

Research Article

Anti-Filarial Antibodies Prevalence after Mass Drug Administration in Two Endemic Areas of Indonesia

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A B S T R A C T

Introduction: Lymphatic filariasis is a public health problem in Indonesia, and efforts to eliminate it began in 2006 in Belitung and in 2007 in Pekalongan City. It is endemic to LF, and MDA treatment has been implemented. The study's goal is to find antifilarial IgG4 antibodies in the community after a lot of drug treatment for filariasis in places where *Brugia malayi* is common, like Belitung district, Kepulauan Bangka Belitung, and Pekalongan city, Central Java, where *Wuchereria bancrofti* is common.

Method: We will conduct this study using serum-stored biological material and immunological techniques such as enzyme-linked immunosorbent assays (ELISA). Using ELISA, we examined a total of 196 serum samples in Belitung and 140 serum samples in Pekalongan.

Results: The study results showed that the antibody prevalence in Belitung was 33.1% (665 out of 196) and 1.43% (2 out of 140). This study discovered a high prevalence of filariasis in children in *Brugia*-endemic communities. However, the study found lower rates of Bancroftian infection compared to adult communities.

Conclusion: In Lassar, children still have a high prevalence of antibodies. In Gemar, the prevalence of antibodies tends to be low across all age groups. The Bm14 antibody test is a promising method for sero-epidemiology. We recommend further research to identify suitable populations for transmission assessments.

Keywords: Lymphatic Filariasis, enzyme-linked immunosorbent assays (ELISA), antibodies, Indonesia

Introduction

Lymphatic Filariasis (LF) is classified within the category of neglected tropical diseases (NTDs). Nematodes called filarial worms, which reside in human lymphatic vessel tissue, cause filariasis. A mosquito bite transmits the infective larvae (L3)

into the skin, causing the infection.¹ The larvae enter the lymphatic system and migrate to the lymph nodes, where they molt and develop into L4 larvae and adult worms. Following copulation, the fully developed female produces viable progeny known as microfilariae, which a mosquito

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can devour, forming L2 and L3 larvae. It is believed that the host's complex relationship with the parasite causes the clinical signs of lymphatic filariasis. After infection, mature worms establish themselves within the lymphatic vessels of the body, thereby impacting the lymphatic system. Millions of larvae circulate in the bloodstream of a person with the infection during the six to eight years that the worms can maintain life.² Filariasis remains a significant issue in public health. Mosquitoes transmit lymphatic filariasis (LF), a disease that *Wuchereria bancrofti*, *Brugia malayi*, and *B. timori* cause. This disease is prevalent globally and may result in significant and enduring physical and psychological impairments. Depending on the species, one can find microfilariae in either peripheral blood or skin tissue. Microfilariae seen in peripheral blood might suggest either nocturnal periodicity, diurnal periodicity, or the absence of any periodicity. The underlying cause of the periodicity remains unclear; however, it might perhaps represent an adaptation to the biting behaviour of the vector. Filarial nematodes have a life cycle that involves two hosts: a definitive host, which is humans, and an intermediate host, which is blood-sucking arthropods.³

According to WHO estimates, filariasis has affected 120 million individuals in 83 countries around the world, primarily in tropical and subtropical regions. Three filariasis parasites, *Wuchereria bancrofti*, *Brugia malayi*, and *Brugia timori*, are found in nine South-East Regional Asia (SEAR) countries: Bangladesh, India, Indonesia, the Maldives, Myanmar, Nepal, Sri Lanka, Thailand, and Timor Leste.⁴ A global baseline estimate shows that in 2021, 882.5 million people in 44 countries needed preventative chemotherapy to stop the spread of lymphatic filariasis. Of these people, 25 million men had hydrocele and over 15 million had lymphoedema.⁵ The World Health Organization advocates for widespread administration of anti-helminthic medicine to eliminate the disease.⁴

Since 1975, Indonesia has implemented efforts to control filariasis, particularly in high filariasis-endemic areas. The World Health Assembly adopted the resolution "Elimination of Lymphatic Filariasis as a Public Health Problem" in 1997, and the WHO decision "The Global Goal of Elimination of Lymphatic Filariasis as a Public Health Problem by the Year 2020" strengthened it in 2000. On April 8, 2002, the Minister of Health launched the Filariasis Elimination Programme Global in Indonesia, focusing on preventative chemotherapy treatment through mass drug administration (MDA) and reducing microfilariae transmission. All filariasis-endemic regions and cities will implement mass drug administration to reduce transmission and ensure that filariasis patients have ongoing access to proper healthcare.⁶

Santoso et al. in Belitung Regency, Indonesia, discovered evidence that, despite 12 years of mass treatment and

the successful completion of TAS-3, transmission was still occurring in the community, particularly in village pockets. The study emphasizes the need for early detection of infection recurrence and potential prevention efforts.⁷ In 2022, Belitung Regency will continue MDA with three-drug regimens (IDA) as a follow-up to post-MDA surveillance.

Pekalongan City comprises four subdistricts. In 2004, the Health City Office documented 12 chronic filariasis cases throughout 11 urban villages within three subdistricts: West Pekalongan, North Pekalongan, and East Pekalongan

The cases dispersed throughout Tegalrejo Village, Karmatsari, Medono, Bendan, Bandengan Subdistrict, and Landungsari Village.⁸ The survey conducted in Kramatsari village revealed a microfilaria rate of 2.4%, as indicated by filarial parasites in the blood. Pekalongan, an endemic city, has partially implemented mass drug administration (MDA) in endemic sub-districts since 2007, according to survey results. In 2008, a prevalence study in Pabean revealed a microfilaria rate of 3.4% (17 out of 495 people)⁸ However, the epidemiological coverage of this partially treated approach is very small, and it is considered ineffective in stopping transmission. Therefore, from 2011 to 2015, Pekalongan City fully implemented MDA, which used two drug regimens: DEC and albendazole.⁹ In the following year, 2016, the city completed a pre-TAS (transmission assessment survey) by the WHO guidelines, and the results classified it as a failed pre-TAS. The WHO criteria required them to continue with the two-year round of MDA (2017–2018). In 2019, we repeated pre-TAS, but the results remained unsatisfactory, with the Mf rate exceeding 1%, indicating continuous active transmission.¹⁰ The Ministry of Health will deploy MDA from 2021–2022, adopting updated WHO recommendation, with three-drug regimens: ivermectin, DEC, and albendazole, also known as IDA.¹¹ Pekalongan City passed pre-TAS after three people tested positive for LF due to a significant decrease in transmission due to multiple rounds of MDA.¹²

According to WHO recommendations, the diagnostic method for the LF elimination program is microscopic examination. Detecting circulating microfilariae in finger-prick blood is an inexpensive and practical tool for mapping lymphatic filariasis endemicity and monitoring mass medication administration. Blood collection must occur at specified periods, based on the microfilariae's periodicity, and is applicable at both the individual and community levels.¹³ The Alere Filariasis Test Strip (FTS) may swiftly and accurately detect *Wuchereria bancrofti* antigens in blood samples, replacing the Binax Now filariasis immunochromatography test (ICT). The WHO recommends this for mapping, monitoring, and transmission assessment surveys (TAS). The WHO also recommends utilizing the *Brugia* Rapid point-of-care cassette test (BRT) to detect IgG4 antibodies against *Brugia* spp. in transmission assessment surveys.¹³

Another test, the Bm14 antibody CELISA, is based on ELISA principles. The recombinant antigen Bm14 is used to detect IgG4 antibodies, a reliable method for diagnosing filariasis, which can indicate ongoing infection and may be detected after MDA, even in individuals who test negative for microfilaria and antigen.¹⁴

The elimination journey in Belitung district and Pekalongan city spans over 16 years, requiring efforts to substantiate any potential transmission following drug administration before proceeding to the next evaluation stage. Therefore, this study aims to identify the antifilarial IgG4 antibodies present in the community following a large-scale drug administration for filariasis in areas commonly affected by *B. malayi* and *W. bancrofti*.

Method

Study Area

The research was carried out from September to December 2023. Samples were taken from serum-stored biological material in Lassar village (n = 196), Belitung Regency, Bangka Belitung Province. This village serves as a sentinel for filariasis elimination programmes.⁷ Sentinel is where mapping is conducted. The survey site is in Gamer urban village (n = 140) in Pekalongan City, Central Java Province. This area has never had finger blood tests done, so it is not a spot check or sentinel village. We collected samples from both areas after administering the IDA treatment. The National Research and Innovation Agency's ethics committee approved the study under reference number 029/KE.03/SK/04/2023.

We employed the microplate enzyme-linked immunosorbent assay (ELISA) method based on antibodies (Filariasis CELISA, Cellabs Pty Ltd, Brookvale, Australia) to identify immunoglobulin G4 antibodies to recombinant filarial antigen Bm-14 in human plasma, examining each sample in a single well. FASD (1x) Dilute in distilled water to 10x concentration, mix buffer, then dilute positive, negative, and test samples 1:100. Place each sample on the workbench. Allow all reagents to equilibrate to room temperature (18-25°C) prior

to utilization., a wash buffer was made, and a FASD sample diluent was created. The test sample was incubated for an hour at 37 °C before conjugate production using FASD and enzyme-conjugated FAPO. Wells were cleaned manually or with an automatic washer by emptying contents, refilling, and drying. 100 microliters of conjugate was poured into each well, incubated for 30 minutes, the SUBSTRATE was prepared within the last 10 minutes of incubation time. 950 L of substrate buffer FASB was mixed with 50µL of substrate chromogen FASC. Wash again as in step 1. 100 µL of substrate was added and incubated in the dark for 15 minutes. After that, 50 µL of FASS stopping solution was added and tapped on the container to mix it. The plate was read visually or using a spectrophotometer at dual wavelengths of 450 and 620 nm, with the results interpreted using the appropriate OD values. We retested the line test results with optical density (OD) values greater than 0.25 on a separate day to confirm their positivity. Positive samples were defined as those with two optical density (OD) values (450/620 nm) greater than 0.25 for serum samples and 0.3 for filter paper blood eluates.

Sampling and Sample Size

The sample size was calculated using a single proportion formula¹⁵: $N = P (1-p) Z^2/d^2$, where P = 0.5 refers to the filariasis study¹⁶; Z = level of confidence, 1.96, and d = 10% marginal error, with a minimum sample size of 96 individuals from each site. The survey included all individuals over 5 years of age of both genders who had lived in the village for more than 1 year. We acquired informed consent from the participants included in the study. We obtained parental approval for children under 18 years of age.

We used a global positioning system (GPS) to precisely record the sample sites' coordinates. Afterwards, we exhibited the spatial distribution of the cases on the maps. We processed and visualised the geographical data using QGIS version 3.28, a free and open-source program. The software is available for access at the following URL: <https://qgis.org/en/site/>.

Results

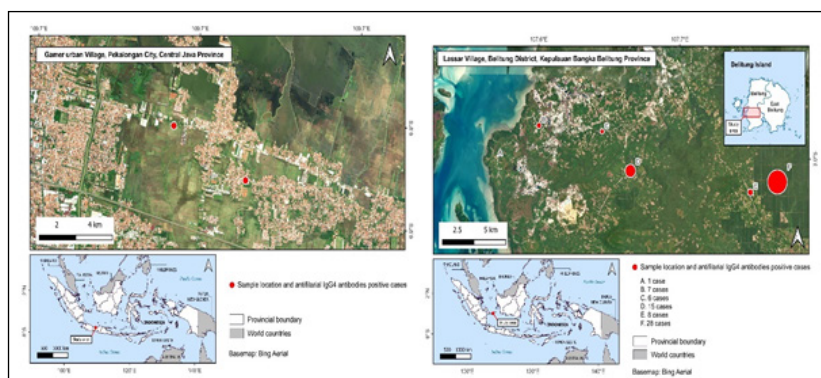


Figure 1. Spatial Distribution of Anti-filarial IgG4 Antibodies in Lassar, Belitung District and Gamer Urban Village, Pekalongan City

A total of 140 samples were in Gamer Village and 196 samples in Lassar Village, Belitung Regency. In Gamer Village, 1.43% (2/140) detected anti-filarial IgG4 antibodies, while in Lassar, 33.16% (65/196). Figure 1 displays the study areas and the distribution of positive anti-filarial IgG4 antibodies in this area.

Over 50% of anti-filarial IgG4 antibody positives in Lassar are male children, while all positive cases in Gemar are male. The sample in Belitung consists of schoolchildren, whereas in Gemar it consists of people over the age of ten.

33% of Lassar's children under the age of ten are antibody-positive. This reveals that despite the IDA treatment's disregard for drug adherence, children still have significant antifilarial IgG4 antibody levels.

Table 1 reveals that most of the sample in Gemar was aged 41–50 years (30.71%), followed by 25.71% aged 31–40 years and 20% aged 51–60 years. The ELISA assay results showed that 1.4% detected positive anti-filarial IgG4 antibodies in the age range of 21–30 years.

Table 1. Characteristics and Positive Samples in Lassar and Gemar Urban Village

Characteristic	Total Sample n (%)	Total Sample n (%)	No. of Positive n (%)	No. of Positive Samples n (%)
	Lassar	Gemar	Lassar	Gemar
Gender				
Male	112 (57.14)	82 (58.57)	48 (52.70)	2 (39.50)
Female	84 (42.85)	58 (41.43)	17 (47.30)	0 (0.00)
Ages (years)				
> 5–10	196 (100.00)	-	65 (33.16)	0 (0.00)
11–20		7 (5.00)		0 (0.00)
21–30		11 (7.86)		2 (100.00)
31–40		36 (25.71)		0 (0.00)
41–50		43 (30.71)		0 (0.00)
51–60		28 (20.00)		0 (0.00)
61–70		14 (10.00)		0 (0.00)
> 70		2 (3.60)		0 (0.00)

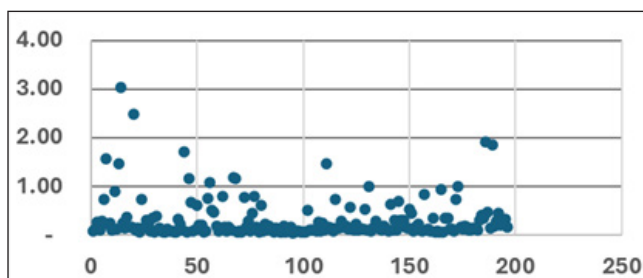


Figure 2. Antibody Results with Optical Densities (OD Values) Obtained with the Filariasis CELISA with Serum Samples (n = 196), Lassar Village, Belitung District

The study utilized the recommended cutoff value of 0.25 for a positive result, identifying samples with mean OD values greater than 0.250 as positive for antibody reactivity. Among the 196 samples from Lassar, Belitung, we identified 65 individuals with an optical density > 0.25, indicating a positive antibody level. The lowest OD value for a positive sample is 0.253, and the highest is 3.031.

In contrast, Figure 3 shows that in Gemar urban village, Pekalongan, only two samples were positive, with OD values of 0.298 and 0.458, respectively. The 138 negative samples had OD values ranging from 0.051 to 0.243.

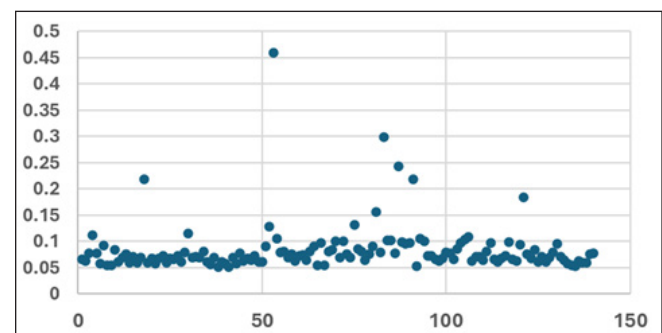


Figure 3. Antibody Results with Optical Densities (OD values) Obtained with the Filariasis CELISA with Serum Samples (n = 140) in Gamer Urban Village, Pekalongan City

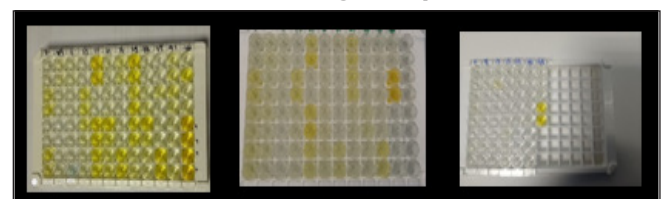


Figure 4. ELISA Machine Spectrophotometer with Two Bands of 450/620 nm Displaying the Sample Test Results

Discussion

The number of children that tested positive for IgG4 antibodies (Abs) following MDA at Lassar may indicate that transmission is still occurring, or that they have had previous filarial infections or exposure. According to a Samoan study, anti-filarial antibody responses in young children may indicate ongoing transmission or recrudescence in post-mass medication administration contexts.¹⁷ The greater of Abs in Lassar, Belitung suggests the presence of microfilaremia or antigenemia in the community. This is consistent with Egypt's research, which indicated that IgG4 antibodies to the recombinant filarial antigen Bm14 were more prevalent than microfilaremia or antigenemia. This means that the test is extremely accurate at identifying those who have been exposed to or infected with filarial parasites.¹⁸

However, we found only two positive antibodies (Abs) in Gamer Pekalongan. The location was not examined for filariasis, and no cases of lymphedema or hydrocele have been reported, indicating low endemicity. Bm14 antibody test sensitivity for *B. malayi* and *W. bancrofti* infection has generally been observed to be positive in over 90% of MF carriers, which was confirmed by a blinded multicentre study.¹⁹

The results are expressed in terms of optical density (OD), which corresponds to the antibody's concentration. There were 14 (7%) samples with OD values close to the cutoff, i.e., OD value ≥ 0.21 in Lassar, Belitung. These were potentially positive and could indicate exposure. There were three (2.2%) samples in Gemar, Pekalongan that were close to the OD value cutoff (≥ 0.21). A study conducted in the South Pacific has revealed a correlation between higher OD values and ongoing transmission.²⁰ It also indicates a potential correlation between antibody titre and the OD value, which is the commonly used measurement method for ELISA.²¹

In Gemar, Pekalongan City, the Abs positive age range is 21–30 years ($n = 2$), whereas in Lassar, Belitung, it is > 5 –10 years ($n = 65$). The fact that Belitung district has implemented MDA indicates that there might be continuous transmission, exposure, or even inadequate adherence to ingesting anti-filarial drugs. Studies show that two or three years of MDA implementation with high coverage ($\geq 80\%$) can effectively reduce LF prevalence compared to the five years recommended for parasite transmission interruption.²² Prolonged administration of treatment, particularly preventive chemotherapy treatment (PCT) poses a significant challenge to the community, often leading to ignorance and misconceptions about their health status.²³

A TAS is crucial for stopping MDA and post-MDA surveillance to detect transmission recrudescence. Repeated surveys should be conducted 2–3 years post-MDA to ensure interruption.²⁴ The TAS survey is specifically designed for children aged 6–7.²⁴ The recent evidence indicates that TAS targeting elementary school children aged 6–7 years fails to detect ongoing Mf-positive transmission, as Mf-positive sufferers are typically adults who are less aware of taking repeated MDA for 5 years.⁷ Despite the WHO's certification of Sri Lanka as having successfully eliminated LF, community surveys still found low levels of LF persistence in several areas.²⁵ Additional research on the target population in surveillance is required because current field evidence contradicts established guidelines.

The recombinant antigen Bm 14 can detect IgG4 antibodies to both *B. malayi* and *W. bancrofti*. Although WHO advises a rapid test in TAS (the post-MDA phase of monitoring), the Bm14 antibody test can be considered an alternative in serology-based surveillance to support LF elimination.

Conclusion

In Lassar village, Belitung district, children's antibody prevalence exceeded 30%, indicating ongoing exposure. In contrast, in Gemar urban, Pekalongan city prevalence is less than 5%. These findings may be useful for supporting post-MDA surveillance and achieving elimination endpoints. The Bm14 antibody test, an ELISA-based approach, is a promising choice for sero-epidemiology to detect residual infection or ongoing transmission. We recommend conducting more research to identify the appropriate populations for transmission assessments, which will help elimination programs develop new strategies.

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Conflict of Interest: None

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