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AIM & SCOPE

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	YEAR	NO OF MANUSCRIPT RECEIVED	DOCUMENTS PUBLISHED	PERCENTAGE (%) OF ACCEPTANCE
Before Scopus Indexed in 2024	2020	90	27	30
	2021	46	16	35
	2022	40	13	33
	2023	54	12	22
Post Scopus-indexed: The number of submitted manuscripts has increased significantly				
After Scopus Indexed in 2024	2024	3472	370	10.7
	2025	5469	475	8.7
	2026	3020 *(For January & April Issue)*	265	8.8
	2026	1746 *(For July Issue)*	To be decided	To be calculated

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Absence of *Paragonimus* DNA and Eggs in Tuberculosis Patients in Indonesia: Implications for Surveillance and Public Health Awareness

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Abstract

Paragonimus spp. are zoonotic trematodes endemic in several Asian countries, often mimicking pulmonary tuberculosis (TB) due to overlapping clinical manifestations. Although Indonesia's geographical proximity to endemic countries and shared culinary practices may increase exposure risk, human paragonimiasis has not been reported since a 1963 case in a Sumatran tiger. This study represents the first molecular screening for human paragonimiasis in Indonesia, aiming to investigate *Paragonimus* DNA and eggs in sputum samples of pulmonary TB patients. This study addresses infectious disease epidemiology and prevention through molecular surveillance. A cross-sectional study was conducted involving 60 sputum samples from TB patients in South Jakarta. Microscopy examination using Ziehl–Neelsen staining and the NaOH concentration method confirmed *Mycobacterium tuberculosis* in all samples, with no *Paragonimus* eggs observed. DNA extracted was performed using a commercial kit and DNA quality was assessed via agarose gel electrophoresis. Positive and negative controls were included to verify assay performance. None of the clinical DNA extracts showed visible *Paragonimus*-specific bands (~221 bp), and therefore PCR amplification was not performed. Although *Paragonimus* was not detected, this does not definitely exclude its presence. Using the zero-case prevalence concept with a 95% confidence level, the findings suggest a prevalence of less than one percent in the study population. Our findings underscore the importance of maintaining diagnostic vigilance for neglected parasitic infections in Indonesia, provide evidence to guide prevention strategies, enhance epidemiological monitoring for zoonotic diseases in TB-endemic areas, supporting the urgency of updating national parasitological surveillance policies and strengthening One Health-based disease monitoring.

Keywords: Molecular, Neglected Tropical Disease, One Health Paragonimiasis, Tuberculosis.

Introduction

Foodborne zoonotic parasites remain a significant public health challenge in many developing countries, particularly in Southeast Asia (1). Changing consumption patterns, increased human mobility, and intensified interactions between humans, animals, and the environment contribute to the emergence and persistence of various often-neglected parasitic diseases. One group of parasites included in this category is the pulmonary trematode of the genus *Paragonimus*, which has received less attention globally than other infectious diseases with similar respiratory manifestations (2). *Paragonimus* infections can cause chronic morbidity, reduce quality of life, and burden the health system due to misdiagnosis and mismanagement (3).

Paragonimus spp., also known as lung flukes, are foodborne trematodes transmitted to humans through the ingestion of raw or undercooked freshwater crustaceans. These parasites cause paragonimiasis, a zoonotic infection (2, 3). The manifestations include chronic cough, hemoptysis, and radiographic abnormalities, which often resemble pulmonary tuberculosis (TB). These similarities lead to frequent diagnostic misclassification in endemic regions where both infections co-occur. Human cases of paragonimiasis have been widely reported in several countries across East and Southeast Asia, including China, the Philippines, Thailand, South Korea, and Vietnam (4, 5).

Several epidemiological and clinical studies in these countries have shown that paragonimiasis is frequently found in populations with traditional dietary habits based on raw or undercooked freshwater crabs or shrimp (6). Paragonimiasis confirmed TB patients in Indonesia, an approach has been found in various reports to be an impor-

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tant cause of chronic cough and hemoptysis in patients initially suspected of pulmonary tuberculosis (7-9). This confirms that the clinical and radiological similarities between paragonimiasis and TB are not merely theoretical phenomena, but rather a real clinical problem with broad diagnostic and therapeutic implications.

Despite sharing cultural, environmental, and ecological conditions and geographical proximity with these endemic countries, Indonesia has not reported a human case of paragonimiasis in modern literature. The last known case was in 1963, involving a Sumatran tiger (*Panthera tigris sumatrae*) (10, 11). Since then, there has been no further surveillance or clinical detection of the parasite in humans. This prolonged absence of documentation raises two possibilities: either *Paragonimus* is indeed absent, or it simply underdiagnosed due to limited clinical awareness and diagnostic capacity.

This situation raises important questions from an epidemiological and public health perspective. Indonesia boasts high biodiversity, extensive freshwater ecosystems, and dietary habits that, in some communities, involve raw or undercooked food. Furthermore, population mobility within and between regions and countries in Southeast Asia is increasing, opening up opportunities for the introduction and spread of parasites across borders (12, 13). The absence of reported human cases for over six decades may reflect successful natural elimination, but it also likely indicates systemic underdiagnosis due to low clinical awareness and limited specific diagnostic facilities.

To date, there is a clear lack of contemporary molecular evidence assessing the presence of *Paragonimus* spp. in human populations in Indonesia, particularly among patients with pulmonary tuberculosis, a group in which clinical overlap may obscure parasitic infections. In endemic settings, the well-documented similarity in clinical presentation between paragonimiasis and TB has made TB-related clinical pathways a practical entry point for identifying previously unrecognized cases of paragonimiasis. Indeed, in several countries, paragonimiasis has been detected among patients initially evaluated for suspected TB, highlighting the usefulness of TB programs as a bridge for human case detection (4, 14).

Importantly, the rationale for focusing on pulmonary TB patients in this study is not to demonstrate or quantify misdiagnosis of paragonimiasis as tuberculosis at the individual level. Rather, TB is used as a pragmatic epidemiological entry point for human surveillance. Furthermore, Indonesia continues to report a substantial number of suspected TB cases that remain bacteriologically unconfirmed, undiagnosed, or unreported within routine surveillance systems. In this context, it is reasonable to hypothesize that a small proportion of these unresolved or presumptive TB cases may represent alternative respiratory diseases with similar clinical presentations, including parasitic infections such as paragonimiasis. Within this broader epidemiological context of unresolved TB suspicion, focusing on bacteriologically confirmed TB patients provides a defined and analytically robust population for exploratory molecular screening.

This study therefore adopts an exploratory approach, using bacteriologically confirmed TB patients as an accessible and clinically relevant population to assess the possible presence of *Paragonimus* spp. in humans, rather than to reclassify or challenge existing TB diagnoses. Addressing this gap, the present study represents the first molecular screening for human paragonimiasis conducted among bacteriologically confirmed pulmonary TB patients in Indonesia. By applying both conventional microscopic examination and molecular detection methods to sputum samples from a high TB-burden urban population in Jakarta, the study generates baseline molecular and epidemiological data that may inform future surveillance strategies, refine diagnostic priorities, and support evidence-based decision-making within national TB control and zoonotic disease monitoring programs.

The primary objective of this study was to assess the potential presence of *Paragonimus* spp. in bacteriologically confirmed pulmonary tuberculosis patients in Indonesia using both conventional microscopic and a molecular approach. The study specifically focused on confirmed TB cases to assess the possibility of *Paragonimus* co-infection within a high TB-burden population, rather than exploring potential misdiagnosis. The novelty of this study lies in the first application of molecular screening for paragonimiasis in a population of

that has not been previously reported. By integrating a zoonotic disease perspective into the context of the national TB program, this study addresses a significant knowledge gap and highlights the need for a more comprehensive diagnostic approach to chronic respiratory diseases in high TB-burden settings.

Methodology

Study Design and Setting

This cross-sectional descriptive study was conducted between February 2023 and July 2024 at four sub-district public health centers in South Jakarta: Pasar Minggu, Tebet, Cilandak, and Pesanggrahan. These sites were selected based on previous reports indicating high TB case notification as reported in the National Tuberculosis Information System (SITB).

Study Population and Sample Size

Participants were selected using simple random sampling from a total of 3,195 smear-positive pulmonary TB patients registered at the four community health centers. The minimum required sample size (calculated using *Statistics and Sample*® software; 95% confidence level; 10% margin of error) was 256. However, only 60 participants provided sputum specimens of sufficient volume for DNA extraction and molecular analysis, as illustrated in Figure 1, which summarizes the overall methodological workflow including participant selection, laboratory screening, and prevalence estimation. Figure 1 summarizes the sequential steps from random sampling of pulmonary TB patients to laboratory screening and prevalence estimation for *Paragonimus* infection.

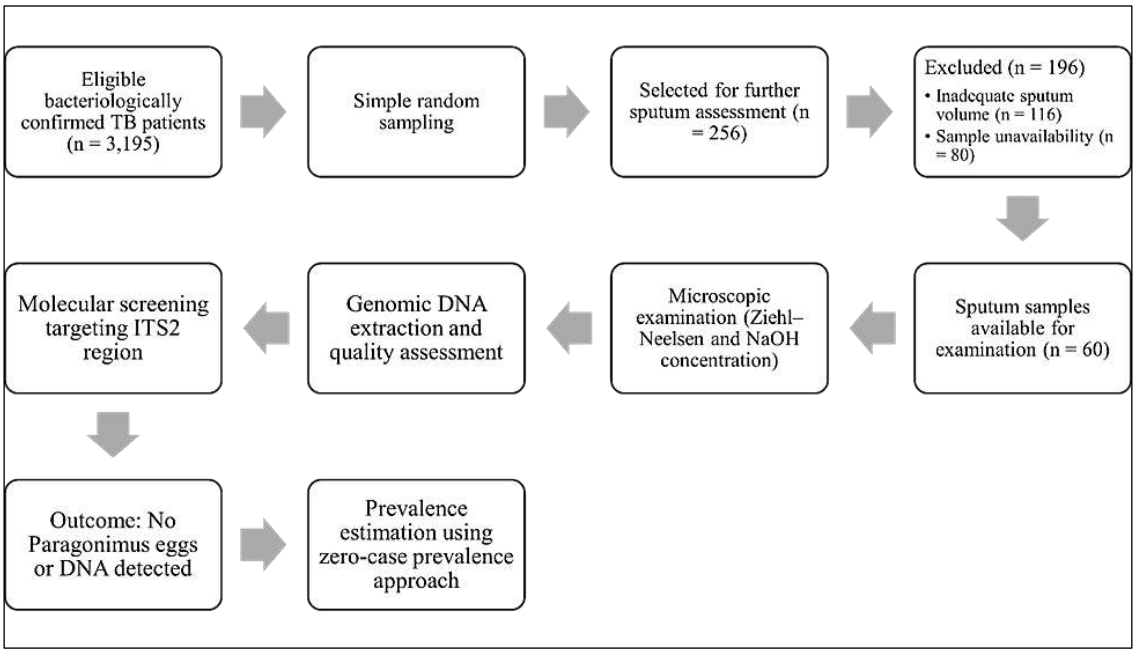

Figure 1: Flowchart of Participant Selection and Analytical Procedures

Inclusion criteria: Age 5–65 years, bacteriologically confirmed pulmonary TB (Ziehl-Neelsen positive), and no anti-TB or antiparasitic treatment in the past six months. Exclusion criteria included extrapulmonary TB, human immunodeficiency virus (HIV) coinfection, or inability to produce adequate sputum.

Sample Collection and Laboratory Processing

Participants submitted two early-morning sputum samples in sterile and wide-mouth containers. Samples were labeled and transported under cold chain to the biomolecular laboratory at Universitas Trisakti.

Microscopic Examination

All sputum specimens were first screened using Ziehl-Neelsen staining to reconfirm TB diagnosis. The NaOH concentration technique was applied to concentrate the samples for helminth eggs detection. Smears were examined under light microscopy at 100× and 400× magnification by two independent parasitologists blinded to PCR results. Any suspected *Paragonimus* egg was documented and re-evaluated under high magnification (1000×) with oil immersion.

DNA Extraction and Gel Electrophoresis

Genomic DNA was extracted from all 60 sputum samples using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's protocol, with minor modifications to optimize yield from mucous-rich specimens. Briefly, 1.5 ml of sputum was examined with 1.5 ml of a mucolytic solution containing 2% sodium hydroxide, 1.45% trisodium citrate, and 0.5% N-acetyl L-cysteine. The mixture was vortexed and incubated at room temperature for 15 minutes, then diluted to a total volume of 50 ml with 1× phosphate-buffered saline (PBS) and centrifuged at 3000 rpm for 20 minutes. The supernatant was discarded, and the pellet was re-suspended in 200 µl PBS, followed by heat treatment at 99 °C for 20 minutes.

DNA concentration and purity were measured using NanoDrop spectrophotometry (Thermo Scientific, USA). Extracted DNA was stored at −20°C until analysis. DNA quality was first assessed by agarose gel electrophoresis (1.5% agarose in 1× TBE buffer, stained with ethidium bromide) and visualized under UV illumination to confirm total DNA integrity. A 100 bp DNA ladder was used as a molecular size marker. The positive control used in this assay was derived from *Paragonimus westermani* adult tissue maintained in the reference collection of the Molecular Parasitology Laboratory, Universitas Trisakti. A no-template negative control was included in all reactions to monitor potential contamination. The expected ~221 bp amplicon corresponds to the internal transcribed spacer 2 (ITS2) region of *Paragonimus* spp., which has been previously validated (4, 7). This fragment size represents the specific PCR product, not the total extracted DNA visualized prior to amplification. Therefore, at this stage, gel electrophoresis was used only to verify the presence and integrity of total genomic DNA, not to identify *Paragonimus*-specific fragments. Validated ITS2 primers [Forward: 5'-GGTACCGGTGGATCACTCGGCTCGTG-3' Reverse: 5'-GGGATCCTGGTTAGTTTCTTTTCCTCCGC-3'] were available; however, PCR amplification was planned as a subsequent analytical step following DNA quality verification. As no *Paragonimus*-specific DNA signals were observed during the preliminary molecular screening stage, further amplification was not pursued.

Analytical Framework

The study followed a predefined stepwise analytical framework to identify potential *Paragonimus* infection among pulmonary TB patients. Following participant selection and sputum collection, all samples underwent microscopic examination and genomic DNA extraction. Molecular screening targeting the ITS2 region was planned as the final analytical step and was contingent upon meeting DNA integrity and quality criteria. In the absence of detectable *Paragonimus* signals by microscopy or molecular screening, prevalence estimation was performed using a zero-case prevalence approach to ensure appropriate epidemiological interpretation.

Data Analysis

The data collected were coded and entered into Microsoft Excel, then analyzed using SPSS software version 25.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics were used to summarize patients' demographics and diagnostic outcomes. Results were expressed as frequencies and percentages. Paragonimiasis prevalence was calculated only if positive cases were identified.

Results

Distribution of Tuberculosis-Positive Cases

During the study period, a total of 3,195 bacteriologically confirmed pulmonary tuberculosis (TB) cases were recorded across four sub-district community health centers in South Jakarta: Pasar Minggu, Tebet, Cilandak, and Pesanggrahan, as summarized in Table 1. The highest proportion of cases occurred in Pasar Minggu (37.8%), followed by Cilandak (31.0%), Tebet (19.2%), and Pesanggrahan. This distribution provides the epidemiological basis for selecting these sites as representative areas for *Paragonimus* screening among confirmed TB patients.

Table 1: Demographic Distribution of Tuberculosis-Positive Patients by Sub-District Health Centers in South Jakarta (N = 3,195)

Characteristics	Community Health Center				Total n (%)
	Pasar Minggu n (%)	Tebet n (%)	Cilandak n (%)	Pesanggrahan n (%)	
Total TB cases	1209 (37.84)	612 (19.15)	991 (31.02)	383 (11.99)	3195 (100.0)
Sex					
Male	918 (75.93)	364 (59.48)	624 (62.97)	211 (55.09)	2117 (66.26)
Female	291 (24.07)	248 (40.52)	367 (37.03)	172 (44.91)	1078 (33.74)
Age group (years)					
0–4	73 (6.04)	36 (5.88)	118 (11.91)	23 (6.01)	250 (7.82)
5–14	122 (10.09)	134 (21.89)	149 (15.04)	17 (4.44)	422 (13.21)
15–30	217 (17.95)	152 (24.84)	205 (20.69)	128 (33.42)	702 (21.97)
31-45	429 (35.48)	248 (40.52)	360 (36.33)	58 (15.14)	1095 (34.27)
46-60	332 (27.46)	42 (6.86)	138 (13.93)	130 (33.94)	642 (20.09)
>60	36 (2.98)	0 (0)	21 (2.12)	27 (7.05)	84 (2.63)

Note: Data are presented as the number and percentage of TB-positive patients within each subdistrict community health center

Microscopic examination of all 60 sputum samples showed no *Paragonimus* eggs. None of the DNA extracts demonstrated detectable amplifiable DNA suitable for PCR amplification. Therefore, PCR testing for *Paragonimus*-specific ITS2 fragments was not performed on clinical samples. Positive and negative controls were run in parallel to validate assay performance.

Gender and Age Profile

The overall gender distribution showed a predominance of male patients (n = 2,117; 66.26%), with a male-to-female ratio of approximately 2:1. This ratio is consistent with national TB surveillance data and aligns with global trends indicating higher TB incidence among men, possibly due to occupational exposure, smoking, and delayed healthcare-seeking behavior.

In terms of age distribution, the highest burden of TB was found in adults aged 31–45 years (n = 1,095; 34.27%), followed by those aged 46–60 years (n = 642; 20.09%) and 15–30 years (n = 702; 21.97%). This trend was consistent across all CHCs, with Pasar Minggu and Cilandak recording particularly high TB burdens among adults aged 31–45 (35.48% and 36.33%, respectively). Pesanggrahan presented a unique distribution, with a substantial proportion of TB cases observed in the 46–60 age group (33.94%). The pediatric population, though less frequent, was not negligible: 672 cases (21.03%) occurred in children under 15 years of age, with the 5–14 age group (n = 422; 13.21%) showing higher case numbers than the 0–4 age group (n = 250; 7.82%).

Microscopic Detection for *Paragonimus* spp. Ova

Despite the clinical suspicion of potential misdiagnosis between TB and paragonimiasis, no *Paragonimus* eggs were detected in a total of 60 sputum specimens that were examined using two conventional methods. The specificity of the NaOH concentration technique in detecting the absence of *Paragonimus* ova was found to be 100% (95% CI: 98.90% to 100%), as confirmed by comparison with the Ziehl-Neelsen staining technique. Images of sputum slides prepared with the NaOH concentration technique and ZNS staining are shown in Figures 2A and 2B, respectively. The absence of egg detection may reflect either the true absence of *Paragonimus* infection in this population or limitations of conventional microscopy, especially in cases of light infections, intermittent egg shedding, or chronic lesions.

Detection of *Paragonimus* DNA by PCR

In this study, DNA extracted from all 60 sputum samples showed no visible amplifiable *Paragonimus*-specific bands when assessed by agarose gel electrophoresis, indicating very low DNA concentration and quality insufficient for PCR amplification. Therefore, PCR testing was not performed on clinical samples. NanoDrop spectrophotometry confirmed the low yield, with absorbance values below the reliable quantification range (<5 ng/μL) and inconsistent A260/A280 ratios.

As shown in Figure 3, the positive control displayed a distinct 221 bp band, while Lanes 3–5 (clinical sputum samples) showed no visible amplification products, confirming the absence of amplifiable *Paragonimus* DNA in all tested specimens.

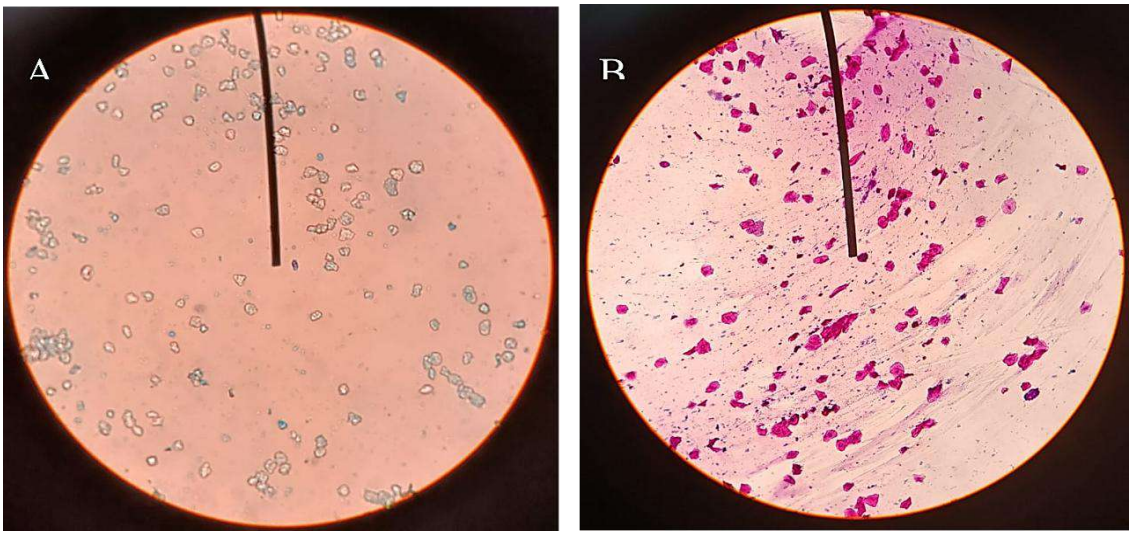

Figure 2: Microscopic Examination of Sputum Demonstrating *Mycobacterium Tuberculosis* Bacilli (Red Rods) and Absence of *Paragonimus* Eggs Using Two Different Techniques at 1000× Magnification (Oil Immersion) (A) Smear Prepared Using the NaOH Concentration Technique (B) Ziehl–Neelsen–Stained Smear

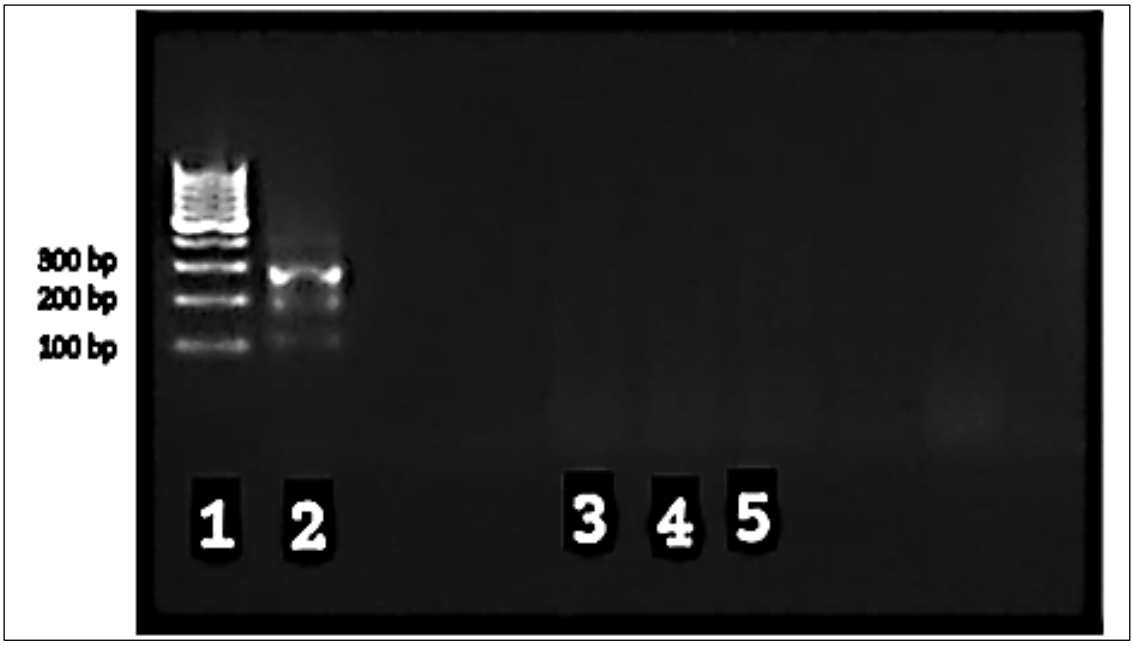

Figure 3. Gel Electrophoresis of Extracted DNA from Sputum Samples. Lane M: 100 Bp DNA Ladder; Lane 1: Negative Control; Lane 2: Positive Control DNA from *Paragonimus westermani*; Lanes 3–5: Clinical Sputum Samples with No Visible *Paragonimus*-Specific Amplification Bands

Discussion

This study explored the potential co-existence of paragonimiasis among bacteriologically confirmed TB patients in South Jakarta. Despite longstanding epidemiological concerns regarding the potential co-infection or clinical overlap between paragonimiasis and TB in endemic areas, our findings revealed no evidence of *Paragonimus* infection. Neither helminth eggs were detected microscopically nor *Paragonimus*-specific DNA identified through molecular analysis targeting the ITS2 region. These findings underscore the significant burden of TB among economically productive adults, reflecting its dual impact on public health and socioeconomic stability. In addition, the notable proportion of pediatric cases highlights the need for strengthened early detection, contact tracing, and screening programs

in household settings to prevent underdiagnosis and disease progression.

The molecular findings further support the absence of *Paragonimus* infection among bacteriologically confirmed TB patients in this study. Based on the zero-case prevalence concept at a 95% confidence level, the absence of detectable *Paragonimus* DNA suggests that the prevalence in this population is below one percent (15). This aligns with the epidemiological context of urban Jakarta, where *Paragonimus* transmission is unlikely due to modernized food practices and limited exposure to freshwater crustacean hosts. These results indicate that the lack of amplification was not a technical failure, as internal positive and negative controls confirmed assay validity, but rather reflect a true absence of *Paragonimus* infection in the sampled population. Nevertheless, the findings highlight the need for continued surveillance in rural or high-risk areas where traditional consumption of undercooked crabs or crayfish persists.

The absence of detectable *Paragonimus* should not be dismissed as inconsequential. The lack of ova in all samples supports the hypothesis that *Paragonimus* is not currently endemic in the area under study. Microscopy using ZNS and NaOH concentration techniques demonstrated high specificity and may continue to serve as a supportive diagnostic tool for differentiating TB from paragonimiasis, especially in endemic regions (4, 16). However, these methods may have limited sensitivity in low-burden settings. This brings substantial epidemiological and clinical significance in the context of evolving disease landscapes and diagnostic practices in tropical medicine. The absence of amplifiable DNA, as confirmed by RNaseP amplification failure, indicates possible challenges in sputum-based molecular diagnostics due to DNA degradation or the presence of inhibitors (17, 18). The limitation underscores the importance of optimizing DNA extraction protocols, particularly for detecting foodborne parasites such as *Paragonimus*.

Our findings provide empirical evidence that the prevalence of pulmonary paragonimiasis among TB-confirmed individuals in urban Jakarta is likely negligible or has markedly declined, challenging previous assumptions of co-endemicity that may have been based on outdated, unreliable, or unverified data. Although our results align with historical records showing the absence of paragonimiasis reporting in Indonesia since 1963 was observed, the potential for re-emergence remains, given increasing seafood imports, travel, and diversification of food culture in the country (10, 11). In settings where paragonimiasis remains endemic, misdiagnosis of pulmonary paragonimiasis as TB may still occur; however, among confirmed TB cases such as ours, the concern shifts toward potential co-infection rather than diagnostic confusion (19).

The clinical overlap between paragonimiasis and TB has been extensively documented. Both conditions may present chronic cough, hemoptysis, pleuritic chest pain, and radiological findings such as cavitary or nodular lesions (19, 20). In resource-limited settings, these overlapping features have historically led to empirical anti-TB therapy in cases where parasitic infections were the actual cause. Studies conducted in Peru, Ecuador, and the Philippines reported that up to 20% of suspected TB patients were eventually diagnosed with paragonimiasis (16, 21). However, those studies were carried out in rural or highland areas with well-documented *Paragonimus* transmission linked to traditional dietary habits such as the consumption of undercooked freshwater crabs or crayfish.

Our study setting in urban Jakarta represents a contrasting environment. Over the past two decades, urbanization, improved food safety awareness, and changing dietary habits have likely contributed to the suppression of *Paragonimus* transmission. Additionally, the absence of *Paragonimus*-specific DNA in our samples, despite employing sensitive molecular assays, supports the notion that urban TB patients are currently not at significant risk for paragonimiasis, even in a country historically regarded as endemic. This finding aligns with recent parasitological mapping efforts in Indonesia, which have reported no active *Paragonimus* foci in recent years (22).

From a methodological standpoint, the strength of our study lies in its dual-layered diagnostic approach, integrating microscopy and DNA-based methods. Conventional microscopy remains valuable and cost-effective but suffers from low sensitivity, particularly when egg output is sparse or intermittent (4, 16). Molecular diagnostics targeting the ITS2 region of *Paragonimus* spp. offer a more reliable alternative with high specificity,

even in pauciparasitic infections (23). The absence of amplifiable *Paragonimus*-specific DNA, therefore, is not a reflection of technical failure but a valid representation of the lack of infection, reinforced by the use of internal controls and reference assays confirming methodological reliability.

Publishing negative or null findings remains an underappreciated yet essential scientific practice (24). Such evidence corrects biased assumptions, prevents redundant investigations, and informs policymakers and clinicians about evolving patterns of disease distribution (25, 26). In our context, it alerts healthcare professionals that routine suspicion of paragonimiasis in urban TB cases—without relevant exposure history or clinical indicators—may no longer be justified. This can optimize diagnostic workflows, reduce empirical antiparasitic treatments, and ensure better allocation resources toward condition with active transmission. Rather than maintaining the status quo, these findings provide evidence-based guidance for rational testing strategies—prioritizing *Paragonimus* screening only in populations or regions with known dietary or ecological risk factors.

This study has several limitations that should be acknowledged. First, sampling was limited to bacteriologically confirmed pulmonary TB patients from an urban population, which may not adequately represent rural or highland communities where *Paragonimus* transmission is more likely. Second, the low yield and variable quality of DNA extracted from sputum specimens could have reduced molecular detection sensitivity. However, the inclusion of internal positive and negative controls verified assay validity, indicating that the absence of amplification was unlikely to be due to technical failure. Finally, the modest sample size may limit the statistical power and the generalizability of the findings beyond the study population.

Lastly, our findings highlight the epidemiological heterogeneity of paragonimiasis and the importance of interpreting historical reports within contemporary contexts. Parasitic infections often exhibit highly focal distributions influenced by ecological and cultural factors, even within countries considered endemic (12, 27). As a result, broad assumptions of co-endemicity may lead to misinterpretation of diagnostic priorities and disease risk (7, 28). The absence of *Paragonimus* spp. in the present study area, despite historical records, illustrates how localized transmission patterns and temporal changes may limit the applicability of older literature.

Implications and Recommendations

Based on the current findings, routine screening for paragonimiasis among urban pulmonary TB patients may not be necessary in low-prevalence settings. However, a targeted diagnostic approach is recommended for patients with atypical clinical features, poor response to anti-tuberculosis treatment, or relevant dietary and environmental exposure histories. Integration of risk-based molecular surveillance into existing TB laboratory networks may enhance early detection of neglected zoonotic infections without imposing unnecessary diagnostic burden. Future research should prioritize high-risk populations, rural or endemic-adjacent regions, and alternative clinical specimens to refine prevalence estimates and inform evidence-based surveillance strategies in Indonesia.

Conclusion

This study presents the first molecular screening for human paragonimiasis among bacteriologically confirmed pulmonary tuberculosis patients in an urban setting in Indonesia. The absence of *Paragonimus* spp. eggs and DNA in sputum samples indicates a minimal risk of paragonimiasis co-infection in the Jakarta study population during the study period. These findings provide contemporary molecular evidence to refine diagnostic priorities, support a targeted risk-based surveillance approach rather than routine screening in urban TB settings, and inform prevention strategies and epidemiological monitoring for neglected zoonotic infections within TB-endemic areas. Molecular diagnostics remain reliable tools for surveillance and should be integrated into broader One Health initiatives; however, these conclusions should be interpreted in light of limitations related to urban-only sampling, modest sample size, and constraints of sputum-based molecular detection. Future research should expand surveillance to rural and highland communities with traditional dietary practices and incorporate environmental and intermediate host sampling to more

comprehensively assess *Paragonimus* transmission risk in Indonesia.

Abbreviations

DNA: Deoxyribonucleic acid, PCR: Polymerase Chain Reaction, SITB: Sistem Informasi Tuberkulosis (the National Tuberculosis Information System), TB: Tuberculosis, ZNS: Ziehl Neelsen-staining.

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Author Contributions

Machrumnizar: conceptualization, methodology, software, validation, funding acquisition, investigation, resources, data curation, original draft writing, review writing, editing, Suriyani Tan: validation, formal analysis, resources, data curation, review writing, editing, visualization, Yuliana: project administration, data collection, field coordinator, proofreading, Rina Kurniasri Kusumaratna: supervision, resources, project administration. All authors have read and approved the final manuscript.

Conflict of Interest

The authors state that the study was performed without any commercial or financial ties that may be seen as a possible conflict of interest during the study.

Declaration of Artificial Intelligence (AI) Assistance

This manuscript was prepared using generative artificial intelligence (AI) tools, specifically Grammarly and QuillBot, for language editing, paraphrasing, and enhancement of academic writing. style. These technologies facilitated the establishment of coherent sentence structures, grammatical precision, and tense consistency throughout the document. The intellectual content, critical analysis, data interpretation, reference selection, and final conclusions in this manuscript were exclusively conceptualized, reviewed, and validated by the authors. No AI technology was employed to produce, analyze, or interpret scientific data, nor to execute any study assignments beyond language support. The authors are completely responsible for the manuscript's originality and accuracy and have ensured that the use of AI-assisted technology follows ethical norms and publication rules.

Ethics Approval

The study involving human participants was reviewed and approved by the Research Ethics Committee of Faculty of Medicine, Universitas Trisakti (Approval No. 056/KEWFK/III2023), and conducted according to the guidelines of the Declaration of Helsinki. Written informed consent to participate in this study was obtained from all participants or their legal guardians.

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Absence of *Paragonimus* DNA and Eggs in Tuberculosis Patients in Indonesia: Implications for Surveillance and Public Health Awareness

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Abstract

Paragonimus spp. are zoonotic trematodes endemic in several Asian countries, often mimicking pulmonary tuberculosis (TB) due to overlapping clinical manifestations. Although Indonesia's geographical proximity to endemic countries and shared culinary practices may increase exposure risk, human paragonimiasis has not been reported since a 1963 case in a Sumatran tiger. This study represents the first molecular screening for human paragonimiasis in Indonesia, aiming to investigate *Paragonimus* DNA and eggs in sputum samples of pulmonary TB patients. This study addresses infectious disease epidemiology and prevention through molecular surveillance. A cross-sectional study was conducted involving 60 sputum samples from TB patients in South Jakarta. Microscopy examination using Ziehl–Neelsen staining and the NaOH concentration method confirmed *Mycobacterium tuberculosis* in all samples, with no *Paragonimus* eggs observed. DNA extracted was performed using a commercial kit and DNA quality was assessed via agarose gel electrophoresis. Positive and negative controls were included to verify assay performance. None of the clinical DNA extracts showed visible *Paragonimus*-specific bands (~221 bp), and therefore PCR amplification was not performed. Although *Paragonimus* was not detected, this does not definitely exclude its presence. Using the zero-case prevalence concept with a 95% confidence level, the findings suggest a prevalence of less than one percent in the study population. Our findings underscore the importance of maintaining diagnostic vigilance for neglected parasitic infections in Indonesia, provide evidence to guide prevention strategies, enhance epidemiological monitoring for zoonotic diseases in TB-endemic areas, supporting the urgency of updating national parasitological surveillance policies and strengthening One Health-based disease monitoring.

Keywords: Molecular, Neglected Tropical Disease, One Health Paragonimiasis, Tuberculosis.

Introduction

Foodborne zoonotic parasites remain a significant public health challenge in many developing countries, particularly in Southeast Asia (1). Changing consumption patterns, increased human mobility, and intensified interactions between humans, animals, and the environment contribute to the emergence and persistence of various often-neglected parasitic diseases. One group of parasites included in this category is the pulmonary trematode of the genus *Paragonimus*, which has received less attention globally than other infectious diseases with similar respiratory manifestations (2). *Paragonimus* infections can cause chronic morbidity, reduce quality of life, and burden the health system due to misdiagnosis and mismanagement (3).

Paragonimus spp., also known as lung flukes, are foodborne trematodes transmitted to humans through the ingestion of raw or undercooked freshwater crustaceans. These parasites cause paragonimiasis, a zoonotic infection (2, 3). The manifestations include chronic cough, hemoptysis, and radiographic abnormalities, which often resemble pulmonary tuberculosis (TB). These similarities lead to frequent diagnostic misclassification in endemic regions where both infections co-occur. Human cases of paragonimiasis have been widely reported in several countries across East and Southeast Asia, including China, the Philippines, Thailand, South Korea, and Vietnam (4, 5).

Several epidemiological and clinical studies in these countries have shown that paragonimiasis is frequently found in populations with traditional dietary habits based on raw or undercooked freshwater crabs or shrimp (6). Paragonimiasis confirmed TB patients in Indonesia, an approach has been found in various reports to be an impor-

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tant cause of chronic cough and hemoptysis in patients initially suspected of pulmonary tuberculosis (7-9). This confirms that the clinical and radiological similarities between paragonimiasis and TB are not merely theoretical phenomena, but rather a real clinical problem with broad diagnostic and therapeutic implications.

Despite sharing cultural, environmental, and ecological conditions and geographical proximity with these endemic countries, Indonesia has not reported a human case of paragonimiasis in modern literature. The last known case was in 1963, involving a Sumatran tiger (*Panthera tigris sumatrae*) (10, 11). Since then, there has been no further surveillance or clinical detection of the parasite in humans. This prolonged absence of documentation raises two possibilities: either *Paragonimus* is indeed absent, or it simply underdiagnosed due to limited clinical awareness and diagnostic capacity.

This situation raises important questions from an epidemiological and public health perspective. Indonesia boasts high biodiversity, extensive freshwater ecosystems, and dietary habits that, in some communities, involve raw or undercooked food. Furthermore, population mobility within and between regions and countries in Southeast Asia is increasing, opening up opportunities for the introduction and spread of parasites across borders (12, 13). The absence of reported human cases for over six decades may reflect successful natural elimination, but it also likely indicates systemic underdiagnosis due to low clinical awareness and limited specific diagnostic facilities. To date, there is a clear lack of contemporary molecular evidence assessing the presence of *Paragonimus* spp. in human populations in Indonesia, particularly among patients with pulmonary tuberculosis, a group in which clinical overlap may obscure parasitic infections. In endemic settings, the well-documented similarity in clinical presentation between paragonimiasis and TB has made TB-related clinical pathways a practical entry point for identifying previously unrecognized cases of paragonimiasis. Indeed, in several countries, paragonimiasis has been detected among patients initially evaluated for suspected TB, highlighting the usefulness of TB programs as a bridge for human case detection (4, 14).

Importantly, the rationale for focusing on pulmonary TB patients in this study is not to demonstrate or quantify misdiagnosis of paragonimiasis as tuberculosis at the individual level. Rather, TB is used as a pragmatic epidemiological entry point for human surveillance. Furthermore, Indonesia continues to report a substantial number of suspected TB cases that remain bacteriologically unconfirmed, undiagnosed, or unreported within routine surveillance systems. In this context, it is reasonable to hypothesize that a small proportion of these unresolved or presumptive TB cases may represent alternative respiratory diseases with similar clinical presentations, including parasitic infections such as paragonimiasis. Within this broader epidemiological context of unresolved TB suspicion, focusing on bacteriologically confirmed TB patients provides a defined and analytically robust population for exploratory molecular screening.

This study therefore adopts an exploratory approach, using bacteriologically confirmed TB patients as an accessible and clinically relevant population to assess the possible presence of *Paragonimus* spp. in humans, rather than to reclassify or challenge existing TB diagnoses. Addressing this gap, the present study represents the first molecular screening for human paragonimiasis conducted among bacteriologically confirmed pulmonary TB patients in Indonesia. By applying both conventional microscopic examination and molecular detection methods to sputum samples from a high TB-burden urban population in Jakarta, the study generates baseline molecular and epidemiological data that may inform future surveillance strategies, refine diagnostic priorities, and support evidence-based decision-making within national TB control and zoonotic disease monitoring programs.

The primary objective of this study was to assess the potential presence of *Paragonimus* spp. in bacteriologically confirmed pulmonary tuberculosis patients in Indonesia using both conventional microscopic and a molecular approach. The study specifically focused on confirmed TB cases to assess the possibility of *Paragonimus* co-infection within a high TB-burden population, rather than exploring potential misdiagnosis. The novelty of this study lies in the first application of molecular screening for paragonimiasis in a population of

that has not been previously reported. By integrating a zoonotic disease perspective into the context of the national TB program, this study addresses a significant knowledge gap and highlights the need for a more comprehensive diagnostic approach to chronic respiratory diseases in high TB-burden settings.

Methodology

Study Design and Setting

This cross-sectional descriptive study was conducted between February 2023 and July 2024 at four sub-district public health centers in South Jakarta: Pasar Minggu, Tebet, Cilandak, and Pesanggrahan. These sites were selected based on previous reports indicating high TB case notification as reported in the National Tuberculosis Information System (SITB).

Study Population and Sample Size

Participants were selected using simple random sampling from a total of 3,195 smear-positive pulmonary TB patients registered at the four community health centers. The minimum required sample size (calculated using *Statistics and Sample*® software; 95% confidence level; 10% margin of error) was 256. However, only 60 participants provided sputum specimens of sufficient volume for DNA extraction and molecular analysis, as illustrated in Figure 1, which summarizes the overall methodological workflow including participant selection, laboratory screening, and prevalence estimation. Figure 1 summarizes the sequential steps from random sampling of pulmonary TB patients to laboratory screening and prevalence estimation for *Paragonimus* infection.

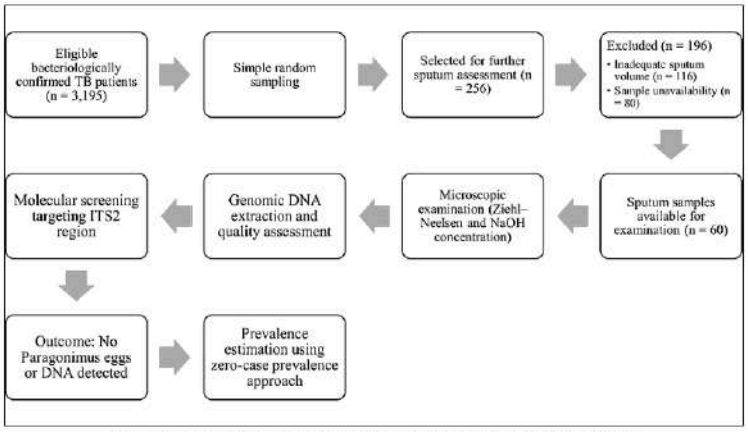

Figure 1: Flowchart of Participant Selection and Analytical Procedures

Inclusion criteria: Age 5–65 years, bacteriologically confirmed pulmonary TB (Ziehl-Neelsen positive), and no anti-TB or antiparasitic treatment in the past six months. Exclusion criteria included extrapulmonary TB, human immunodeficiency virus (HIV) coinfection, or inability to produce adequate sputum.

Sample Collection and Laboratory Processing

Participants submitted two early-morning sputum samples in sterile and wide-mouth containers. Samples were labeled and transported under cold chain to the biomolecular laboratory at Universitas Trisakti.

Microscopic Examination

All sputum specimens were first screened using Ziehl-Neelsen staining to reconfirm TB diagnosis. The NaOH concentration technique was applied to concentrate the samples for helminth eggs detection. Smears were examined under light microscopy at 100× and 400× magnification by two independent parasitologists blinded to PCR results. Any suspected *Paragonimus* egg was documented and re-evaluated under high magnification (1000×) with oil immersion.

DNA Extraction and Gel Electrophoresis

Genomic DNA was extracted from all 60 sputum samples using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's protocol, with minor modifications to optimize yield from mucous-rich specimens. Briefly, 1.5 ml of sputum was examined with 1.5 ml of a mucolytic solution containing 2% sodium hydroxide, 1.45% trisodium citrate, and 0.5% N-acetyl L-cysteine. The mixture was vortexed and incubated at room temperature for 15 minutes, then diluted to a total volume of 50 ml with 1× phosphate-buffered saline (PBS) and centrifuged at 3000 rpm for 20 minutes. The supernatant was discarded, and the pellet was re-suspended in 200 µl PBS, followed by heat treatment at 99 °C for 20 minutes.

DNA concentration and purity were measured using NanoDrop spectrophotometry (Thermo Scientific, USA). Extracted DNA was stored at −20°C until analysis. DNA quality was first assessed by agarose gel electrophoresis (1.5% agarose in 1× TBE buffer, stained with ethidium bromide) and visualized under UV illumination to confirm total DNA integrity. A 100 bp DNA ladder was used as a molecular size marker. The positive control used in this assay was derived from *Paragonimus westermani* adult tissue maintained in the reference collection of the Molecular Parasitology Laboratory, Universitas Trisakti. A no-template negative control was included in all reactions to monitor potential contamination. The expected ~221 bp amplicon corresponds to the internal transcribed spacer 2 (ITS2) region of *Paragonimus* spp., which has been previously validated (4, 7). This fragment size represents the specific PCR product, not the total extracted DNA visualized prior to amplification. Therefore, at this stage, gel electrophoresis was used only to verify the presence and integrity of total genomic DNA, not to identify *Paragonimus*-specific fragments. Validated ITS2 primers [Forward: 5'-GGTACCGGTGGATCACTCGGCTCGTG-3' Reverse: 5'-GGGATCCTGGTTAGTTTCTTTTCCTCCGC-3'] were available; however, PCR amplification was planned as a subsequent analytical step following DNA quality verification. As no *Paragonimus*-specific DNA signals were observed during the preliminary molecular screening stage, further amplification was not pursued.

Analytical Framework

The study followed a predefined stepwise analytical framework to identify potential *Paragonimus* infection among pulmonary TB patients. Following participant selection and sputum collection, all samples underwent microscopic examination and genomic DNA extraction. Molecular screening targeting the ITS2 region was planned as the final analytical step and was contingent upon meeting DNA integrity and quality criteria. In the absence of detectable *Paragonimus* signals by microscopy or molecular screening, prevalence estimation was performed using a zero-case prevalence approach to ensure appropriate epidemiological interpretation.

Data Analysis

The data collected were coded and entered into Microsoft Excel, then analyzed using SPSS software version 25.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics were used to summarize patients' demographics and diagnostic outcomes. Results were expressed as frequencies and percentages. Paragonimiasis prevalence was calculated only if positive cases were identified.

Results

Distribution of Tuberculosis-Positive Cases

During the study period, a total of 3,195 bacteriologically confirmed pulmonary tuberculosis (TB) cases were recorded across four sub-district community health centers in South Jakarta: Pasar Minggu, Tebet, Cilandak, and Pesanggrahan, as summarized in Table 1. The highest proportion of cases occurred in Pasar Minggu (37.8%), followed by Cilandak (31.0%), Tebet (19.2%), and Pesanggrahan. This distribution provides the epidemiological basis for selecting these sites as representative areas for *Paragonimus* screening among confirmed TB patients.

Table 1: Demographic Distribution of Tuberculosis-Positive Patients by Sub-District Health Centers in South Jakarta (N = 3,195)

Characteristics	Community Health Center				Total n (%)
	Pasar Minggu n (%)	Tebet n (%)	Cilandak n (%)	Pesanggrahan n (%)	
Total TB cases	1209 (37.84)	612 (19.15)	991 (31.02)	383 (11.99)	3195 (100.0)
Sex					
Male	918 (75.93)	364 (59.48)	624 (62.97)	211 (55.09)	2117 (66.26)
Female	291 (24.07)	248 (40.52)	367 (37.03)	172 (44.91)	1078 (33.74)
Age group (years)					
0–4	73 (6.04)	36 (5.88)	118 (11.91)	23 (6.01)	250 (7.82)
5–14	122 (10.09)	134 (21.89)	149 (15.04)	17 (4.44)	422 (13.21)
15–30	217 (17.95)	152 (24.84)	205 (20.69)	128 (33.42)	702 (21.97)
31–45	429 (35.48)	248 (40.52)	360 (36.33)	58 (15.14)	1095 (34.27)
46–60	332 (27.46)	42 (6.86)	138 (13.93)	130 (33.94)	642 (20.09)
>60	36 (2.98)	0 (0)	21 (2.12)	27 (7.05)	84 (2.63)

Note: Data are presented as the number and percentage of TB-positive patients within each subdistrict community health center

Microscopic examination of all 60 sputum samples showed no *Paragonimus* eggs. None of the DNA extracts demonstrated detectable amplifiable DNA suitable for PCR amplification. Therefore, PCR testing for *Paragonimus*-specific ITS2 fragments was not performed on clinical samples. Positive and negative controls were run in parallel to validate assay performance.

Gender and Age Profile

The overall gender distribution showed a predominance of male patients (n = 2,117; 66.26%), with a male-to-female ratio of approximately 2:1. This ratio is consistent with national TB surveillance data and aligns with global trends indicating higher TB incidence among men, possibly due to occupational exposure, smoking, and delayed healthcare-seeking behavior.

In terms of age distribution, the highest burden of TB was found in adults aged 31–45 years (n = 1,095; 34.27%), followed by those aged 46–60 years (n = 642; 20.09%) and 15–30 years (n = 702; 21.97%). This trend was consistent across all CHCs, with Pasar Minggu and Cilandak recording particularly high TB burdens among adults aged 31–45 (35.48% and 36.33%, respectively). Pesanggrahan presented a unique distribution, with a substantial proportion of TB cases observed in the 46–60 age group (33.94%). The pediatric population, though less frequent, was not negligible: 672 cases (21.03%) occurred in children under 15 years of age, with the 5–14 age group (n = 422; 13.21%) showing higher case numbers than the 0–4 age group (n = 250; 7.82%).

Microscopic Detection for *Paragonimus* spp. Ova

Despite the clinical suspicion of potential misdiagnosis between TB and paragonimiasis, no *Paragonimus* eggs were detected in a total of 60 sputum specimens that were examined using two conventional methods. The specificity of the NaOH concentration technique in detecting the absence of *Paragonimus* ova was found to be 100% (95% CI: 98.90% to 100%), as confirmed by comparison with the Ziehl-Neelsen staining technique. Images of sputum slides prepared with the NaOH concentration technique and ZNS staining are shown in Figures 2A and 2B, respectively. The absence of egg detection may reflect either the true absence of *Paragonimus* infection in this population or limitations of conventional microscopy, especially in cases of light infections, intermittent egg shedding, or chronic lesions.

Detection of *Paragonimus* DNA by PCR

In this study, DNA extracted from all 60 sputum samples showed no visible amplifiable *Paragonimus*-specific bands when assessed by agarose gel electrophoresis, indicating very low DNA concentration and quality insufficient for PCR amplification. Therefore, PCR testing was not performed on clinical samples. NanoDrop spectrophotometry confirmed the low yield, with absorbance values below the reliable quantification range (<5 ng/μL) and inconsistent A260/A280 ratios.

As shown in Figure 3, the positive control displayed a distinct 221 bp band, while Lanes 3–5 (clinical sputum samples) showed no visible amplification products, confirming the absence of amplifiable *Paragonimus* DNA in all tested specimens.

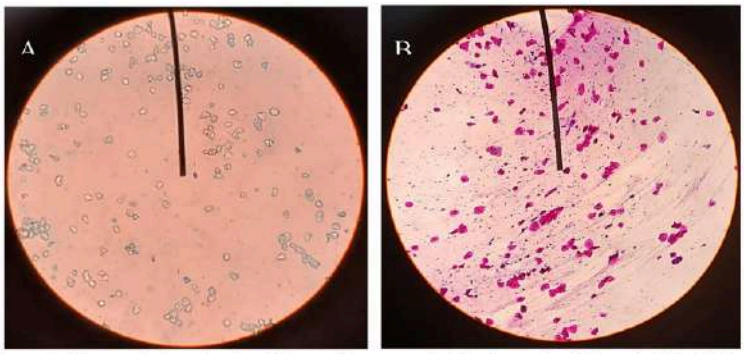

Figure 2: Microscopic Examination of Sputum Demonstrating *Mycobacterium Tuberculosis* Bacilli (Red Rods) and Absence of *Paragonimus* Eggs Using Two Different Techniques at 1000× Magnification (Oil Immersion) (A) Smear Prepared Using the NaOH Concentration Technique (B) Ziehl–Neelsen–Stained Smear

Figure 3. Gel Electrophoresis of Extracted DNA from Sputum Samples. Lane M: 100 Bp DNA Ladder; Lane 1: Negative Control; Lane 2: Positive Control DNA from *Paragonimus westermani*; Lanes 3–5: Clinical Sputum Samples with No Visible *Paragonimus*-Specific Amplification Bands

Discussion

This study explored the potential co-existence of paragonimiasis among bacteriologically confirmed TB patients in South Jakarta. Despite longstanding epidemiological concerns regarding the potential co-infection or clinical overlap between paragonimiasis and TB in endemic areas, our findings revealed no evidence of *Paragonimus* infection. Neither helminth eggs were detected microscopically nor *Paragonimus*-specific DNA identified through molecular analysis targeting the ITS2 region. These findings underscore the significant burden of TB among economically productive adults, reflecting its dual impact on public health and socioeconomic stability. In addition, the notable proportion of pediatric cases highlights the need for strengthened early detection, contact tracing, and screening programs

in household settings to prevent underdiagnosis and disease progression.

The molecular findings further support the absence of *Paragonimus* infection among bacteriologically confirmed TB patients in this study. Based on the zero-case prevalence concept at a 95% confidence level, the absence of detectable *Paragonimus* DNA suggests that the prevalence in this population is below one percent (15). This aligns with the epidemiological context of urban Jakarta, where *Paragonimus* transmission is unlikely due to modernized food practices and limited exposure to freshwater crustacean hosts. These results indicate that the lack of amplification was not a technical failure, as internal positive and negative controls confirmed assay validity, but rather reflect a true absence of *Paragonimus* infection in the sampled population. Nevertheless, the findings highlight the need for continued surveillance in rural or high-risk areas where traditional consumption of undercooked crabs or crayfish persists.

The absence of detectable *Paragonimus* should not be dismissed as inconsequential. The lack of ova in all samples supports the hypothesis that *Paragonimus* is not currently endemic in the area under study. Microscopy using ZNS and NaOH concentration techniques demonstrated high specificity and may continue to serve as a supportive diagnostic tool for differentiating TB from paragonimiasis, especially in endemic regions (4, 16). However, these methods may have limited sensitivity in low-burden settings. This brings substantial epidemiological and clinical significance in the context of evolving disease landscapes and diagnostic practices in tropical medicine. The absence of amplifiable DNA, as confirmed by RNaseP amplification failure, indicates possible challenges in sputum-based molecular diagnostics due to DNA degradation or the presence of inhibitors (17, 18). The limitation underscores the importance of optimizing DNA extraction protocols, particularly for detecting foodborne parasites such as *Paragonimus*.

Our findings provide empirical evidence that the prevalence of pulmonary paragonimiasis among TB-confirmed individuals in urban Jakarta is likely negligible or has markedly declined, challenging previous assumptions of co-endemicity that may have been based on outdated, unreliable, or unverified data. Although our results align with historical records showing the absence of paragonimiasis reporting in Indonesia since 1963 was observed, the potential for re-emergence remains, given increasing seafood imports, travel, and diversification of food culture in the country (10, 11). In settings where paragonimiasis remains endemic, misdiagnosis of pulmonary paragonimiasis as TB may still occur; however, among confirmed TB cases such as ours, the concern shifts toward potential co-infection rather than diagnostic confusion (19).

The clinical overlap between paragonimiasis and TB has been extensively documented. Both conditions may present chronic cough, hemoptysis, pleuritic chest pain, and radiological findings such as cavitary or nodular lesions (19, 20). In resource-limited settings, these overlapping features have historically led to empirical anti-TB therapy in cases where parasitic infections were the actual cause. Studies conducted in Peru, Ecuador, and the Philippines reported that up to 20% of suspected TB patients were eventually diagnosed with paragonimiasis (16, 21). However, those studies were carried out in rural or highland areas with well-documented *Paragonimus* transmission linked to traditional dietary habits such as the consumption of undercooked freshwater crabs or crayfish.

Our study setting in urban Jakarta represents a contrasting environment. Over the past two decades, urbanization, improved food safety awareness, and changing dietary habits have likely contributed to the suppression of *Paragonimus* transmission. Additionally, the absence of *Paragonimus*-specific DNA in our samples, despite employing sensitive molecular assays, supports the notion that urban TB patients are currently not at significant risk for paragonimiasis, even in a country historically regarded as endemic. This finding aligns with recent parasitological mapping efforts in Indonesia, which have reported no active *Paragonimus* foci in recent years (22).

From a methodological standpoint, the strength of our study lies in its dual-layered diagnostic approach, integrating microscopy and DNA-based methods. Conventional microscopy remains valuable and cost-effective but suffers from low sensitivity, particularly when egg output is sparse or intermittent (4, 16). Molecular diagnostics targeting the ITS2 region of *Paragonimus* spp. offer a more reliable alternative with high specificity,

even in pauciparasitic infections (23). The absence of amplifiable *Paragonimus*-specific DNA, therefore, is not a reflection of technical failure but a valid representation of the lack of infection, reinforced by the use of internal controls and reference assays confirming methodological reliability.

Publishing negative or null findings remains an underappreciated yet essential scientific practice (24). Such evidence corrects biased assumptions, prevents redundant investigations, and informs policymakers and clinicians about evolving patterns of disease distribution (25, 26). In our context, it alerts healthcare professionals that routine suspicion of paragonimiasis in urban TB cases—without relevant exposure history or clinical indicators—may no longer be justified. This can optimize diagnostic workflows, reduce empirical antiparasitic treatments, and ensure better allocation resources toward condition with active transmission. Rather than maintaining the status quo, these findings provide evidence-based guidance for rational testing strategies—prioritizing *Paragonimus* screening only in populations or regions with known dietary or ecological risk factors.

This study has several limitations that should be acknowledged. First, sampling was limited to bacteriologically confirmed pulmonary TB patients from an urban population, which may not adequately represent rural or highland communities where *Paragonimus* transmission is more likely. Second, the low yield and variable quality of DNA extracted from sputum specimens could have reduced molecular detection sensitivity. However, the inclusion of internal positive and negative controls verified assay validity, indicating that the absence of amplification was unlikely to be due to technical failure. Finally, the modest sample size may limit the statistical power and the generalizability of the findings beyond the study population.

Lastly, our findings highlight the epidemiological heterogeneity of paragonimiasis and the importance of interpreting historical reports within contemporary contexts. Parasitic infections often exhibit highly focal distributions influenced by ecological and cultural factors, even within countries considered endemic (12, 27). As a result, broad assumptions of co-endemicity may lead to misinterpretation of diagnostic priorities and disease risk (7, 28). The absence of *Paragonimus* spp. in the present study area, despite historical records, illustrates how localized transmission patterns and temporal changes may limit the applicability of older literature.

Implications and Recommendations

Based on the current findings, routine screening for paragonimiasis among urban pulmonary TB patients may not be necessary in low-prevalence settings. However, a targeted diagnostic approach is recommended for patients with atypical clinical features, poor response to anti-tuberculosis treatment, or relevant dietary and environmental exposure histories. Integration of risk-based molecular surveillance into existing TB laboratory networks may enhance early detection of neglected zoonotic infections without imposing unnecessary diagnostic burden. Future research should prioritize high-risk populations, rural or endemic-adjacent regions, and alternative clinical specimens to refine prevalence estimates and inform evidence-based surveillance strategies in Indonesia.

Conclusion

This study presents the first molecular screening for human paragonimiasis among bacteriologically confirmed pulmonary tuberculosis patients in an urban setting in Indonesia. The absence of *Paragonimus* spp. eggs and DNA in sputum samples indicates a minimal risk of paragonimiasis co-infection in the Jakarta study population during the study period. These findings provide contemporary molecular evidence to refine diagnostic priorities, support a targeted risk-based surveillance approach rather than routine screening in urban TB settings, and inform prevention strategies and epidemiological monitoring for neglected zoonotic infections within TB-endemic areas. Molecular diagnostics remain reliable tools for surveillance and should be integrated into broader One Health initiatives; however, these conclusions should be interpreted in light of limitations related to urban-only sampling, modest sample size, and constraints of sputum-based molecular detection. Future research should expand surveillance to rural and highland communities with traditional dietary practices and incorporate environmental and intermediate host sampling to more

comprehensively assess *Paragonimus* transmission risk in Indonesia.

Abbreviations

DNA: Deoxyribonucleic acid, PCR: Polymerase Chain Reaction, SITB: Sistem Informasi Tuberkulosis (the National Tuberculosis Information System), TB: Tuberculosis, ZNS: Ziehl Neelsen-staining.

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Author Contributions

Machrumnizar: conceptualization, methodology, software, validation, funding acquisition, investigation, resources, data curation, original draft writing, review writing, editing, Suriyani Tan: validation, formal analysis, resources, data curation, review writing, editing, visualization, Yuliana: project administration, data collection, field coordinator, proofreading, Rina Kurniasri Kusumaratna: supervision, resources, project administration. All authors have read and approved the final manuscript.

Conflict of Interest

The authors state that the study was performed without any commercial or financial ties that may be seen as a possible conflict of interest during the study.

Declaration of Artificial Intelligence (AI) Assistance

This manuscript was prepared using generative artificial intelligence (AI) tools, specifically Grammarly and QuillBot, for language editing, paraphrasing, and enhancement of academic writing. style. These technologies facilitated the establishment of coherent sentence structures, grammatical precision, and tense consistency throughout the document. The intellectual content, critical analysis, data interpretation, reference selection, and final conclusions in this manuscript were exclusively conceptualized, reviewed, and validated by the authors. No AI technology was employed to produce, analyze, or interpret scientific data, nor to execute any study assignments beyond language support. The authors are completely responsible for the manuscript's originality and accuracy and have ensured that the use of AI-assisted technology follows ethical norms and publication rules.

Ethics Approval

The study involving human participants was reviewed and approved by the Research Ethics Committee of Faculty of Medicine, Universitas Trisakti (Approval No. 056/KEWFK/III2023), and conducted according to the guidelines of the Declaration of Helsinki. Written informed consent to participate in this study was obtained from all participants or their legal guardians.

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IRJMS Iquz Galaxy Publisher <editor@irjms.com> Sat, Nov 8, 2025 at 10:13 AM
To: machrumnizar_md@trisakti.ac.id

Article Title: ABSENCE OF PARAGONIMUS DNA AND EGGS IN TUBERCULOSIS PATIENTS IN INDONESIA: IMPLICATIONS FOR SURVEILLANCE AND PUBLIC HEALTH AWARENESS

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Manuscript_IRJMS/08660: Send Back to Author: Request to Revised Submission

Machrumnizar Machrumnizar <machrumnizar_md@trisakti.ac.id> Sat, Nov 8, 2025 at 1:39 PM

To: IRJMS Iquz Galaxy Publisher <editor@irjms.com>

Dear Editor,

Thank you for your email and for providing the editorial feedback regarding my manuscript entitled *"ABSENCE OF PARAGONIMUS DNA AND EGGS IN TUBERCULOSIS PATIENTS IN INDONESIA: IMPLICATIONS FOR SURVEILLANCE AND PUBLIC HEALTH AWARENESS."*

I would like to ask for clarification about the revision submission process. Should I send the revised manuscript and cover letter directly via email, or should I resubmit it through the journal's submission system as a new submission? Currently, I am unable to log in to the journal's website, and I cannot find an editorial manager or manuscript tracking system to follow up on the submission.

For your reference, I have attached the revised version of the manuscript and the updated cover letter according to the editorial comments.

I would greatly appreciate your guidance on the correct procedure for submitting the revised version.

Thank you for your attention and kind assistance.

Best regards,

Machrumnizar

Corresponding author

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IRJMS Iquz Galaxy Publisher <editor@irjms.com> Fri, Dec 19, 2025 at 3:13 PM
To: Machrumnizar Machrumnizar <machrumnizar_md@trisakti.ac.id>

Submission Title: ABSENCE OF PARAGONIMUS DNA AND EGGS IN TUBERCULOSIS PATIENTS IN INDONESIA: IMPLICATIONS FOR SURVEILLANCE AND PUBLIC HEALTH AWARENESS

Dear Machrumnizar,

We appreciate your submission to the "International Research Journal of Multidisciplinary Scope" with the online ISSN of 2582-631X. Your manuscript has been reviewed, and **Minor revisions** are necessary (see attached/below for comments). It is advised that you thoroughly review the manuscript and note any modifications that were made.

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Machrumnizar Machrumnizar <machrumnizar_md@trisakti.ac.id> Sat, Dec 20, 2025 at 1:11 PM

To: IRJMS Iquz Galaxy Publisher <editor@irjms.com>

Dear Editor,

We are pleased to resubmit our revised manuscript entitled *"Absence of Paragonimus DNA and Eggs in Tuberculosis Patients in Indonesia: Implications for Surveillance and Public Health Awareness"* for your consideration.

We sincerely thank you and the reviewers for the constructive comments and valuable suggestions. All comments have been carefully addressed, and the manuscript has been revised accordingly. Detailed, point-by-point responses to the Editor and Reviewers are provided, and all revisions are highlighted using different colors for each reviewer to facilitate review.

We hope that the revised version now meets the journal's requirements and look forward to your consideration.

Thank you for your time and effort in handling our manuscript.

Sincerely,

Machrumnizar
On behalf of all authors

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IRJMS Iquz Galaxy Publisher <editor@irjms.com> Fri, Jan 2, 2026 at 7:53 PM
To: Machrumnizar Machrumnizar <machrumnizar_md@trisakti.ac.id>

Dear Author,

Thank you for sharing your revised manuscript. After careful revision, there are a few minor corrections that need to be done. **This is the last chance for correction; any mistake found next time, it will be directly rejected. Please do the correction in the attached file (do not use any file from your personal storage).**

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Machrumnizar Machrumnizar <machrumnizar_md@trisakti.ac.id> Sat, Jan 3, 2026 at 9:00 PM

To: IRJMS Iquz Galaxy Publisher <editor@irjms.com>

Dear Editor,

We are pleased to resubmit the revised version of our manuscript entitled “Absence of Paragonimus DNA and Eggs in Tuberculosis Patients in Indonesia: Implications for Surveillance and Public Health Awareness."

The manuscript has been further revised in response to the reviewers’ comments. All comments have been addressed, and detailed responses are provided in the attached “Response to Reviewers” file.

Thank you for your continued consideration of our manuscript.

Sincerely,

Machrumnizar

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IRJMS Iquz Galaxy Publisher <editor@irjms.com> Sun, Jan 11, 2026 at 12:54 AM

To: Machrumnizar Machrumnizar <machrumnizar_md@trisakti.ac.id>

Dear Machrumnizar.,

Congratulations!! We are very pleased to inform you that your manuscript entitled: **"**ABSENCE OF PARAGONIMUS DNA AND EGGS IN TUBERCULOSIS PATIENTS IN INDONESIA: IMPLICATIONS FOR SURVEILLANCE AND PUBLIC HEALTH AWARENESS**"** has been approved for publication in "International Research Journal of Multidisciplinary Scope (IRJMS)" ISSN (ONLINE) : 2582-631X.

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