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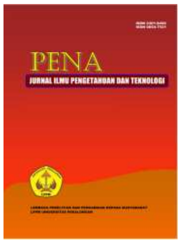

Detection of Mycobacteria in Poultry Farming Areas in Malang Regency, East Java: A One Health Approach

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ABSTRACT

Background:Members of the genus Mycobacterium are notable pathogens of animals and humans. Their ability to persist in farm environments makes them a potential source of zoonotic infection.
Objective: This study investigated the prevalence of Mycobacteria in poultry farm environments in Malang Regency, East Java, using a One Health framework to understand implications for animal and human health. Methods: Soil samples (n = 25) were collected purposively from poultry farms across Malang Regency. Samples were decontaminated according to World Health Organization protocols. Acid-fast bacilli were detected using the Ziehl–Neelsen staining technique. Results: Two of the 25 samples (8 %) were positive for acid-fast bacilli suggestive of Mycobacteria. This low prevalence indicates the presence of Mycobacteria in the environment but suggests limited dissemination within the sampled farms.
Conclusions: The detection of Mycobacteria in farm soils highlights potential risks for poultry and farm workers. Regular environmental surveillance and species-level identification are warranted to clarify transmission pathways and develop targeted control strategies.

Keywords: *Mycobacteria, Poultry farm, Soil, One Health, Environmental*

INTRODUCTION

The One Health paradigm recognizes the interdependence of human, animal and environmental health. One of the pressing challenges within this framework is zoonosis associated with Mycobacteria, a diverse genus that includes both obligate pathogens and opportunists. Zoonotic infections caused by Mycobacteria demand cross-disciplinary attention because they threaten public health, animal welfare and environmental integrity (Mackenzie et al., 2014; Jones et al., 2016). Mycobacterial infections contribute to significant morbidity in livestock and may spill over to humans. Previous work has documented non-tuberculous Mycobacteria (NTM) and members of the *Mycobacterium tuberculosis* complex (MTBC) in various animal hosts (Holt et al., 2018).

In East Java, Saptawati et al. (2022) reported multiple NTM isolates in clinical samples, with a substantial proportion of infections occurring in males. Malang Regency is an agricultural region with a high density of poultry farms, creating conditions conducive to pathogen exchange between livestock and people. The farm environment, animal health management and interactions between humans and animals are critical factors shaping disease transmission (Cosivi et al., 2015; (Gagneux, 2018). Members of the MTBC such as *M. tuberculosis, M. bovis, M. cannetii* and *M. africanum* can cause tuberculosis in animals and humans. In addition, opportunistic NTM species like *M. fortuitum, M. abscessus* and *M. marinum* may infect immunocompromised hosts ((Kurniawati et al., 2018; Satta et al., 2019; Kurniawati et al., 2021).

Despite recognition of these risks, environmental surveillance for Mycobacteria in Indonesian livestock settings remains limited. Understanding the presence and distribution of Mycobacteria in farm soils is essential for evaluating exposure risks and informing control measures. This study applied a One Health perspective to detect Mycobacteria in poultry farming areas in Malang Regency, East Java. We hypothesised that Mycobacteria would be present at detectable levels in soils collected from farm environments (Müller et al. 2013; Harrisson et al. 2016; Griffith et al., 2017; Honda et al., 2018).

RESEARCH METHODS

Study area and sampling strategy

The study was conducted in Malang Regency, East Java, Indonesia, an area characterized by intensive poultry production. A purposive random sampling strategy was used to capture ecological variation across farms. Twenty-five soil samples were collected from the top layer of ground in different poultry farms distributed throughout the region. Sampling points were selected to represent variations in management practices and environmental conditions.

Sample processing and decontamination

Upon collecting, soil samples were transferred to sterile containers and transported on ice to the laboratory. Decontamination followed World Health Organization guidelines (1998b). Each soil sample was mixed with an equal volume of sterile distilled water, vortexed and centrifuged at 3000 × g for 10 minutes. The supernatant was discarded, leaving a small volume for further analysis.

Acid-fast staining

The presence of acid-fast bacilli (AFB) was assessed using the Ziehl–Neelsen method. A smear of the sediment was air-dried and heat-fixed on a glass slide. Slides were flooded with carbol fuchsin, gently heated for five minutes, and rinsed with water. Decolorization was performed with acid alcohol for 2–3 minutes, followed by rinsing and counterstaining with methylene blue. Slides were examined under 100× oil immersion for acid-fast organisms (WHO, 1998a; Kurup et al., 2014; Kurniawati et al., 2018). All staining procedures and microscopy were carried out at the Institute of Tropical Diseases, Universitas Airlangga.

RESULTS AND DISCUSSION

From 25 soil samples analyzed, two samples were positive for acid-fast bacilli, yielding an estimated prevalence of 8 %. The remaining 23 samples showed no detectable acid-fast organisms. Positive samples were recovered from farms located in different sub-districts, suggesting that environmental contamination is not confined to a single area. However, the overall low prevalence implies that widespread dissemination of Mycobacteria in poultry soils was not evident at the time of sampling.

This study provides preliminary evidence of Mycobacteria in poultry farm soils in Malang Regency. The detection of acid-fast bacilli, albeit in a small fraction of samples, indicates the potential relevance of environmental reservoirs in the epidemiology of mycobacterial diseases. Previous studies have demonstrated that Mycobacteria can survive for extended periods under diverse environmental conditions, contributing to their persistence and transmission (Falkinham, 2009). Inhalation of contaminated dust, ingestion of polluted feed or water, or exposure of skin lesions to contaminated materials are plausible routes of infection for animals and humans (Whipps et al., 2017; Wassilew et al., 2016).

The low prevalence observed may reflect effective hygiene practices or limited contamination at the sampled farms. Nevertheless, even low-level environmental exposure could pose risks, especially for immuno-compromised individuals or animals. Comparisons with other regions are instructive: Cosivi et al. (1998) documented zoonotic tuberculosis caused by *M. bovis* in developing countries, while Sattar et al. (2021) isolated Mycobacterium avium and other NTM from chickens and captive birds in Malaysia. These studies illustrate the diversity of mycobacterial species circulating in farm environments and highlight the importance of surveillance.

Our findings should be interpreted considering several limitations. The sample size was modest and may not capture the full variability of mycobacterial contamination across the region. Ziehl–Neelsen staining is practical but lacks the ability to differentiate at the species level. Future work should incorporate culture and molecular methods to characterize isolates, quantify bacterial loads and assess antimicrobial resistance profiles. Longitudinal sampling would also help determine temporal dynamics and evaluate the impact of interventions.

Conclusions

The presence of Mycobacteria in soils from poultry farms in Malang Regency demonstrates that environmental reservoirs of these bacteria exist East Java, Indonesia. Although only 8 % of samples were positive, the findings warrant attention because of potential risks to animal and human health. Continued monitoring, coupled with species-specific identification and One Health collaboration, is essential to inform risk assessments and guide preventative measures.

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