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RESEARCH ARTICLE

Detection of Zoonotic Potential Non-Tuberculous Mycobacteria (NTM) in Cats in the Special Region of Yogyakarta, Indonesia

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ABSTRACT

Mycobacterium spp. infection in cats presents an important veterinary public health issue because of its zoonotic potential. One significant group of Mycobacterium is Non-tuberculous mycobacteria (NTM), a facultative pathogen that can cause diseases in animals and humans. The high cat population in the Special Region of Yogyakarta, Indonesia, increases the risk of NTM transmission in cats and causes a zoonotic risk to humans. However, case reports of this infectious agent in the region are currently unavailable, indicating the need for more study. This study aimed to detect and identify the presence of NTM infection in cats in the Special Region of Yogyakarta, Indonesia. Swab specimens (nasal, oropharyngeal, and wound) were collected from 70 cats with clinical signs of skin and respiratory tract infections. All specimens were analyzed using microscopic examination, bacteria culture (HEYM), PCR using IS6100, and sequencing DNA. Five colonies were found with typical characteristics of Mycobacterium species, after confirmation Microscopic examination with Ziehl-Neelsen (ZN) and PCR examination using IS6110, only three isolates were positive Mycobacterium spp. Two isolates were identified as *Mycobacterium fortuitum,* and one isolate was identified as *Mycobacterium chelonae*. This study suggests the potential epidemiological relevance of NTM infections in cats, which warrants further investigation regarding their implications for public health.

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INTRODUCTION

Mycobacterium spp. infection presents a severe threat to veterinary health, including that of cats. Mycobacterium species that infect cats are classified into three groups include Mycobacterium Tuberculosis Complex (MTBC), Feline Leprosy Syndrome (FLS), and Non-tuberculous mycobacteria (NTM) (Lioret *et al.,* 2013; Gunn-Moore, 2014; Didkowska *et al.,* 2024). Among these three groupings, Non-tuberculous mycobacteria (NTM) is primarily concerned because of the rising number of case reports in recent years (Dahl *et al.,* 2022). NTM showed significant environmental distribution and presented a potential zoonotic risk (Didkowska *et al.,* 2024).

Non-tuberculous mycobacteria (NTM) is a group of facultative pathogens that are opportunistic saprophytic bacteria commonly found in the environment, including soil, water, and organic matter (Gunn-Moore, 2014; KuÈntzel *et al.,* 2018). Current studies have shown that NTM infections occur in immunocompromised animals and healthy animals without evident immune deficiencies (Pekkarinen *et al.,* 2018; Ricotta *et al.,* 2021). Infections can be detected via clinical signs, including respiratory tract infections, skin and soft tissue lesions, osteoarticular infections, and systemic indications in immuno-compromised animals (Gunn-Moore, 2014; Barandiaran *et al.,* 2017; Munro *et al.,* 2021; Ricotta *et al.,* 2021; Mitchell *et al.,* 2022). In severe cases, NTM infections may result in mortality (Gunn-Moore *et al.,* 2011; Pekkarinen *et al.,* 2018; Munro *et al.,* 2021).

Interactions between cats and humans are carefully intense because many people adopt them as pets. This may have the potential to increase the risk of increasing the spread of zoonotic diseases, such as infection with Mycobacterium spp. from the NTM group (Afzal *et al.,* 2021; Aljohani, 2023; Didkowska *et al.,* 2024). Several

studies indicate that interactions between people and cats can increase the risk of NTM infection (Ngan *et al.,* 2005; Minato *et al.,* 2021; Shah *et al.,* 2023; Holanda, 2024; Salem *et al.,* 2024). The direct connection between humans and animals in the transmission of NTM infections requires a comprehensive strategy for preventing, controlling, and eliminating these infections.

Although significant evidence of NTM infection in cats and its zoonotic potential in other nations, there are yet to be any documented instances in the Special Region of Yogyakarta and Indonesia. This indicates a gap in our comprehension of the distribution and prevalence of NTM infections in cats within the region. Consequently, more study is required to confirm the existence of NTM infections in cats in the Special Region of Yogyakarta. This detection is needed to identify the presence of NTM infection in cats and determine the risk factors that can cause this disease transmission in the area where it occurs. The results of this study have the potential to contribute to knowledge of the epidemiology of NTM infection in cats and to design effective management methods in the Special Region of Yogyakarta and throughout Indonesia.

MATERIALS AND METHODS

Study period and location: This study was conducted from June to October 2024. Samples were collected from the four districts (Sleman, Yogyakarta city, Bantul, Kulon Progo) in the Special Region of Yogyakarta, Indonesia. This research was conducted using equipment at the National Research and Innovation Agency of Indonesia and Universitas Gadjah Mada, funded by the Inter-University Center of Excellence Program (PUAPT).

Sample collection: A total of 70 cats with clinical signs of skin and respiratory tract infections were used for animals sampled. The specimens were aseptically collected by swabbing the nasal, oropharyngeal, or wound regions from animals sampled, then placed in sterile containers for storage.

Microscopic examination: Microscopic examination of swab specimens or bacterial culture isolates was performed using the Ziehl-Neelsen staining method (Holani *et al.,* 2014; Desire *et al.,* 2024) and examined under a light microscope at 100x magnification.

Mycobacterium spp. Isolation: Bacterial culture was performed using Herrold's Egg Yolk Medium (HEYM) (Küntzel *et al.,* 2018). Swab specimens were suspended in PBS, centrifuged at 10,000 rpm for 5 minutes, and 100µL of the sediment was collected. NaOH was added to eliminate contaminants, and the solution was then neutralized with HCl (Chatterjee *et al.,* 2013; Verma and Kashyap, 2021). A specimen suspension of 100µL was inoculated onto HEYM with a Pasteur pipette and incubated at 35-37°C for 4-7 days in a tilted orientation with the lid slightly loosened. The media was thereafter secured, the lid was firmly sealed, and it was incubated at 37°C for up to 12 weeks. Weekly assessments of the bacterial growth were performed (Adji and Nuradji, 2023).

Extraction of DNA from swabs and isolates: DNA was extracted using the Genomic DNA Mini Kit (Geneaid®) according to the manufacturer's instructions. After all the extraction steps, the specimens were subsequently utilized in the PCR process or stored at -20°C (Adji and Nuradji, 2023).

DNA amplification: The primer uses IS6110, with the forward primer IS6110_F1 (5'-CAG-GAC-CAC-GAT-CGC-TGA-TC) and the reverse primer IS6110_R1 (5'-CTG-CCC-AGG-TCG-ACA-CAT-AG) (Dykema *et al.,* 2016). PCR reaction mixtures were formulated in a total volume of 20µL, comprising 7.2µL of nuclease-free water, 10µL of 2X KAPA Taq ReadyMix, 0.4µL of each primer and 2µL of DNA template. The PCR protocol comprised an initial denaturation at 95°C for 3 minutes, succeeded by 35 cycles of denaturation at 95°C for 15 seconds, annealing at 60°C for 15 seconds, and extension at 72°C for 30 seconds, concluding with a final extension at 72°C for 5 minutes (Dykema *et al.,* 2016). For species-level identification, PCR amplification targeting the Mycobacterium 16S rRNA gene was then performed on PCR-positive samples using the forward primer 16S-5F (5′-TTGGAGAGTTTGATCCTGGCTC-3) and the reverse primer 16S-534R (5′-TACCGCGGCTGCTGGCAC-3). The expected amplicon size is approximately 500 bp (Simmon *et al*., 2010). Amplification results were analyzed using electrophoresis on a 2% agarose gel, stained with ethidium bromide, and seen using a UV transilluminator.

Sequencing and data analysis: Sequencing was applied to the PCR result obtained from the amplification of the 16S rRNA gene in samples that tested positive in the PCR to confirm identification at the level of species (Adékambi and Drancourt, 2004; Simmon *et al.,* 2010). After sequencing, phylogenetic analysis was performed using Basic Local Alignment Search Tool (BLAST) for sequence analysis and phylogenetic analysis using Molecular Evolutionary Genetics Analysis (MEGA) software version 11.0. All acquired data were presented in tables and figs and analyzed descriptively.

Ethical clearance and informed consent: The Ethical Commission of Faculty of Veterinary Medicine Universitas Gadjah Mada has approved this research, No: 05/EC-FKH/Int./2024, and the pet owners agreed as respondents. It gave a permit before collecting the rectal swab sample.

RESULTS

The investigated cats showed clinical signs of respiratory and skin infections, such as sneezing, mucous discharge from the nasal passages and eyes, chronic cough, enlarged submandibular and prescapular lymph nodes, skin nodules, and ulceration. Additional documented symptoms include cachexia, anorexia, anemia, vomiting, and diarrhea. A visual representation of these clinical signs is shown in (Fig.1). Laboratory examinations included microscopic examination, bacterial culture, and PCR analysis of swab specimens (nasal/oropharyngeal/wound). Microscopic examination of the swab specimens yielded negative results for all samples, while PCR analysis showed a positive result in one sample (K84) (Fig. 2).

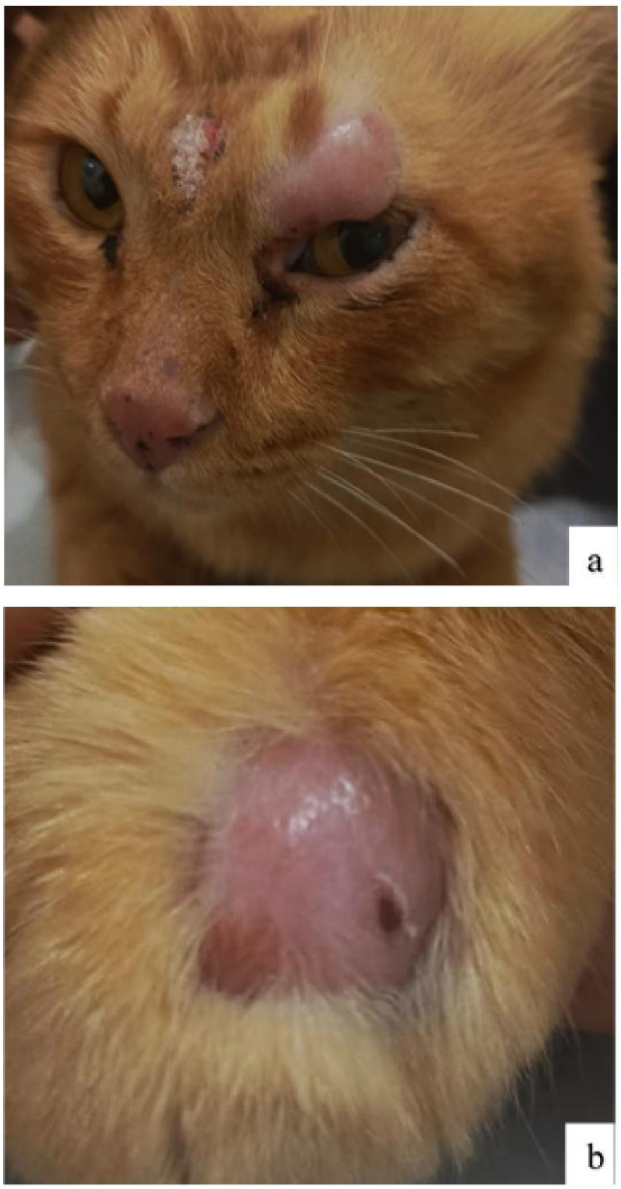

Fig. 1: Lesions on the skin area of the sample; (a). Dermal nodule on the face area, (b) Dermal nodule on the extremity area.

The five samples on HEYM showed the morphology characteristic of Mycobacterium colony growth, appearing as small, dry, and solitary with a yellow-gray color (Tkachenko *et al.,* 2020). Bacterial colonies that grew on HEYM media were subsequently confirmed through microscopic examination using Ziehl-Neelsen (ZN) staining (Fig. 3). The microscopic examination results revealed the presence of acid-fast bacteria in samples K07, K30, K36 and K84. The morphological characteristics of the bacteria observed under the microscope consisted of rod-shaped bacteria, approximately 7.9µM in length, with a distinctive red color in the ZN staining results (Fig. 4). In addition, confirmation of the bacterial culture by PCR testing revealed three positive samples (samples K30, K36, and K84). Fig. 5 displays the results of the PCR examination of bacterial culture isolates from cat samples.

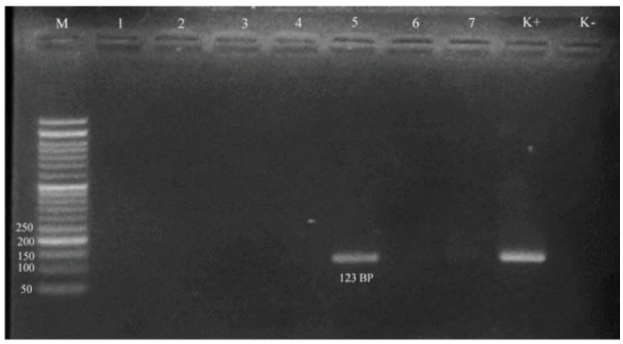

Fig. 2: Results of examination of swab specimens using IS6110 primers. Positive results were found in column 5 with a 123 bp DNA band (sample code K84). Negative results were found in columns 1,2,3,4,6, and 7 (Sample codes K29, K30, K36, K83, A55, and A56). M = marker, K.+= positive control Mycobacterium bovis (Sunday market isolate from BRIN Veterinary Research Centre), K-= negative control

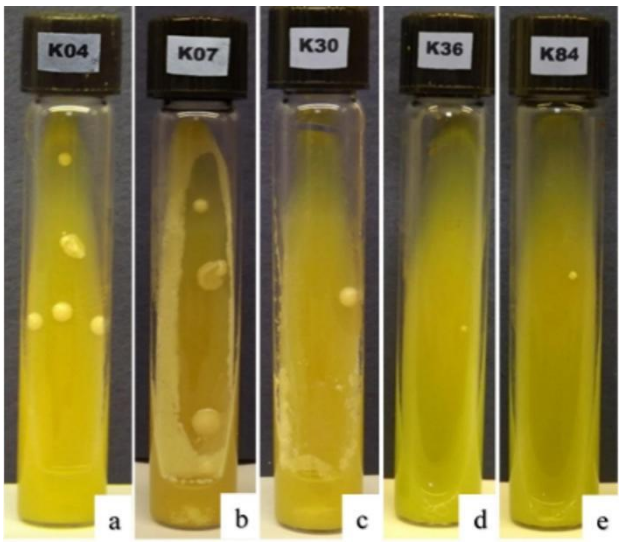

Fig. 3: Morphological colonies of Mycobacterium spp. on HEYM media. a = Sample code K04, b = Sample code K07, c = Sample code K30, d = Sample code K36, e = Sample code K84.

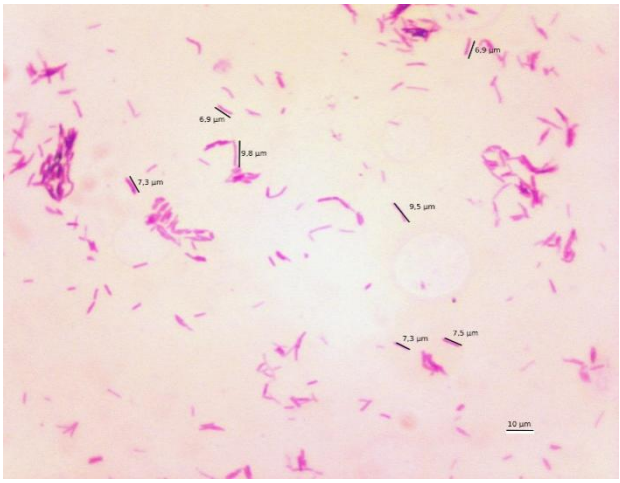

Fig. 4: Microscopic image of culture isolate (sample code K84) with Ziehl-Neelsen (ZN) staining, showing rod-shaped bacteria with an average size of 7.9µM, red in colour typical of acid-fast bacteria (100X magnification).

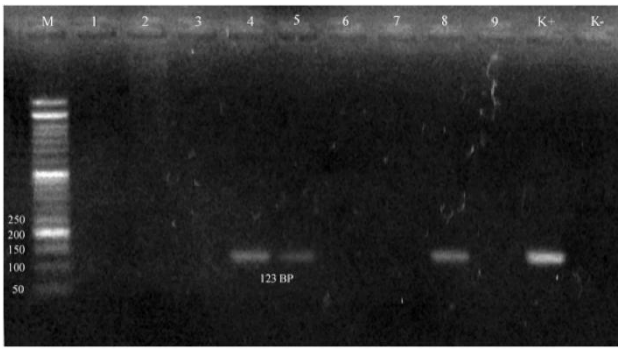

Fig. 5: PCR results on culture specimens using IS6110 primers. Positive results were found in columns 4, 5, and 8 (sample codes K36, K30, and K84) with 123 bp DNA bands. Negative results were found in columns 1, 2, 3, 6, 7, and 9 (sample codes K04, K07, K26, K39, K42, and K82). M = marker, K.+= positive control Mycobacterium bovis (Pasar minggu isolate from BRIN Veterinary Research Centre), K-= negative control.

DISCUSSION

Microscopic examination of all samples showed negative results, while culture and PCR tests identified three positive samples (sample K30, K36, and K84). Microscopic examination with Ziehl-Neelsen staining has lower sensitivity compared to bacterial culture or PCR (Desire *et al.,* 2024). Several factors can affect the sensitivity of microscopic examination, including the prevalence of infection, the quality and quantity of specimens examined, the staining method used, and the skill level of laboratory staff conducting the examination (Holani *et al.,* 2014). Arora and Dhanashree (2020) mention the bacterial count in the specimen can affect the sensitivity of microscopic examination, with a minimum of 10,000 bacteria per mL required for detection.

Confirmation of bacterial culture revealed that of the five colonies with Mycobacterium morphology, four were acid-fast bacteria, while one was not. Researchers have identified some bacteria, such as *Aeromonas sp.* and *Pseudomonas sp.,* that grow on egg-yolk-based media like HEYM but are not acid-fast bacteria (Lysyk *et al.,* 2002; Andriyanov *et al.,* 2023), both are also known to infect cats (Płókarz *et al.,* 2022). Among the four acid-fast bacteria that grew on HEYM, only three were PCR-positive and identified as Mycobacterium spp. The remaining acid-fast bacteria, which did not belong to the Mycobacterium group like other bacteria, such as *Nocardia sp*. (Huang *et al.,* 2021; Spiliopoulou *et al.,* 2023).

This study used the IS6110 primer, previously designed to detect members of the Mycobacterium tuberculosis complex (MTBC); however, this primer can also amplify several Non-tuberculous mycobacteria (NTM) species due to sequence homology (Coros *et al*., 2008; Müller *et al.,* 2015). Previous studies have reported that IS6110 can cross-react with several NTM species, which may affect its specificity. Thacker *et al*. (2011) reported that the IS6110 primer was capable of detecting *Mycobacterium bovis* from the MTBC group and, additionally, *Mycobacterium peregrinum* and *Mycobacterium chelonae* from the NTM group. In this study, the IS6110 primer was used as an initial screening tool to detect Mycobacterium spp. To improve specificity and ensure accurate identification, the samples were subsequently analyzed using 16S rRNA gene amplification followed by sequencing, which provides more reliable species-level identification (Zou *et al.,* 2014). Therefore, this combined approach allows for initial detection followed by confirmatory identification, thereby reducing the risk of misclassification. However, the limitations of IS6110 specificity must be considered when interpreting the results.

Based on 16S rRNA gene sequencing, two species were identified within the three PCR-positive samples classified as Mycobacterium spp., such as *Mycobacterium chelonae* (sample K30) and *Mycobacterium fortuitum* (samples K36 and K84). The sequencing results are shown in Table 1. The sequencing results are then analyzed using phylogenetic analysis by comparing the target gene sequences found in the GenBank genetic database. Phylogenetic study results indicated that Sample K30 is similarly related to *Mycobacterium chelonae* and *Mycobacterium abscessus*, but samples K36 and K84 exhibit significant similarity to *Mycobacterium fortuitum*. Fig. 6 displays the outcomes of the phylogenetic analysis.

Fig. 6: Phylogenetic tree structure of 3 positive samples of Mycobacterium spp.

Table 1: Results of sample sequencing analysis using BLAST analysis

No	Sample code	Breed	GenBank	E-Value	Percent identity
1	K30	Cat	*Mycobacterium chelonae*	0.0	100%
2	K36	Cat	*Mycobacterium fortuitum*	0.0	100%
3	K84	Cat	*Mycobacterium fortuitum*	0.0	100%

The close similarity between *Mycobacterium abscessus* and *Mycobacterium chelonae* is due to their highly homologous genetic sequences, particularly the 16S rRNA gene used in this study, with a reported sequence variation ranging from 0 to 11 bp (Turenne, 2019; Dibaj *et al*., 2020; Zhang *et al*., 2024). Although this limited variation may reduce the ability of 16S rRNA gene sequencing to clearly differentiate between these closely related species, this method remains widely accepted for reliable identification of Mycobacterium at the genus level and for confirming the presence of Non-tuberculous mycobacteria (NTM) (Zou *et al.,* 2014). Therefore, the findings in this study strongly indicate the presence of NTM. However, the use of additional molecular markers, such as hsp65 or rpoB, would improve discrimination at the species level and should be considered in future studies (Peterhans *et al*., 2021; Zhang *et al*., 2024).

Non-tuberculous mycobacteria (NTM) infections specifically *Mycobacterium chelonae* and *Mycobacterium fortuitum* in cats will present multiple clinical signs. Couto and Artacho (2007) reported clinical signs in animals

infected with *Mycobacterium fortuitum*, including dyspnea lasting 3 weeks, weight loss, and significant periodontal disease, with thoracic radiographs showing significant diffuse alveolar infiltration that obscured the cardiac overview. Another article by Munro *et al.* (2020) records that clinical signs observed in animals infected with NTM can be respiratory signs, specifically chronic cough, or skin signs, such as ulcers, in many regions, especially in the ventral abdomen or inguinal area in infected cats. Other clinical signs caused by *Mycobacterium fortuitum* infection include skin nodules located in the lower abdomen, in proximity to the right pelvic limb and inguinal region, with histological analysis identifying pyogranulomatous panniculitis (Morgado *et al.* 2022). These reports align with the study's findings, which indicated respiratory and skin infections. Clinical signs of respiratory infection, such as chronic cough, sneezing, and purulent discharge from the nose or eyes, were observed in samples K30 and K36. Simultaneously, clinical signs of skin infection, such as persistent non-healing ulcers and skin nodules on the face, head, and limbs, were noted in samples K36 and K84. The presentation of clinical signs and various additional variables (Table 2).

Non-tuberculous mycobacteria (NTM) infection of the respiratory and skin is transmitted via multiple routes, including direct contact with infected organs or systemic transmission via blood circulation. Bacteria can penetrate the respiratory tract by inhaling dust or aerosols contaminated with Mycobacterium. After entering the respiratory tract, these bacteria may induce inflammation and persistent infection (Falkinham, 2015; Ratnatunga *et al.,* 2020). Skin infections are frequently caused by contact with NTM bacteria and the skin surface. Following entrance, the bacteria can proliferate at the infection site and disseminate locally (Franco-Paredes *et al.,* 2018).

During NTM bacteria infiltrate the body, the immune system identifies them via immune cells, such as macrophages, which use toll-like receptors (TLRs) to identify components of the bacterial cell wall. The interaction of NTM with TLRs initiates an inflammatory response, causing macrophages to secrete pro-inflammatory cytokines that activate the immune system, including CD4+ T cells that assist macrophages in eliminating bacteria and CD8+ T cells that destroy infected cells. NTM can persist within macrophages via circumventing annihilation. If the immune response is insufficient, the infection may progress to a chronic place, resulting in tissue damage and chronic inflammation. The persistent inflammatory response results in granuloma formation, structures that allow bacterial persistence and may cause skin ulceration and the development of hard nodules on the skin or in the lungs (Méndez-Samperio, 2010; Feng *et al.,* 2020; Ratnatunga *et al.,* 2020).

The study showed that all three positive cases were identified in adult animals with outdoor access, with two confirmed cases being male. Previous studies have indicated that NTM infections frequently occur in adult males aged 1–6 years (Couto and Artacho, 2007; Gunn-Moore, 2014; Morgado *et al.,* 2022). Furthermore, multiple reports of NTM infections in cats confirm the significance of outdoor access as a risk factor for this bacterial disease (Malik *et al.,* 2004; Couto and Artacho, 2007; Munro *et al.,* 2020; Morgado *et al.,* 2022). Lioret *et al.* (2013) and Gunn-Moore (2014) reported that Mycobacterium spp. Infections are more prevalent in adult males with outdoor access. Male animals are more likely to explore and hunt in the wild, which increases their exposure to environmental pathogens and heightens their susceptibility. More significant outdoor activity and exploratory behaviour elevate the likelihood of contact with Mycobacterium spp. through interactions with wildlife or contaminated environments, subsequently increasing the risk of disease transmission (Pekkarinen *et al.,* 2018).

The body condition scores (BCS) are 2/5 and 3/5. A BCS of 2/5 signifies that the animal is thin, and a BCS of 3/5 indicates a generally normal body. Thinness may be associated with infection by Mycobacterium spp. Munro *et al.* (2020) stated that weight loss is one of the indications detected in animals infected with NTM, leading to thinness. In animals infected with Mycobacterium spp., thinness can result from increased production of inflammatory cytokines, which accelerate metabolism and eliminate muscle tissue and fat for energy. Chronic infection can reduce appetite, leading to insufficient calorie and protein consumption, weight loss, and muscular atrophy (Méndez-Samperio, 2010; Feng *et al.,* 2020; Nielsen *et al.,* 2023).

The study showed that all cats testing positive for Non-tuberculous mycobacteria (NTM) received a varied diet, including dry, wet, raw food, or human food scraps. Biet and Boschiroli (2014) and Salem *et al.* (2024) find that NTM can infect animals through contaminated feed, especially from raw materials (Dziedzinska *et al.,* 2016). Feed can get contaminated with NTM when it comes into contact with diseased animals or from soil, water, or biofilms that hold these bacteria (Küntzel *et al.,* 2018). When animals ingest contaminated feed, microorganisms may infiltrate the body and induce infection.

The confirmed positive cases in this study are limited (3 out of 70 samples), which may restrict broader epidemiological conclusions. However, based on the principles of disease surveillance, the detection of even a single positive case indicates the presence of the disease within a population (Cameron, 2012). Therefore, the identification of positive cases in this study indicates the presence of NTM in the cat population in the study area.

Zoonotic potential of *Mycobacterium chelonae* and *Mycobacterium fortuitum*: The study's results indicated that two animals, specifically samples K36 and K84, confirmed positive for *Mycobacterium fortuitum* and were associated with owners diagnosed with tuberculosis (TB). Meanwhile, the K30 sample came from Yogyakarta City, a region with a

Table 2: Clinical presentation information of positive cases of *Mycobacterium fortuitum* and *Mycobacterium abscessus* in cats

No	Code	Age (year)	Sex	BCS	Outdoor access	Positive tests	Feed	Clinical signs noted by the owner
1	K30	2	M	2	Yes	Culture	Mix	Lethargy, hyporexia, and Chronic cough
2	K36	5	M	2	Yes	Culture	Mix	Chronic cough, Ulcerated skin mass on the head, living in households with tuberculosis patients
3	K84	1,7	F	3	Yes	Culture and PCR	Mix	Ulcerated skin mass on the face and extremities, living in households with tuberculosis patients

significant incidence of human tuberculosis, reporting over 1,300 cases in 2023 (Bappeda DIY, 2024). These results suggest a possible epidemiological link between NTM infections in cats and human TB cases in the same geographic region. However, this study does not provide direct evidence of transmission between animals and humans. The observed coexistence may reflect shared environmental exposure, as NTM is commonly found in soil and water, rather than direct zoonotic transmission. An overview of NTM infection areas and their association with human cases (Fig. 7).

Microscopic and radiographic examinations are often used as an early method for diagnosing tuberculosis, however, they do not always confirm that the noted signs are related to bacteria from the Mycobacterium Tuberculosis Complex (MTBC), including *Mycobacterium tuberculosis*. Comparable clinical signs can appear from bacteria related to the Non-tuberculous mycobacteria (NTM) group (Vashisht *et al.,* 2023). Haseeb *et al.* (2022) investigated patients with likely pulmonary TB. In terms of 215 cases, 55 have been attributed to species within the Non-tuberculous mycobacteria (NTM) group, which includes *Mycobacterium avium* in 27 cases (49%), followed by *Mycobacterium abscessus* (13 cases or 23.6%), *Mycobacterium kansasii* (9 cases or 16.3%), and *Mycobacterium intracellulare* (6 cases or 10.9%). This presents the potential for an association between the incidents identified in this case and suspected human TB cases in the region.

The correlation between NTM cases in animals and people is paralleled by a rising incidence of NTM infections in both populations (Biet and Boschiroli, 2014; Dahl *et al.,* 2022). The global incidence of NTM patients increased to 4.0% yearly (Dahl *et al.,* 2022). In some Asian nations, including Japan, the rate of NTM infection is higher than that of tuberculosis, with significant increases observed in recent years. The incidence of NTM infection in South Korea grew fivefold from 2007 to 2016 (Hamed and Tillotson, 2023).

The incidence of Non-tuberculous mycobacteria (NTM) infection in cats has not been reported in Indonesia. However, NTM infections among humans have been reported in several regions, indicating a potential concern that requires more attention. Saptawati *et al.* (2019) reported that of the 1,636 acid-fast bacteria cultures examined, 334 cases (15%) were identified as NTM. Saptawati *et al.* (2022) next reported that the most common NTM species identified in Indonesia are the *Mycobacterium fortuitum* and *Mycobacterium abscessus.*

Several studies show the relationship NTM bacterial infections in people and animals. Shah *et al.* (2023) reported a *Mycobacterium chelonae* infection in a 78-year-old male who had a pyoderma gangrenosum skin lesion on his left hand. The condition arose subsequent to a cat scratch. Minato *et al.* (2021) reported a *Mycobacterium chelonae* infection in a 77-year-old male following a dog bite. The individual established inflammation and swelling of his hand. Bacterial culture results indicated

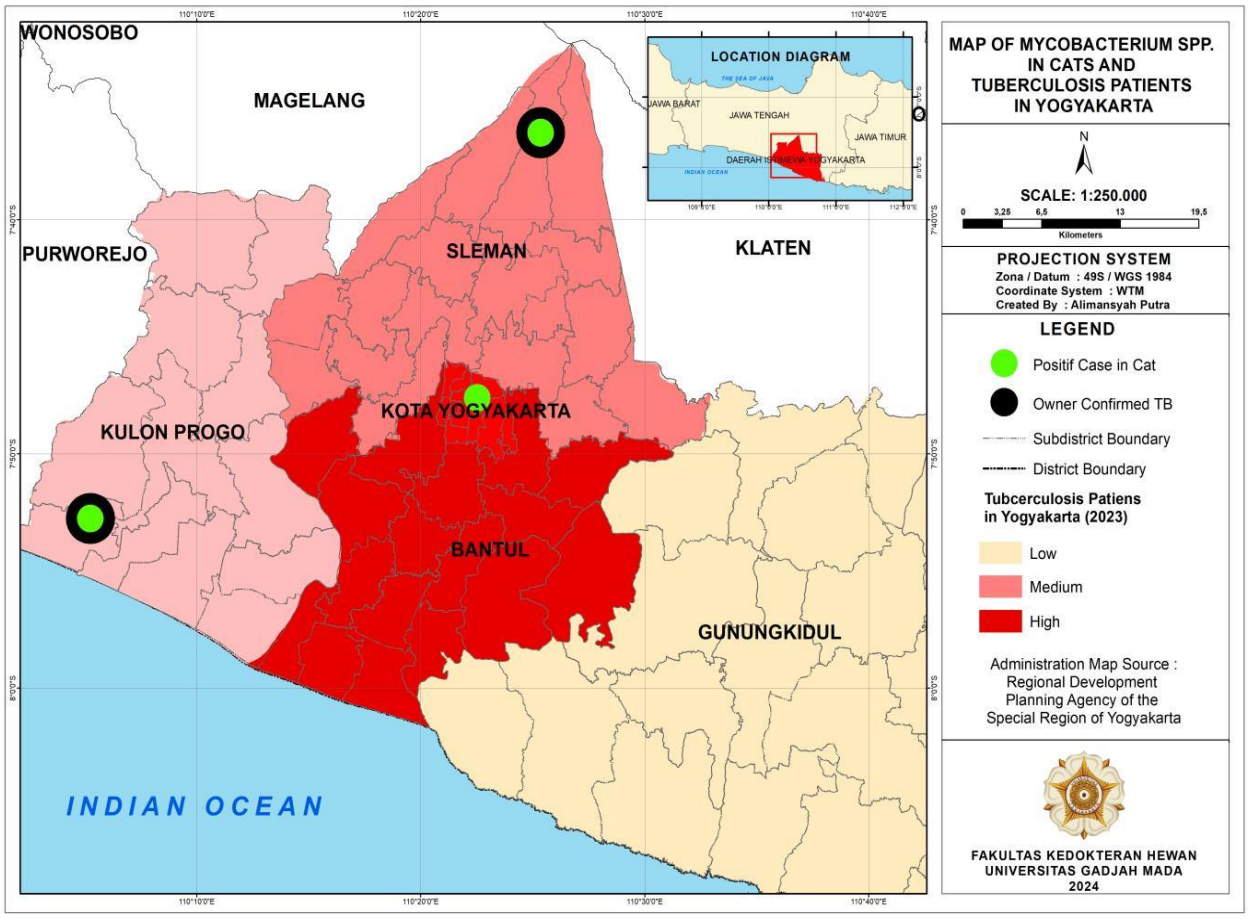

Fig. 7: Map of the distribution of *Mycobacterium chelonae* and *Mycobacterium fortuitum* infections in cats, TB cases in cat owners, and distribution of TB cases in humans in the Special Region of Yogyakarta. (Source: Map Prepared by the corresponding author).

Mycobacterium chelonae infection in the extensor tendons of the hand, indicating extensor tenosynovitis. Dibaj *et al.* (2020) found that house cats are usually more predisposed to NTM infection than wild cats. This suggests zoonotic potential for NTM infection transmission from cats to humans.

This study has several limitations, including the small number of confirmed positive cases and the lack of direct evidence of transmission between animals and humans. Therefore, the interpretation of these findings as a zoonosis remains limited. However, the detection of NTM in cats indicates the presence of this organism in the environment and underscores the need for further investigation.

Conclusions: This study detected three cases of Non-tuberculous mycobacteria (NTM) infection in cats in the Special Region of Yogyakarta, specifically *Mycobacterium chelonae* and *Mycobacterium fortuitum*. These findings indicate the presence of NTM infection in cats and suggest a risk of zoonosis, which warrants further investigation.

Conflict of Interest: The authors declare no conflict of interest.

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Author's Contribution: AP: sample collection, laboratory diagnosis, article drafting; WSN: research design, coordinating the research project, data analysis, article drafting; IT: supervise sample collection and clinical examination, article review; RSA: molecular diagnosis and analysis, article review.

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