using multiple collection methods to capture diverse vector species.

3.1. Ecological Variations and Transmission Risks in Cerucuk and Lassar

The ecological diversity of Cerucuk and Lassar villages in Belitung Regency plays a critical role in shaping the dynamics of LF transmission. These villages represent distinct ecological settings, each with unique environmental characteristics influencing mosquito breeding, vector density, and human-vector contact. Our mosquito collection results support these ecological observations. In Cerucuk, the dominant species were *C. gelidus*, *A. letifer*, and *A. subalbatus*, while in Lassar, *A. letifer*, *C. vishnui*, and *C. quinquefasciatus* were abundant. These genus-level differences are further illustrated in Table 1, which shows the relative abundance of Culex, Anopheles, Armigeres, and other groups across the two villages. Furthermore, mosquito yields varied by capture method, with resting catches producing mainly Culex and Armigeres, human landing catches capturing predominantly Anopheles, and BG-Sentinel traps more effective for Aedes. These findings indicate that the ecological characteristics of each village (swampy habitats, plantations, and brackish water zones) strongly shaped species composition and vector abundance. By explicitly linking our field results with ecological context, the data provide insight into potential LF transmission risks in Cerucuk and Lassar.

Cerucuk Village is characterized by a diverse ecological landscape that provides ideal breeding grounds for mosquito vectors, particularly Mansonia and Anopheles species, which are known vectors of *B. malayi* [16]. The village features numerous swamps and small rivers, which serve as primary breeding sites for mosquitoes [17][18]. These water bodies are often stagnant or slow-moving, creating optimal conditions for mosquito larvae development [4]. Additionally, the extensive oil palm and rubber plantations in Cerucuk provide ample resting sites for adult mosquitoes, further contributing to the high vector density in the area [3]. The proximity of residential areas to these breeding sites increases the risk of human-vector contact, a key factor in LF transmission. Studies have shown that areas with high human activity near mosquito breeding sites are more likely to experience sustained transmission of filarial parasites [10][19]. In Cerucuk, abundant breeding sites, favorable resting areas, and close human settlements create an environment where LF transmission can persist, even after multiple rounds of MDA. In contrast, Lassar Village presents a more rural and forested environment, with mixed vegetation and brackish water zones that support a wide range of mosquito species. The village's ecological conditions are particularly favorable for Culex and Anopheles species, also vectors of *B. malayi*. The presence of swamps and brackish water zones in Lassar provides suitable habitats for these mosquito species, which thrive in freshwater and saline environments [13].

Cattle farming areas in Lassar further contribute to the mosquito population dynamics. Cattle serve as alternative blood meal sources for mosquitoes, potentially increasing the vector population density [20]. This phenomenon, known as zooprophylaxis, can either reduce or increase human-vector contact, depending on mosquito feeding preferences [11]. In Lassar, cattle farming areas near human settlements may increase the risk of LF transmission, as mosquitoes that feed on both humans and animals can act as bridge vectors, facilitating the spread of filarial parasites. The ecological heterogeneity between Cerucuk and Lassar villages highlights the importance of tailoring surveillance and control strategies to local environmental conditions. In Cerucuk, the dominance of Mansonia and Anopheles species in swamp and plantation areas suggests that vector control efforts should focus on these habitats. For example, environmental management strategies, such as draining stagnant water bodies and clearing vegetation around residential areas, could reduce mosquito breeding and resting sites [21]. In Lassar, the prevalence of Culex species in brackish water zones and cattle farming areas indicates that vector control measures should target these specific habitats. Using larvicides in brackish water zones [22] and implementing zooprophylaxis strategies, such as placing cattle pens away from human settlements, could help reduce mosquito populations and minimize human-vector contact [20][23].

The ecological variations between Cerucuk and Lassar also influence the effectiveness of MX as a post-MDA surveillance tool. In Cerucuk, the high density of Mansonia and Anopheles species in swamp and plantation areas increases the likelihood of detecting filarial DNA in mosquito vectors, provided that transmission is ongoing. However, this study's absence of detectable *B. malayi* DNA suggests that MDA efforts may have successfully interrupted transmission in these areas. In Lassar, the presence of Culex species in brackish water zones and cattle farming areas presents a different challenge. While these species are known vectors of *B. malayi*, their feeding preferences and habitat distribution may affect the sensitivity of MX. For example, if Culex species primarily feed on cattle rather than humans, the likelihood of detecting filarial DNA in these mosquitoes may be lower, even if transmission is ongoing [11]. This underscores the need for comprehensive sampling strategies for ecological variations and vector behavior when implementing MX. Seasonal fluctuations may have altered mosquito population dynamics and, thus, the finding of *B. malayi* DNA in the present study. Temperature, humidity, and rainfall patterns can all influence mosquito breeding, survival rates, and feeding habits, thereby impacting the abun danceof infected vectors at the time of collection. Future research should look on longitudinal sampling throughout several seasons to account for seasonal fluctuations in mosquito abundance and increase the accuracy of xenomonitoring results

3.2. Mosquito Collection and Analysis Results

The mosquito collection and analysis conducted in Cerucuk and Lassar villages provide critical insights into the vector population dynamics and the

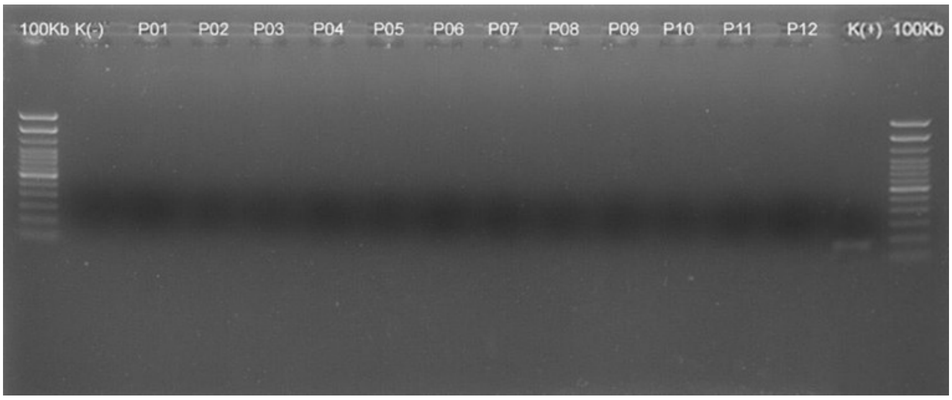

Figure 2. PCR results for pooled mosquito samples collected from Cerucuk and Lassar villages.

potential for ongoing LF transmission in these areas. The results of this study are particularly significant in the context of post-MDA surveillance, as they help assess the effectiveness of previous elimination efforts and identify areas where transmission risks may persist. Mosquito collection was carried out in Cerucuk and Lassar villages using a combination of methods to ensure comprehensive sampling. These methods included indoor and outdoor resting captures, human bait techniques, and mosquito traps such as CDC Light Traps and BG-Sentinel Traps. The collection focused on households with confirmed LF cases and targeted specific habitat types known to support mosquito breeding and resting.

In Cerucuk Village, a total of 502 mosquitoes were collected, with the dominant species being *C. gelidus* (99), *Culex tritaeniorhynchus* (132), *A. letifer* (22), and *A. subalbatus* (81). These species are known vectors of *B. malayi*, the primary causative agent of LF in Belitung Regency [4]. The distribution of these species varied across different habitat types, with Culex species predominantly found in village and livestock-related habitats. In contrast Anopheles and Mansonia species were more abundant in forest-edge and swamp areas. In Lassar Village, a total of 768 mosquitoes were collected, with *A. letifer* (249), *C. vishnui* (151), *C. quinquefasciatus* (138), and *Mansonia uniformis* (39) being the most prevalent species. The high density of *A. letifer* in both village and mixed vegetation habitats suggests a strong presence of this species in human settlements, increasing the risk of human-vector contact. Meanwhile, *C. vishnui* and *C. quinquefasciatus* were mainly detected in brackish swamp and village habitats, highlighting the role of specific ecological conditions in shaping mosquito population dynamics [9].

Mosquito species' ecological and behavioral characteristics play a significant role in the collection and analysis results. In Cerucuk Village, the dominance of Culex species in village and livestock-related habitats reflects their preference for breeding in stagnant water bodies and resting in areas with high human and animal activity [24]-[26]. In contrast, the high density of *A. letifer* in Lassar Village suggests that this species is well-adapted to–village and mixed vegetation habitats, increasing the risk of human-vector contact in these areas [27][28]. Multiple collection methods, including human bait and mosquito traps, were crucial for capturing a representative sample of the mosquito population. Human bait techniques are particularly effective for collecting anthropophilic species such as Anopheles [29], which are more likely to transmit *B. malayi* to humans [9][30]. However, the reliance on human bait can also introduce bias, as it may underestimate the population of zoophilic species that prefer animal hosts. The inclusion of mosquito traps helped mitigate this bias by capturing a wider range of species, including those that may act as bridge vectors between animals and humans [29].

3.3. Molecular Analysis: PCR Results and Implications

Molecular analysis using conventional PCR was performed on 144 mosquito pools (65 from Cerucuk and 79 from Lassar) to detect the presence of *B. malayi* DNA. The PCR protocol involved 35 cycles of amplification, with specific primers targeting a 322 bp fragment of the *B. malayi* genome. Although many vector mosquitoes were collected, no *B. malayi* DNA was detected in any mosquito pool (Figure 2). This finding suggests that active LF transmission may have been interrupted in these areas, likely due to the success of previous MDA campaigns. Amplified DNA fragments were separated by electrophoresis on a 2% agarose gel and visualized using SYBR® Safe staining. Lane M is 100 bp DNA ladder, lane P is positive control (*B. malayi* DNA, expected band size 322 bp), lane N is negative control (nuclease-free water), and lanes 1–20 are representative pooled mosquito samples from Cerucuk and Lassar. The absence of bands at 322 bp in all tested pools indicates no detectable *B. malayi* DNA in the mosquito populations. However, the absence of detectable *B. malayi* DNA does not necessarily imply the complete absence of transmission. Low-level or sporadic transmission may still occur below the detection threshold of current methods, particularly in areas with persistent ecological risk factors [11]. Additionally, the sensitivity of PCR-based detection can be influenced by factors such as the quality of DNA extraction, the efficiency of primer binding, and the presence of inhibitors in mosquito samples [7].

Therefore, while the results are encouraging, they should be interpreted caustiously, and continued surveillance is essential to confirm the interruption of transmission.

3.4. Challenges and Limitations

Despite the comprehensive sampling strategy, several challenges and limitations were encountered during mosquito collection and analysis. One major challenge was the variability in mosquito density across different habitats and seasons. In Cerucuk Village, the high density of Culex species in swamp and livestock areas made it easier to collect large numbers of mosquitoes, while in Lassar Village, the distribution of *A. letifer* across mixed vegetation and brackish water zones required more extensive sampling efforts. Another limitation was the potential for false-negative results in PCR analysis. Factors such as low parasite load in mosquito populations, inefficient DNA extraction, and the presence of PCR inhibitors can reduce the sensitivity of molecular detection methods [7]. To address these limitations, future studies could consider using more sensitive molecular techniques, such as quantitative PCR (qPCR), which can detect lower levels of parasite DNA and provide more accurate results [31][32]. Pooling mosquitoes up to 25 individuals may reduce the sensitivity of detecting low-prevalence infections, as a single infected mosquito could be diluted among many uninfected ones. Previous studies have noted this limitation, emphasizing the value of qPCR for increased sensitivity [33][34]. Despite this, conventional PCR remains widely used for MX and provides reliable results when transmission intensity is moderate to high.

3.5. Implications for Post-MDA Surveillance and LF Elimination

The results of this study have important implications for post-MDA surveillance and LF elimination efforts in Belitung Regency and other endemic regions. The absence of *B. malayi* DNA in mosquito vectors suggests that MDA campaigns effectively reduced transmission in Cerucuk and Lassar villages. However, the persistence of suitable mosquito habitats and the presence of potential vector species highlight the need for continued surveillance to prevent the resurgence of LF transmission. In comparison to host-based detection methods, MX offers several unique advantages and limitations. TAS, which rely on antigen detection in children, are widely used to determine whether MDA can be stopped; however, they may miss low-level transmission when prevalence is below the detection threshold [7][9][35]. Microfilaria (Mf) surveys through night blood smears provide direct evidence of infection but are invasive, less acceptable to communities, and logistically challenging for large-scale surveillance [10]. In contrast, MX is non-invasive, community-wide, and can be more sensitive in detecting residual transmission, since a single infected mosquito may indicate ongoing parasite circulation [13][36]. MX has proven to be a valuable tool for post-MDA surveillance, particularly in areas with persistent ecological risk factors. By detecting parasite DNA in mosquito vectors, MX provides an indirect measure of transmission that complements traditional human-based surveys such as the TAS [7]. However, implementing MX at an operational scale requires careful consideration of cost-effectiveness, laboratory capacity, and the availability of trained personel [7][8][37].

4. CONCLUSIONS

This study demonstrates that MX can be a useful tool for post-MDA surveillance, particularly in areas with persistent ecological risk factors. The absence of *B. malayi* DNA in mosquitoes from Cerucuk and Lassar suggests that LF transmission may have been interrupted. Importantly, this represents the first field validation of MX in Indonesia, highlighting its feasibility and potential as a complementary surveillance tool in national LF elimination efforts.

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Conflicts of Interest

The authors declare that they have no conflict of interests.

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DECLARATION OF GENERATIVE AI

During the preparation of this work, the authors used ChatGPT and DeepSeek to assist in generating text, summarizing literature, and refining language. After using this tool/service, the authors reviewed and edited the content as needed and takes full responsibility for the content of the publication.

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