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Research

Rapid Identification Prevalence Zoonotic Tuberculosis (*Mycobacterium bovis*) in West Bandung and Pangalengan

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ABSTRACT

Bovine tuberculosis is caused by the bacterium *Mycobacterium bovis* (*M. bovis*). Bovine tuberculosis in dairy cows is thought to contribute to an increase in tuberculosis cases because it can be transmitted to humans (zoonosis), hence the term Zoonotic Tuberculosis. Transmission through the mouth can occur by consuming milk or dairy products contaminated with bacteria. The objective of this study was to identify the prevalence of *M. bovis* infection as part of efforts to mitigate the risk of foodborne disease in milk. The study design was descriptive qualitative, using AFB staining and PCR with the MPB70, RD1, CSB1, CSB2, and CSB3 genes as DNA amplification targets. The results showed that the prevalence of infection in 60 milk samples tested using the AFB staining method was 0% (negative), and in 6 samples (10% of all samples) tested using PCR, the prevalence was also 0%. The conclusion is there is no *M. bovis* in milk, indicating a currently low risk, but ongoing surveillance, improved diagnostics, and interdisciplinary collaboration are needed to reduce zoonotic transmission and economic impacts. Efforts to prevent and control Bovine Tuberculosis in Indonesia focus on promoting community practices: avoiding contact with infected animals, cooking meat thoroughly, and consuming processed milk.

Keywords: risk, zoonotic tuberculosis, *Mycobacterium bovis*, milk, bovine

ABSTRAK

Tuberkulosis sapi disebabkan oleh bakteri *Mycobacterium bovis* (*M. bovis*). Tuberkulosis sapi pada sapi perah diyakini berkontribusi terhadap peningkatan kasus tuberkulosis karena dapat menular ke manusia (zoonosis), sehingga disebut Tuberkulosis Zoonotik. Penularan melalui mulut dapat terjadi dengan mengonsumsi susu atau produk susu yang terkontaminasi bakteri. Tujuan penelitian ini adalah untuk mengidentifikasi prevalensi infeksi *M. bovis* sebagai bagian dari upaya mitigasi risiko penyakit bawaan makanan pada susu. Metode yang digunakan adalah bersifat deskriptif kualitatif, menggunakan pewarnaan BTA dan PCR dengan gen MPB70, RD1, CSB1, CSB2, dan CSB3 sebagai target amplifikasi DNA. Hasil menunjukkan bahwa prevalensi infeksi pada 60 sampel susu yang diuji dengan metode pewarnaan BTA adalah 0% (negatif), dan pada 6 sampel (10% dari semua sampel) yang diuji dengan PCR, prevalensi juga 0%. Simpulan penelitian ini adalah tidak ada *M. bovis* dalam sampel susu, menunjukkan risiko yang saat ini rendah, tetapi pemantauan berkelanjutan, diagnostik yang ditingkatkan, dan kolaborasi interdisipliner diperlukan untuk mengurangi penularan zoonotik dan dampak ekonomi. Upaya pencegahan dan pengendalian Bovine Tuberculosis di Indonesia berfokus pada promosi praktik kesehatan masyarakat seperti menghindari kontak dengan hewan terinfeksi, memasak daging secara menyeluruh, dan mengonsumsi susu olahan.

Kata kunci: risiko, tuberkulosis zoonotic, *Mycobacterium bovis*, susu, sapi perah

INTRODUCTION

Bovine tuberculosis is a zoonotic disease known as "Zoonotic Tuberculosis" caused by the mycobacteria *Mycobacterium bovis* (*M. bovis*), which can infect both humans and animals. Bovine tuberculosis mainly affects cattle, but other animals can also be affected (Khairullah et al., 2024). Mycobacteria are a group of rod-shaped bacteria that multiply for a relatively long time and can cause various diseases. Certain mycobacteria that cause tuberculosis (TB) disease in humans and animals are grouped into MTBC (Mycobacterium Tuberculosis Complex). Although most cases of TB in humans are caused by *Mycobacterium tuberculosis* (*M. tuberculosis*), other MTBC members such as *M. bovis*, *M. africanum*, *M. canetti*, and *M. microti* can also cause TB disease in humans (Zhang et al., 2022).

Bovine tuberculosis is present worldwide, except Antarctica (Ramos et al., 2020). Worldwide, more than 50 million cattle are exposed to Bovine Tuberculosis(Srinivasan et al., 2021). There are risk mitigation efforts underway to eradicate Bovine Tuberculosis in the United States, New Zealand, Japan and some European countries (Borham et al., 2022). India has the largest number of infected livestock worldwide with an estimated Bovine Tuberculosis prevalence of 7.3% in dairy and beef cattle (Ramanujam & Palaniyandi, 2023), followed by Brazil (2.5%) (Rodrigues et al., 2022), and China (2.4%) (Gong et al., 2021).

Cattle infected with Bovine Tuberculosis suffer from a chronic and disabling disease. In the early stages of the disease, there are no symptoms. Depending on its local location in the infected animal, *M. bovis* can be found in respiratory secretions, milk, urine, feces, vaginal secretions, semen, and exudates from lesions (such as lymph node drainage and some skin lesions). These diseases generally reduce livestock productivity and can have a negative financial impact on the livestock business, particularly the dairy industry (Khairullah et al., 2024). The main factors contributing to zoonotic *M. bovis* infection in humans are consumption of raw or unpasteurized animal products (foodborne disease), inhalation of droplets containing the bacteria, and contact with diseased carcasses (Luciano & Roess, 2020).

To successfully control and eradicate Bovine Tuberculosis, developed countries have implemented routine testing and culling of infected animals (Khairullah et al., 2024). Mitigating the risk of foodborne disease is necessary because sick animals lose productivity and humans are at risk of contracting Bovine Tuberculosis. However, control efforts are not implemented well enough in developing countries due to financial constraints, lack of skilled professionals, and low awareness of the importance of zoonotic diseases and One-Health by governments (Ramanujam & Palaniyandi, 2023).

Diagnosis to detect the presence of *M. bovis* can be done by various methods. Ziehl-Neelsen staining is the gold standard method for microscopic examination of Bovine Tuberculosis in cattle (Daulay et al., 2017). The Polymerase Chain Reaction (PCR) approach was developed for direct detection of *M. bovis* in bovine milk samples and used effectively to identify MTBC members (Santos et al., 2021).

Data from the World Organization for Animal Health (WOAH) in 2024 stated that in Indonesia, including West Java Province, there were no clinically reported cases of Bovine Tuberculosis. In 2013 in Bangli District of Bali Province a study reported that the seroprevalence of Bovine Tuberculosis was 2.22%, Bogor West Java with Bovine Tuberculosis prevalence of 21.78% (Daulay et al., 2017), and East Java 4-6.8% (Desire et al., 2024).

Until now, there have been no reports or research case studies on the spread of Bovine Tuberculosis in dairy cows in West Bandung and Pangalengan Districts based on information from the Food Security and Livestock Services of West Java Province. According to data from the Central Bureau of Statistics in 2023, West Java is the second largest cow milk contributor province in Indonesia after East Java. In West Java, the districts with the largest cow's milk production are West Bandung (Lembang, Cisarua and Parongpong) and Pangalengan Districts (Sudrajat et al., 2022).

Based on the description above, this study aims to identify the prevalence of *M. bovis* infection with the approach of Ziehl-Neelsen / Acid Fast Bacilli (AFB) staining and PCR in milk from dairy cows in West Bandung and Pangalengan Districts.

MATERIALS AND METHOD

Time and Place of Research

This study was conducted from February to May 2025 at the Laboratory Center for Biotechnology and Bioinformatics Research, Padjadjaran University and Subang Veterinary Center.

Preparation of dairy milk sampling

Dairy milk samples were collected randomly from two districts representing the research locations (Cisarua and Pangalengan). The sample size was determined using purposive sampling method with consideration of the selection of the research location is the area with the most cow's milk production. Based on this method, 30 milk samples were taken in Cisarua, West Bandung, and 30 milk samples were taken in Pangalengan, Bandung. The total amount of milk samples is 60 samples.

Sampling was conducted directly by researchers and sampling officers from KUD Sarwa Mukti Cisarua, West Bandung and Koperasi Peternakan Bandung Selatan (KPBS) Pangalengan. Ethical clearance was obtained from the Research Ethics Committee of the Faculty of Medicine, Padjadjaran University, Bandung, registration number: 2412031566. A total of 60 lactating cows were used for milk sampling. Milk sampling was done aseptically, and samples were collected in sterile storage bags. Sampling activities were carried out in the morning milking. Each sample was labeled with information on sampling time, location name, cow identity and owner identity.

Samples were transported with a cold chain system within 2 x 24 hours using a coolbox to carry out for Ziehl-Neelsen staining at the Padjadjaran University Biotechnology and Bioinformatics Research Center Laboratory, Singaperbangsa Rd. No. 2 Bandung. As for PCR testing, it was carried out at the Laboratory of the Subang Veterinary Center (BVET), Terusan garuda Rd. Blok Werasari RT 33 RW 11 Kel. Dangdeur, Kec. Subang, Subang Regency - West Java. The *M. bovis* bacterial isolate used for the positive control was a pure freeze-dried isolate from BVET Subang. The *M. tuberculosis* bacterial isolate used for the positive control was a pure freeze-dried isolate belonging to BVET Subang also. BVET is a veterinary center in Indonesia that has conducted testing on Bovine Tuberculosis so that it is used as a reference and source of bacterial isolates.

Preparation of DNA extract

Thirty milliliters of milk sample was taken from each cow. The sample was then centrifuged at 3000× g for 10 min (Tomy MX-305 Centrifuge, Japan). The pellet was used to prepare smears and extract DNA. Acid-fast Bacillus (AFB) staining was performed by the Ziehl-Neelsen method which has high sensitivity and specificity and is a simple staining method performed according to the manufacturer's instructions using the Ziehl-Neelsen Acid-fast Bacillus Staining Kit *(IndoReagen, Indonesia). The preparations were dried on a drying rack and then examined using a light microscope (Olympus, Japan) at 1000x magnification.*

PCR testing procedure

Total DNA in milk samples was extracted using a DNA minikit (QIAamp®, Germany), according to the manufacturer's instructions. The results of DNA extraction were then amplified using the multiplex PCR method. The target pathogen detected in this study was the presence of *M. tuberculosis* and *M. bovis* genetic material in the samples. The primers used in this study were MPB70 forward (5′-TGACCAGCATCCTGACCTACC-3′), MPB70 reverse (5′-CGGCGTTACCGACCTTGA-3′) (CN103981260B - A Kind of Method Detecting Mycobacterium Bovis and Mycobacterium Tuberculosis in Aerosol - Google Patents.Html, n.d.), RD1 forward (5'-TTCTGGTCGACGATTGGCA-3′), RD1 reverse (5'-AGGGATGAGTATTACCAGGGCC-3′) (RU2689801C1 - Method for Detecting Mycobacterium Bovis Bcg Strains in Real Time Format - Google Patents.Html, n.d.), and a commonly used forward primer, CSB1 (5'-TTCCGAATCCCTTGTGA-3'), as well as two reverse primers, CSB2 (5'-GGAGAGCGCCGTTGTA-3') which is specific to *M. bovis*, and CSB3 (5'- AGTCGCGTGGCTTCTTTTA-3') which is specific to *M. tuberculosis* (Daulay et al., 2017) (Putra et al., 2023).

PCR amplification was 25 µl in volume, consisting of 12.5 µl Bioline master mix (MyTaq HS Mix, USA), primers, nuclease free water and DNA samples. DNA amplification stages were carried out on a Thermal Cycler machine (BIO RAD T100TM, USA). The PCR program used was 1 cycle at 95°C for 1 minute for initial denaturation, followed by 35 cycles at 95°C for 15 seconds for denaturation, 58°C for 15 seconds for annealing, and 72°C for 15 seconds for extension, then followed by 1 cycle at 72°C for 5 minutes for final extension.

PCR products were then electrophoresed using 1.5% agarose, 100 volts 34 minutes and visualized using a UV transilluminator (Invitrogen, USA). This study used primer concentrations of 10µM derived from a 100µM stock primer, while the PCR annealing temperature used was 58°C, calculated based on (Tm-5)°C, from the average of the forward and reverse primers.

Data Analysis

The data collected in this study were primary data from observations of the specific amplicons of primer MPB70 which can detect both *M. tuberculosis* & *M. bovis*

in the same band (135 bp). Primer RD1, specifically detects only *M. tuberculosis*.

The specific amplicons of primers CSB1, CSB2, and CSB3 of M. tuberculosis were 168 bp (base pairs) and M. bovis 264 bp, and both control. The data obtained were qualitative. Data from the study were analyzed descriptively.

RESULTS

AFB staining results on 60 milk samples taken from dairy farms in West Bandung and Pangalengan districts showed negative results (Table 1). PCR testing on 6 milk samples (10%) of the total samples, showed negative results also (Table 2). These results indicate that no M. bovis bacteria were detected in the milk samples examined by the PCR method (Fig. 1,2,3).

Table 1. AFB staining results of milk samples from dairy farms in West Bandung and Pangalengan Districts

Location	Sampel size (n=60)	Result
West Bandung	30	Negative
Pangalengan	30	Negative

Table 2. PCR results of milk samples from dairy farms in West Bandung and Pangalengan Districts.

Location	Sampel size (n=6)	Result
West Bandung	3	Negative
Pangalengan	3	Negative

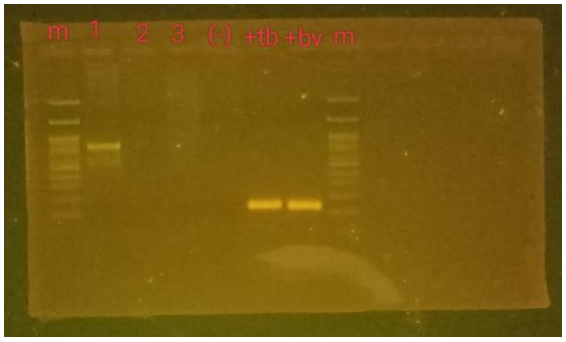

Figure 1. Electrophoresis of PCR products on 1.5% agarose gel. M: 100 bp molecular size marker; lanes 1, 2, and 3 indicate samples (duplo, negative results); lane (-) indicates negative control; +tb is positive control for *M. tuberculosis*; +bv is positive control for *M. bovis* (both at the same band 135 bp) of the MPB70 gene.

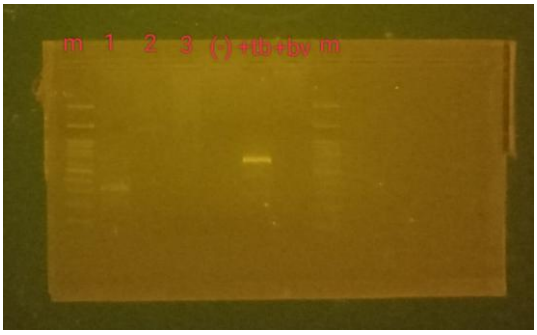

Figure 2. Electrophoresis of PCR products on 1.5% agarose gel. M: 100 bp molecular size marker; lanes 1, 2, and 3 indicate samples (duplo, negative results); lane (-) indicates negative control; +tb is a positive control for *M. tuberculosis* (specifically detects only *M. tuberculosis*); +bv is a positive control for *M. bovis* (not detected) of the RD1 gene.

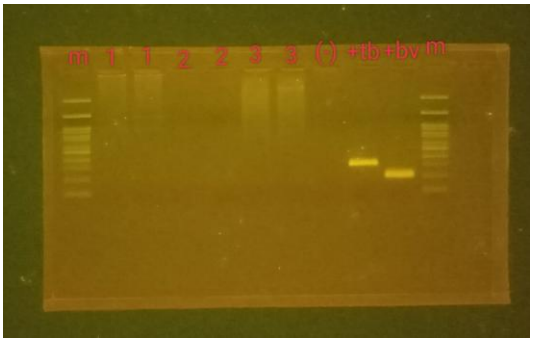

Figure 3. Electrophoresis of PCR products on 1.5% agarose gel. M: 100 bp molecular size marker; lanes 1, 2, and 3 indicate samples (duplo, negative results); lane (-) indicates negative control; +tb is a positive control for *M. tuberculosis* (168-bp band); +bv is a positive control for *M. bovis* (264-bp band) of CSB1, CSB2, and CSB3 genes.

DISCUSSION

Globally, there are an estimated 147,000 new cases of zoonotic tuberculosis in humans and 12,500 deaths from the disease, with the highest burden reported in Africa. Tuberculosis cases in humans in Indonesia are still reported to be high. More than 724,000 new tuberculosis cases were found in 2022, and the number increased to 809,000 cases in 2023 (Global Tuberculosis Report 2023, 2023).

This number is much higher when compared to cases before the pandemic, which averaged less than 600,000 per year. It is noted that globally, individuals at high risk of contracting zoonotic tuberculosis include farmers, animal market workers, veterinarians, slaughterhouse workers, and individuals who are HIV-AIDS positive.

Indonesia's commitment to combating tuberculosis is demonstrated by improving its detection and reporting systems, resulting in the highest number of cases ever reported in 2022 and 2023 (Kemenkes, Dinkes KBB, 2024).

The most common route of transmission of *M. bovis* to humans is through unpasteurized milk products, undercooked meat products, drinking contaminated water, and through the air via close contact with infected animals or animal products. Cattle are the most important animals as a source of tuberculosis transmission in relation to zoonotic exposure in humans, but the disease can affect many other species and spread to wildlife sources.

West Bandung and Pangalengan are among the districts with the second highest dairy cattle population in West Java Province and the third highest incidence of tuberculosis in humans. The practice of raw milk consumption in West Bandung and Pangalengan and the condition of dairy farms, which are dominated by traditional farms, allow the transmission of Zoonotic Tuberculosis (*M. bovis*) from animals, especially dairy cows, to humans in this region. Therefore, it is necessary to identify prevalence infection as preparedness measure and to ensure sustainable cooperation between humans, animals, and related sectors to address the threat of Zoonotic Tuberculosis.

West Bandung and Pangalengan are two of the districts with the highest dairy cattle population in West Java Province and the third highest incidence of tuberculosis in humans. As of September 2024, the number of tuberculosis cases in West Bandung District remains quite high at around 2,962 reported cases, while the government's target is to reduce the incidence of tuberculosis to 60 per 100,000 population. Therefore, hard work is needed to reduce the number (Kemenkes, Dinkes KBB, 2024) (Sudrajat et al., 2021).

Bovine tuberculosis is caused by *Mycobacterium tuberculosis var. bovis*, often referred to as *Mycobacterium bovis* (*M. bovis*). Bovine tuberculosis in dairy cows is thought to contribute to an increase in tuberculosis cases because it can be transmitted to humans (zoonosis), hence it is known as Zoonotic Tuberculosis. Generally, this bacterium can only survive for a few weeks outside its host because it is not resistant to heat, direct sunlight, or dry conditions. This bacterium is non-motile, does not form spores, and is aerobic/microaerophilic.

This disease is persistent and chronic because clinical symptoms only appear when it is already severe, but sometimes it can be acute and progressive, especially in young animals. Symptoms include respiratory disorders such as shortness of breath, emaciation, and weakness. Cough caused by bacteria in cattle (*M. bovis*) is very difficult to distinguish from cough caused by bacteria in humans (*M. tuberculosis*), as both show very similar symptoms such as persistent coughing and, in severe cases, vomiting blood. A total of 10 million active tuberculosis cases occurred in humans globally in 2019, with 140,000 (range 69,800–235,000) of these estimated to be new cases of zoonotic tuberculosis (1.4%) and approximately 11,400 (8.1%, range 4,470–21,600) resulted in death (Kock et al., 2021).

Indonesia ranks second in the world for the number of tuberculosis cases (*Global Tuberculosis Report 2023*, 2023). This disease is a public health burden and causes significant economic losses because it disrupts animal health, reduces production, increases eradication program costs, and restricts trade (Singhla & Boonyayatra, 2022).

Rapid Identification of *M. bovis* Prevalence in West Bandung and Pangalengan related to Zoonotic Tuberculosis was conducted to determine mitigation and risk management of Zoonotic Tuberculosis cases in the West Bandung and Pangalengan Regencies. This is because West Bandung and Pangalengan Regencies have the highest dairy cattle population in West Java.

Based on data from the Central Statistics Agency / Badan Pusat Statistik (BPS) in 2023, West Bandung Regency has the largest dairy cattle population in West Java Province after Pangalengan (Bandung Regency). The total dairy cattle population in West Bandung Regency in 2023 is 38.491, with 5.598 farmers (accounting for 34,6% of the total dairy cattle population in West Java Province). The three subdistricts with the highest dairy cow populations are: Lembang, Cisarua, and Parongpong. Meanwhile, Pangalengan has the second largest dairy cattle population of 25.000 in West Bandung Province (*Statistik-Indonesia-2023*, n.d.).

Additionally, data on tuberculosis cases in West Bandung Regency up to August 2024 shows approximately 2,700 cases. There are 126 patients

diagnosed with tuberculosis also in Pangalengan Hospital (57 men and 69 women) spread across 21 villages in Pangalengan Regency. This condition has the potential for the transmission of Zoonotic Tuberculosis from animals to humans or vice versa.

Most people in West Bandung and Pangalengan Districts keep dairy cows as their main livelihood. The rearing system applied in cages is intensive. Feed is provided by farmers in the form of forage hay in the form of hay or silage and other grasses. Concentrates come from various regions both within and outside West Bandung and Pangalengan. The feeding schedule averages 2-3 times a day with a milking system of 2 times a day in the morning and evening.

Dairy farming is oriented in the form of smallholder farming with an average work output of 8 hours/day. Dairy cattle ownership ranges from between 4-5 cows for each household (average 4 cows). Cage conditions are generally humid, with poor lighting and good air circulation systems. Floors are plastered, with wooden cages. Biosecurity and biosafety systems in dairy farms are generally not running optimized.

Milk milked on smallholder farms is carried in buckets and collected at the collection point of each livestock group using a milk can. The milk is then transported to the cooperative's milk collection unit and collected into milk containers. From the milk collection unit, fresh milk is transported using cooling units to milk processing business units (milk processing companies within and outside West Bandung and Pangalengan). In addition, fresh milk is also distributed to Milk Small and Medium Enterprises (MSMEs) in West Bandung and Pangalengan, and process milk into pasteurized milk, yoghurt, cheese, milk crackers, ice cream, milk sticks, milk pudding, milk candy and milk dodol.

Six (10%) out of 60 dairy cows in West Bandung and Pangalengan Districts were negative for *M. bovis* and *M. tuberculosis*. These results are representative electropherogram of PCR testing for the entire sample showing no readable target DNA bands when compared to the positive control DNA bands of *M. bovis* and *M. tuberculosis* at each amplification.

Laboratory diagnosis of animal TB against *M. bovis* can be done by various methods such as tuberculin test, mycobacteria isolation/culture, Ziehl-Neelsen staining, and PCR (Szacawa et al., 2025). The test with negative results is similar to the results of research by (Putra et al., 2023) on cow's milk samples in the Central and Eastern Regions of Java Island, Indonesia which showed negative results in the PCR test without being preceded by a tuberculin test. PCR test results on milk samples also did not find *M. tuberculosis* bacteria. The test for the presence of *M. tuberculosis* was conducted because *M. tuberculosis* and *M. bovis* are the main causes of TB which are highly pathogenic, can infect many animal species and are possible sources of human TB infection.

This research provides essential information on the prevalence and distribution of *M. bovis* infections in Indonesia, particularly in the West Bandung and Pangalengan regencies. This information is crucial for controlling this zoonotic disease. The results support efforts to mitigate the risk of foodborne diseases by detecting and controlling the infection early on, specifically in dairy cow milk, thereby preventing the spread of tuberculosis in humans and livestock. This research assists farmers and the government in formulating policies to control and prevent disease, as well as raise public awareness of the importance of consuming safe, healthy milk. In the long term, this will improve public health, promote sustainable livestock farming, and reduce the economic burden caused by this zoonotic disease.

Other possible causes of negative results are milk collection units (MCU) in West Bandung Regency and Pangalengan, which already have NKV (Veterinary Control Number) certificates. In addition, data from animal traffic information system (Lalin iSIKHNAS) shows no data on the import and export of dairy cows to and from provinces other than West Java (no applications for Veterinary Certificates / Animal Import Recommendations). So that cattle in West Java are not exposed to cattle infected with *M. bovis* that were found in Pasuruan and Surabaya, East Java. Meanwhile, the dairy cattle population in Central Java in 2021 was 142,513, and no positive cases of *M. bovis* (zoonotic tuberculosis) have been found. From routine post-mortem examinations also at the West Bandung District Slaughterhouse, no cases of lung nodules have ever been found.

Biosecurity issue that needs to be considered is the possibility of unmonitored entry and exit of dairy cattle, as farmers do not apply for permits and there is easy access via the toll road. The government must require that livestock and livestock origins be free of *M. bovis* (zoonotic tuberculosis) for cattle to be imported into Bandung, West Java Province.

M. bovis must be included in the priority animal disease control program in Bandung, West Java Province, so that there are regulations regarding testing and slaughter. Prevention and control measures for *M. bovis* in Bandung are still in the biosecurity implementation stage, which began at the time of FMD (15% of 5,779 farmers in West Bandung).

The health of cows and the quality of milk samples at KPBS Pangalengan are relatively similar, with milk quality above Indonesian National Standard / SNI (2011), except for the total plate count, which is still above SNI standards. Total Plate Count (TPC) is the total number of microbes present in milk that can affect milk quality, which states that equipment can be a source of milk contamination if it is not thoroughly cleaned, especially the parts that come into direct contact with milk. KPBS Pangalengan member farmers are on average 46 years old, have an elementary school education level, an average of 3 family members, an average of 19 years of farming experience, and an average of 4 cows, with milk production and quality still within the normal range according to SNI standards (Sudrajat et al., 2021).

Practical implications for farmers and at the slaughterhouses are personnel hygiene must be optimized (using personal protective equipment (masks, aprons, gloves), and workers must wear boots). It is recommended that ante-mortem and post-mortem examinations be conducted at the slaughterhouses, and the results be reported to the local authorities. Farmers must pay attention to the condition of the cages, it is not damp, have adequate lighting, and a good air circulation system. The cage floors are plastered, or the cages are made of wood so easy to clean.

The biosecurity and biosafety systems in dairy farms are generally not yet operating optimally. Practical implications for dairy cooperatives to implement biosecurity One Health measures to prevent zoonotic tuberculosis are that milk collection units (MCU) must have an NKV (Veterinary Control Number) certificate. NKV stands for Veterinary Control Number, which is an official certificate proving that an animal product business unit has met hygiene and sanitation requirements. This certificate serves as a guarantee of animal product safety, ensuring that the products are safe, healthy, intact, and halal (ASUH) for consumption. Therefore, it has met food safety guarantees. NKV is written proof that animal products are safe and do not contain biological, chemical, or physical hazards.

The practical implication for consumers is the importance of pasteurization. In terms of community practices, avoid consuming fresh (raw) milk directly from farmers. Fresh (raw) milk sometimes also sold freely on social media without clear descriptions, and not all of them come with instructions for pasteurizing or cooking the milk.

The research results were reinforced by field data. The status of bovine tuberculin testing in the region in 2023, surveillance of *M. bovis* Bovine Tuberculosis in dairy cattle was conducted in West Bandung District (Lembang, Parongpong, Cisarua and Ngamprah), with a sample size of 1.240 samples, 216 farms, with negative results. Routine Post Mortem examinations at the West Bandung Abattoir have never found humped lung. This proves that the farm's health history support a negative interpretation.

Rapid Identification of the prevalence of Zoonotic Tuberculosis (*M. bovis*), showed that all 60 dairy milk samples (30 from West Bandung and 30 from Pangalengan) examined by AFB staining and PCR methods, were negative (0%). This means that no cases of *M. bovis* infection were found in the milk samples examined. Local milk from both regions is relatively safe for consumption and does not show the potential to transmit TB to humans. The husbandry practices adopted by local farmers may have supported the low risk of zoonotic transmission, although there are still challenges in implementing comprehensive biosecurity, underscores the need for ongoing surveillance, improved diagnostics, and public awareness to prevent zoonotic transmission and economic impacts.

Tuberculin test method (as the gold standard macroscopic) has not been used. It can be combined using advanced molecular techniques such as Whole Genome Sequencing. Screening for the presence of *M. bovis* or other contaminants in processed products such as yoghurt, cheese, or pasteurized milk, and seroprevalence studies at high risk (farmers, dairy cooperative workers, butchers) needs to prevent Zoonotic Tuberculosis.

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"The author declare that there is no conflict of interest with the parties involved in this research."

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