



EOSINOPHILIA WITHOUT MICROFILARIAE: A CASE STUDY OF POTENTIAL HIDDEN FILARIASIS TRANSMISSION IN CENTRAL BENGKULU DISTRICT, INDONESIA

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Abstract

Lymphatic filariasis (LF) is a parasitic infection causing chronic disability and disfigurement, commonly affecting tropical countries like Indonesia. Although Bengkulu Province, particularly Central Bengkulu District, has been classified as non-endemic after mass drug administration (MDA) campaigns, the presence of chronic cases suggests the need for continued surveillance. This study aimed to explore the presence of hidden or subclinical LF transmission in a non-endemic area by assessing eosinophil levels as a potential early indicator of filarial infection, particularly when microfilariae are undetectable by microscopy. A cross-sectional study was conducted from August to October 2022 in three villages of Central Bengkulu. A total of 262 finger-prick blood samples were examined for microfilariae using Giemsa-stained smears, while 82 venous blood samples from Tiambang Village were analyzed for eosinophil levels using a Neubauer hemocytometer and Dunger's solution. Sociodemographic data were also recorded. No microfilariae were detected in any of the samples. However, eosinophilia (>350 cells/mm 3) was observed in a significant portion of participants in Tiambang Village, with an average eosinophil count of 436 cells/mm 3 . Elevated eosinophil levels were more common among females and individuals aged 26–45 years, who are typically more exposed to mosquito bites due to outdoor activities. Although microfilariae were not detected, elevated eosinophil levels may suggest hidden LF transmission or residual immune responses in the population. These findings highlight the importance of integrating eosinophil profiling as a complementary surveillance tool for early detection of LF in post-MDA or low-prevalence settings.

Keywords: Lymphatic filariasis, eosinophil count, microfilariae detection, post-MDA surveillance, Central Bengkulu

INTRODUCTION

Lymphatic filariasis (LF) is a neglected tropical disease caused by filarial parasites, primarily *Wuchereria bancrofti*, *Brugia malayi*, and *Brugia timori*, transmitted through mosquito bites, leading to chronic conditions such as lymphedema, elephantiasis, and hydrocele (WHO, 2023; WHO, 2025; Simonsen et al., 2025). Globally, LF remains a major cause of permanent disability, social stigma, and impaired quality of life, particularly in tropical and subtropical countries such as Indonesia (Abdulmalik et al., 2018). The

infection may be symptomatic or asymptomatic, with many individuals silently harboring microfilariae or circulating filarial antigens (CFA) in their peripheral blood (Dreyer et al., 2000; Chesnais et al., 2016).

As of 2023, approximately 863 million people in 47 countries remain at risk of LF, with Southeast Asia accounting for about 60% of the total cases (WHO, 2023; WHO, 2025; Bishani et al., 2021). In Indonesia, Bengkulu Province is among the endemic areas, with Central Bengkulu District reporting a considerable number of chronic cases as an indicator of ongoing or previous transmission (Ministry of Health Republic of Indonesia, 2022).

To address this, the Global Programme to Eliminate Lymphatic Filariasis (GPELF) implements mass drug administration (MDA) to interrupt transmission and provides morbidity management for affected individuals (Kamgno & Djeunga, 2020). Traditionally, LF diagnosis relies on microscopic detection of microfilariae using thick or thin blood smears stained with Giemsa (Mathison et al., 2019). However, this method may not detect all cases, especially during latent or prepatent phases of infection.

One biological marker of parasitic infection including LF is eosinophilia, defined as an eosinophil count exceeding 350 cells/mm³ in peripheral blood. Elevated eosinophil levels may result from helminthic infections, allergic diseases, autoimmune conditions, or neoplastic disorders (Wen & Rothenberg, 2016). LF is known to cause secondary eosinophilia due to immune response to filarial antigens, especially in early or hidden stages of infection. Although the prevalence of eosinophilia due to parasitic infections has declined with the widespread distribution of albendazole during MDA campaigns, unexplained eosinophilia in non-endemic or post-MDA areas may suggest undetected or re-emerging transmission (Ramirez et al., 2018).

This study aims to explore a potential anomaly: the presence of elevated eosinophil counts without microfilariae detection in a community classified as non-endemic for LF. By examining eosinophil profiles alongside standard microscopic screening, this research investigates the possibility of hidden or subclinical LF transmission in Central Bengkulu District, contributing to more sensitive surveillance and early detection strategies in post-MDA or low-prevalence settings.

MATERIALS AND METHODS

Research Location

The study was conducted in Bengkulu Tengah Regency from August to October 2022. Bengkulu Tengah Regency is a non-endemic area with 7 cases of chronic filariasis. The research location was in three villages that were selected purposively with consideration of the village where the filariasis case resided,

namely the City Point Village located between $3^{\circ}37'26''$ S South Latitude and $102^{\circ}16'16''$ E East Longitude, Talang Panjang Village was located between $3^{\circ}37'44''$ S South Latitude South and $102^{\circ}17'55''$ E East Longitude and Tiambang Village is located between $3^{\circ}34'19''$ S South Latitude and $102^{\circ}19'31''$ E East Longitude (Figure 1). The research has received ethical approval from the Bengkulu Polytechnic of Ministry Health Ethics Committee Number. KEPK/395/08/20

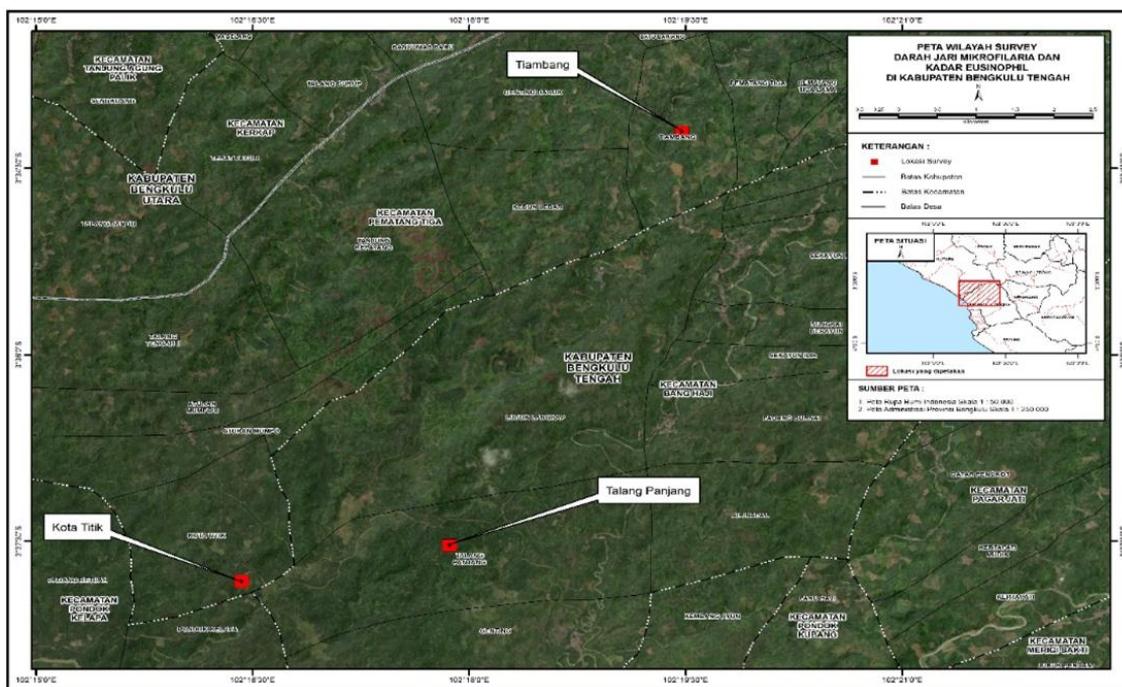


Figure 1 Study Area

Population and Sample

The study population comprised residents aged five years and older from three villages in Bengkulu Tengah District: Kota Titik, Talang Panjang, and Tiambang. Eligibility criteria included having resided in the village for at least one year and not having traveled to filariasis-endemic areas outside the study sites within the past six months, in order to ensure accurate assessment of local transmission.

A total of 262 finger-prick blood samples were collected for microscopic examination of microfilariae: 107 from Kota Titik, 73 from Talang Panjang, and 82 from Tiambang. In addition, 82 venous blood samples were obtained from Tiambang Village for eosinophil blood profile analysis, as it recorded the highest number of chronic filariasis cases among the three villages.

Sample size was calculated using the CSurvey 2.0 application, suitable for two-stage cluster sampling designs. In the first stage, clusters were selected using the probability proportional to size (PPS) method, following WHO guidelines. In the second stage, a simple random sampling technique was applied to select individual participants from each environmental unit within the selected clusters.

Data Collection

Sample Data collection was conducted between August and October 2022. Finger-prick blood samples for microfilariae detection were collected during nighttime hours (21:00 to 01:00 WIB) to match the nocturnal periodicity of filarial parasites. The samples were processed and examined microscopically at the Bengkulu Provincial Health Laboratory.

Venous blood samples for eosinophil analysis were collected from Tiambang Village using standard venipuncture techniques. Samples were stored at 8–10°C and analyzed within 24 hours. Eosinophil counts were performed using the visual method with an Improved Neubauer hemocytometer on EDTA-treated blood samples. A 2% eosin solution mixed with acetone in a 1:1 ratio (Dunger's solution) was used to stain eosinophil granules, with distilled water used for red blood cell lysis and acetone for eosinophil fixation (Madan et al., 2001).

Data Analysis

Detection of microfilariae was conducted using Giemsa staining for 30 minutes, followed by microscopic examination at 100× magnification. The presence of microfilariae in peripheral blood smears confirmed filariasis infection.

Eosinophil counts were classified into two categories: normal (50–350 cells/µL) and abnormal (<50 cells/µL or >350 cells/µL), in accordance with standard hematological reference ranges (15).

RESULTS AND DISCUSSION

Result

Sample Characteristics Based On Age And Sex Group

Sample characteristics based on age and sex are presented in Figure 2A and B. The highest sample percentage was in the 26-45 years age group in Talang Panjang village. In general, the sample percentage of women was higher in all villages.

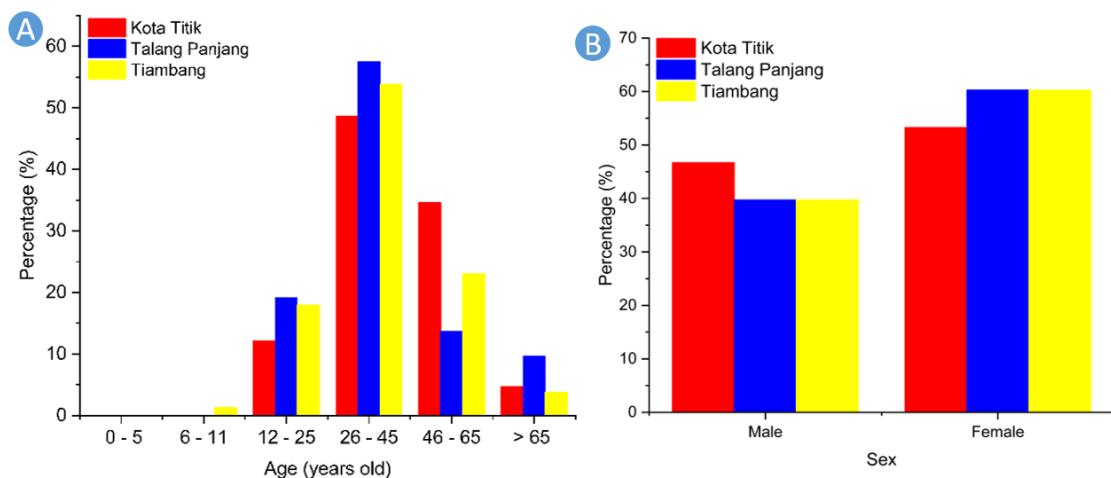


Figure 2 Characteristic sample based on age (A) and sex (B)

Detection Of Microfilaria Based On Microscopic Examination Result

The percentage of samples examined for microfilariae in the Central Bengkulu district was in Kota Titik (41%), followed by Tiambang and Talang Panjang villages, respectively, 31% and 28% (Figure 3). The results of the examination on all samples tested did not detect microfilariae.

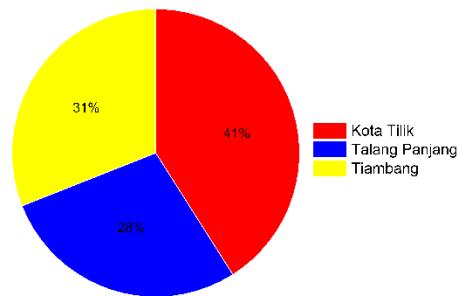


Figure 3 Percentage of samples based on villages for microfilariae detection

Eosinophil Profile

Eosinophil levels from 82 samples in Tiambang Village, Central Bengkulu Regency, is presented in Figure 4. There was an increase in eosinophil levels (> 350 cells/ mm^3) with an average eosinophil level of 436 cells/ mm^3 . Elevated eosinophil level were common found in female. The highest eosinophil levels, at 351-1000 cells/ mm^3 , were observed with percentages of 20% in male and 26% in female.

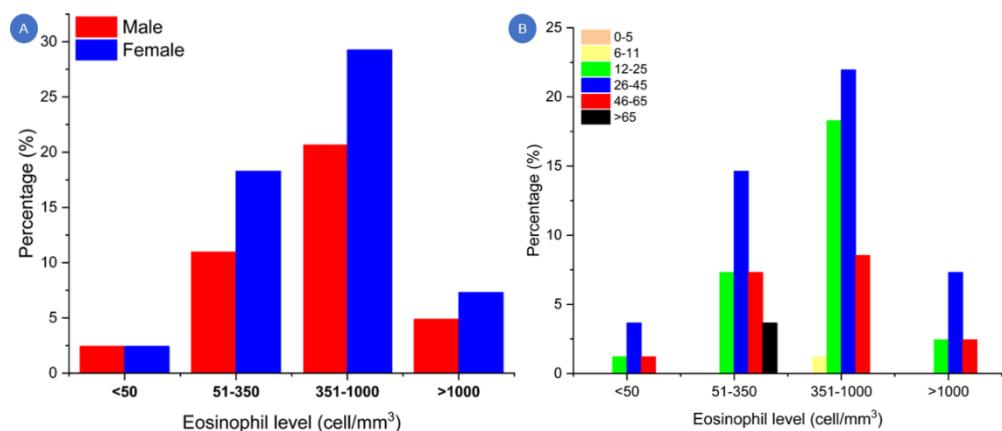


Figure 4 Eosinophil Levels in Tiambang Village, Central Bengkulu Regency

Discussion

Sample Characteristics by Age and Gender: Sociodemographic Indications of Filariasis Risk

The distribution of samples based on age showed a predominance of individuals aged 26–45 years, particularly in Talang Panjang Village. This age group represents the productive population who are generally engaged in outdoor activities such as working in fields, plantations, or other environments that potentially increase exposure to filariasis vector mosquito bites, including *Mansonia* spp., *Anopheles* spp., and *Culex* spp. This finding is consistent with the study by Gyapong et al. (Onyeneho et al., 2021), which noted that the productive age group is at a higher risk of filariasis transmission due to their increased exposure to endemic environments.

In addition, the proportion of female participants was higher than that of males across all study sites. This phenomenon may reflect greater participation of women in survey activities or easier access to women during sampling times. From both biological and sociocultural perspectives, women in rural areas often play significant roles in outdoor tasks such as fetching water, food processing, and engaging in community interactions, all of which can elevate their risk of exposure to filarial vectors. Onyeneho et al. (Ministry of Health Republic of Indonesia, 2023) highlighted the importance of incorporating gender dimensions in the design of community-based disease control interventions.

Absence of Microfilariae: Indicator of Successful Elimination or Hidden Transmission?

This study aimed to comprehensively evaluate the potential for sustained transmission of lymphatic filariasis in areas officially declared as non-endemic, by investigating early immunological indicators that may not be detected by conventional diagnostic methods. The selection of Tiambang Village in Central Bengkulu Regency as the study site is particularly significant, given that this area was administratively declared free from filariasis following the implementation of five consecutive rounds of mass drug

administration (MDA) (Simonsen et al., 2025). However, the ecological conditions of the village—characterized by shrub vegetation, open water channels, and high humidity—strongly justify a re-examination of its elimination status, especially when considered alongside biological findings that indicate potential ongoing transmission.

One of the key findings of this study was the absence of microfilariae in night blood smears examined by microscopy. Nevertheless, microscopy is known to have limited sensitivity, particularly in detecting low-density infections or subpatent cases (Rao et al., 2018; Simonsen et al., 2021). In contrast, complete blood count results revealed mild to moderate eosinophilia in most individuals, with an average eosinophil count of 436 cells/mm³. Eosinophilia has long been associated with parasitic infections, including filariasis, especially in prepatent or chronic phases when microfilariae are not yet or no longer detectable in peripheral blood (Nutman, 2013; Babu & Nutman, 2020).

Similar findings have been reported in several countries. For instance, a study in India showed that many individuals with positive filarial antigen tests had negative microfilaria results, yet exhibited immunological markers such as eosinophilia and specific antibodies to *Wuchereria bancrofti* (Babu et al., 2019). In Ghana and Papua New Guinea, increased eosinophil levels were found among children during the post-MDA period despite the absence of microfilariae, indicating repeated exposure to filarial infection (Budge et al., 2013; King et al., 2020). In Haiti, active surveillance revealed that individuals with elevated eosinophil levels were more likely to test positive via antigen or PCR assays despite negative microscopy results (Sriwichai et al., 2020).

The ecological context of Tiambang Village positions it as a critical area for reassessing filariasis elimination status. The local environmental conditions closely resemble those of other Southeast Asian regions, such as parts of southern Thailand and Myanmar, where residual transmission persists despite years of MDA (Nasir et al., 2023). The presence of shrub vegetation, temporary water puddles, and local *Mansonia* mosquito populations supports the hypothesis that the environment continues to provide suitable habitats for the filarial vector lifecycle.

Furthermore, this study proposes eosinophilia as a potential early screening marker to identify at-risk groups within the population, especially during the post-elimination phase. This approach has been adopted in Sri Lanka, where biomarker-based surveillance (including eosinophilia and antibody tests) has been used to detect potential hidden transmission in areas with official elimination status (WHO, 2021). Similar strategies have been employed in Malawi and eastern Indonesia, where rapid antigen diagnostic tests and molecular PCR tools are selectively applied in areas with suspicious immunological profiles (Gyapong et al., 2022).

Venous blood samples for eosinophil analysis were collected from Tiambang Village using standard venipuncture techniques. Samples were stored at 8–10°C and analyzed within 24 hours. Eosinophil counts were performed using the visual method with an Improved Neubauer hemocytometer on EDTA-treated blood samples. A 2% eosin solution mixed with acetone in a 1:1 ratio (Dunger's solution) was used to stain eosinophil granules, with distilled water used for red blood cell lysis and acetone for eosinophil fixation (Silumbwe et al., 2020; WHO, 2023).

The distribution of samples based on age showed a predominance of individuals aged 26–45 years, particularly in Talang Panjang Village. This age group represents the productive population who are generally engaged in outdoor activities such as working in fields, plantations, or other environments that potentially increase exposure to filariasis vector mosquito bites, including *Mansonia* spp., *Anopheles* spp., and *Culex* spp. This finding is consistent with the study by Gyapong et al. (Gunawardena et al., 2019), which noted that the productive age group is at a higher risk of filariasis transmission due to their increased exposure to endemic environments.

In addition, the proportion of female participants was higher than that of males across all study sites. This phenomenon may reflect greater participation of women in survey activities or easier access to women during sampling times. From both biological and sociocultural perspectives, women in rural areas often play significant roles in outdoor tasks such as fetching water, food processing, and engaging in community interactions, all of which can elevate their risk of exposure to filarial vectors. Onyeneho et al. (2021) highlighted the importance of incorporating gender dimensions in the design of community-based disease control interventions.

The policy implications of these findings are significant, as they demonstrate that relying solely on microscopy is insufficient for detecting latent infections or residual transmission. Therefore, a multi-method surveillance approach including circulating filarial antigen (CFA) detection, antibody assays, and molecular tools such as PCR is warranted. This recommendation aligns with the WHO's 2023 guidelines for post-elimination surveillance, which emphasize adaptive, evidence-based monitoring and risk mapping incorporating both ecological and immunological data (WHO, 2023).

In conclusion, this study not only highlights the potential for covert transmission of lymphatic filariasis in Tiambang Village but also underscores the importance of context-specific approaches in evaluating elimination status. Moving forward, community-based surveillance focused on immunological indicators such as eosinophilia may serve as an early warning system to prevent the re-emergence of the disease, particularly in areas that are administratively considered non-endemic yet ecologically and socially

remain conducive to filarial transmission.

Elevated Eosinophil Profile: A Clinical Indicator of Chronic Filarial Infection or Other Parasitic Diseases

This study aimed to comprehensively evaluate the potential for sustained transmission of lymphatic filariasis in areas officially declared as non-endemic, by investigating early immunological indicators that may not be detected by conventional diagnostic methods. The selection of Tiambang Village in Central Bengkulu Regency as the study site is particularly significant, given that this area was administratively declared free from filariasis following the implementation of five consecutive rounds of mass drug administration (MDA) (Simonsen et al., 2025). However, the ecological conditions of the village characterized by shrub vegetation, open water channels, and high humidity strongly justify a re-examination of its elimination status, especially when considered alongside biological findings that indicate potential ongoing transmission.

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An analysis of 82 blood samples from Tiambang Village revealed a consistently elevated eosinophil count, averaging 436 cells/mm³ and exceeding the normal reference range of 50–350 cells/mm³. Most individuals exhibited eosinophil counts ranging from 351 to 1000 cells/mm³, with the condition more frequently observed among female participants. This pattern suggests an immune response to parasitic antigens, either from active or chronic infections. These findings align with King and Nutman (2014), who reported that elevated eosinophil levels reflect host reactions to migrating or lysed filarial larvae in tissues, persisting even in the absence of detectable microfilariae.

However, eosinophilia is not specific to filariasis. Other parasitic infections—such as *Ascaris lumbricoides*, *Strongyloides stercoralis*, and *Trichuris trichiura*—can also elevate eosinophil levels, especially in areas with poor sanitation. The widespread prevalence of soil-transmitted helminths (STH) in rural Indonesian settings complicates interpretation, as co-infections may contribute to the observed immunological profiles. The high prevalence of eosinophilia in Tiambang Village may therefore reflect a syndemic interaction between filariasis and other parasitic diseases, facilitated by environmental factors

such as inadequate sanitation, open defecation practices, and high humidity. The predominance among females could be linked to domestic and agricultural exposures, consistent with reports from Nigeria and Papua New Guinea, where women exhibited higher parasitic burdens due to sociobehavioral risks (Onyeneho et al., 2021; King et al., 2020).

Similar findings have been reported internationally. In India, many individuals with positive filarial antigen tests but negative microfilaria results exhibited immunological markers such as eosinophilia and antibodies against *Wuchereria bancrofti* (Babu et al., 2019). In Ghana and Papua New Guinea, eosinophilia persisted among children in post-MDA settings despite the absence of microfilariae, suggesting repeated exposure to infection (Budge et al., 2013; King et al., 2020). In Haiti, surveillance showed that individuals with high eosinophil counts were more likely to test positive by antigen or PCR assays, even when microscopy was negative (Sriwichai et al., 2020).

The ecological context of Tiambang Village positions it as a critical area for reassessing filariasis elimination status. Local conditions mirror regions of southern Thailand and Myanmar where residual transmission persists post-MDA (Nasir et al., 2023). Shrub vegetation, temporary puddles, and *Mansonia* mosquito populations sustain environments conducive to filarial vectors.

To strengthen interpretation, further diagnostic refinement is required. Studies have demonstrated that eosinophilia may coexist with positive CFA or antifilarial antibodies despite negative microfilaria results (Babu et al., 2019). Therefore, integration of serological methods (ELISA, CFA assays) and molecular tools (PCR for parasite DNA) is essential to confirm etiology. This aligns with WHO's 2023 surveillance guidelines, which recommend multi-method approaches combining ecological and immunological data (WHO, 2023).

Venous blood samples for eosinophil analysis in this study were collected using standard venipuncture. Samples were stored at 8–10°C and analyzed within 24 hours. Eosinophil counts were determined with the Improved Neubauer hemocytometer on EDTA-treated blood, using Dunger's solution for staining, distilled water for RBC lysis, and acetone for fixation (Madan et al., 2001).

Sample distribution revealed a predominance of participants aged 26–45 years, especially in Talang Panjang Village. This age group, representing the productive workforce, engages in outdoor agricultural activities and thus faces greater exposure to vector mosquitoes (*Mansonia*, *Anopheles*, *Culex*). This trend echoes findings by Gyapong et al. (Gunawardena et al., 2019), who noted increased risk of filariasis in productive-age groups due to occupational exposure.

The higher proportion of female participants may reflect easier access during survey times, but also highlights gender-based vulnerabilities. In rural contexts, women's involvement in water collection, food processing, and farming activities increases vector exposure. Onyeneho et al. (2021) emphasize the necessity of incorporating gender perspectives in community-based control efforts.

Policy implications are clear: reliance on microscopy alone is insufficient to capture latent infections or residual transmission. A tiered surveillance strategy combining eosinophil monitoring, CFA detection, antibody assays, and PCR is recommended, especially in areas classified as non-endemic but ecologically at risk. Integrating eosinophil counts into surveillance may provide a cost-effective screening tool to identify high-risk groups and guide targeted testing, as implemented in Sri Lanka and Zambia (Gyapong et al., 2022; Silumbwe et al., 2020)

In conclusion, elevated eosinophil counts in Tiambang Village highlight possible covert transmission of lymphatic filariasis amid overlapping parasitic infections. While eosinophilia is a useful early immunological marker, its interpretation must consider environmental exposures and potential co-infections. Context-specific, multi-method surveillance will be critical to sustain elimination gains and prevent disease re-emergence in vulnerable rural populations.

CONCLUSION

This study demonstrates that sociodemographic characteristics, such as being in the productive age group and a higher proportion of female participants, contribute to an increased risk of exposure to filariasis vectors in Tiambang Village. Although no microfilariae were detected through microscopic examination, significantly elevated eosinophil profiles in most participants indicate an immune response to parasitic infections, including possible occult filarial infection. These findings underscore the importance of implementing a multiparameter surveillance approach including antigen detection, antibody assays, and molecular methods during the post-elimination phase to detect potential residual transmission. The use of eosinophilia as a potential early warning marker is also recommended as part of a community based early detection system, particularly in areas that are administratively classified as non-endemic but still exhibit ecological and social conditions conducive to the lifecycle of filariasis vectors and other parasitic infections.

ACKOWLEDMENT

The author extends sincere gratitude to all residents of Tiambang Village, healthcare workers, and community leaders for their support and participation in the implementation of this study. Special appreciation is also given to the laboratory and technical team from the Provincial Health Laboratory of

Bengkulu for their valuable contributions in sample analysis. This research was supported by the Human Resource Development Scholarship Program of the Ministry of Health of the Republic of Indonesia. The author also acknowledges Poltekkes Kemenkes Bengkulu and the Bengkulu Provincial Health Office for their administrative and logistical support.

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