

A meta-analysis of bovine tuberculosis in Indonesia: a concerning zoonotic disease

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ABSTRACT

Bovine tuberculosis (bTB) is a zoonotic disease with high economic impact and public health concern. However, the understanding of bTB in Indonesia is limited. Thus, this study aims to estimate the pooled prevalence of bTB in cattle in Indonesia using a meta-analysis. Five online databases were searched for related articles. The articles were screened by two independent reviewers following the inclusion criteria. The included studies went through quality assessment using five pre-determined questions. The necessary data were extracted and analysed using random effect model in R studio 4.3.0 with "meta" R package to estimate the pooled prevalence, heterogeneity level, and risk of publication bias. Subgroup meta-analysis was conducted for subgroup diagnostic method and *Mycobacterium* species. The results showed that six studies fit in inclusion criteria, representing 711 samples. The quality assessment showed the included studies were good quality. The estimated pooled prevalence of bTB in Indonesia was 9.71% (95% CI, 1.47-43.69%) with high heterogeneity. Based on subgroup meta-analysis, no significant difference was found in between non-molecular and molecular diagnostic methods (10.55% [95% CI, 0.48-42.03] vs 6.43 [95% CI, 1.64-22.11], $p = 0.92$). Similarly, between *M. bovis* and *M. tuberculosis*, no significant difference in prevalence was found (2.04% [95% CI, 0.38-10.13] vs 6.55% [95% CI, 0.15-76.17], $p = 0.57$). The risk of bias assessment showed no indication of severe publication bias. This study indicates the necessity of bTB active surveillance and epidemiological study in Indonesia. Furthermore, a control and preventive strategy for zoonotic transmission mitigation should be developed.

Introduction

Bovine tuberculosis (bTB) is a zoonotic infectious disease primarily originating from cattle, caused predominantly by *Mycobacterium bovis*, a member of the *Mycobacterium tuberculosis* complex (MTBC) (El-Sayed *et al.*, 2016). Meanwhile, in humans, *M. tuberculosis* is the main causative agent (Bañuls *et al.*, 2015). However, both *M. bovis* and *M. tuberculosis* are capable to infect human and animal which increases the difficulty of controlling the pathogen and its zoonotic potential. In livestock, bTB results in significant economic loss following the reduction of the product up to 18% of milk and 15% of meat and the death of the livestock (Azami & Zinsstag, 2018). Additionally, the economic loss can also occur due to limitation in trade and test-and-slaughter policy which have been implemented in many developed countries as an effort to decrease the bTB and zoonotic potency (Azami & Zinsstag, 2018).

Some cases of *M. bovis* infecting humans have been reported in several countries, and conversely, *M. tuberculosis* has also been detected in animals (Olea-Popelka *et al.*, 2017). *Mycobacterium* can be transmitted between animals through direct contact with the agent via respiratory and oral tracts or through indirect transmission by contaminated food or water, especially in shared water and feed areas (Ahmad *et al.*, 2023). It is common in semi-intensive and extensive farming systems, where animals from different farms interact in the same grazing area, as seen in some regions of Indonesia. bTB in animals can cause various clinical signs such as cough, dyspnea, diarrhea, or constipation, depending on the complexity of the infected organs (Ramos *et al.*, 2020). However, in many cases, animals show no symptoms of tuberculosis and become sources of disease transmission to other animals and humans (Olea-Popelka *et al.*, 2017). bTB can be transmitted to humans through the consumption of contaminated food, specifically raw milk and untreated meat. Additionally, direct contact with the agent through the respiratory tract and contact with infected tissue can also transmit the disease to humans, increasing the

complexity of disease control (Pérez-Lago *et al.*, 2014). The highest incidence of zoonotic tuberculosis is found in impoverished, marginalized, and remote populations that live in close proximity to animals and have limited access to hygienic food and medical care (Ahmad *et al.*, 2023).

In Indonesia, the reports regarding bTB in livestock is scarce, thus it is difficult to understand the disease completely. According to the World Health Organization (WHO), Indonesia is among the eight countries that collectively account for one-third of the world's tuberculosis cases (World Health Organization, 2022). Tuberculosis ranks among the top five leading causes of death in Indonesia overall and is the number one cause of death in children under five (World Health Organization, 2022). Additionally, tuberculosis rates increased in 2022 compared to 2021, indicates the significance of this disease in Indonesia (World Health Organization, 2022). A lack of understanding regarding *M. bovis* as a pathogen in human tuberculosis hinders a comprehensive understanding of the true burden of tuberculosis. Although *M. tuberculosis* is the primary cause of tuberculosis in humans, studies in several countries have shown that *M. bovis* is also linked to some human tuberculosis cases (Taye *et al.*, 2021). Surveillance data are necessary for disease eradication, assessment of disease burden, minimalizing risk, and monitoring the risk factors in the vulnerable population for both humans and animals (Ramos *et al.*, 2020). However, the information regarding bTB in Indonesia is limited. Therefore, this study aims to estimate the pooled prevalence of bTB in cattle in Indonesia to have a better understanding of bTB in Indonesia.

Materials and methods

Literature Search

The literature search was conducted systematically in five online databases, PubMed (<https://pubmed.ncbi.nlm.nih.gov/>), Scopus (<https://www.scopus.com/>), ScienceDirect (<https://sciencedirect.com/>), Garuda

(<https://garuda.kemdikbud.go.id/>), and Google Scholar (<https://scholar.google.com>) using the combination of the following terms: 'bovine tuberculosis', cattle, *Mycobacterium*, and Indonesia. Specifically for the GARUDA website, Indonesian keywords were used. A total of 428 articles were retrieved (Table 1, Fig. 1). The retrieved articles were selected following the preferred reporting items for systematic reviews and meta-analysis (PRISMA) guidelines (Page *et al.*, 2021). A protocol was designed prior to the study and published in Open Science Framework (OSF) (<https://osf.io/x6thj/>).

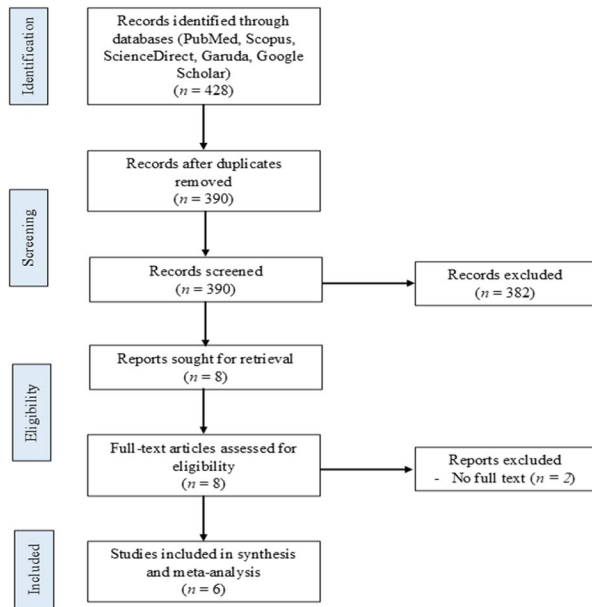


Fig. 1. PRISMA flowchart of studies selection for systematic review and meta-analysis of bovine tuberculosis in Indonesia.

Selection criteria and data extraction

The selection was based on several criteria: cross-sectional study, peer-reviewed original articles or conference proceedings, reporting bovine tuberculosis in Indonesia's cattle, full paper availability, and published in English or Bahasa Indonesia. Reports with other study designs, no full paper, or not conducted in Indonesia were excluded. Due to lack of study, no minimum sample size was required for the inclusion criteria. Two independent reviewers performed the initial screening using Rayyan-Intelligent Systematic Review (<https://www.rayyan.ai/>). The discrepancy between reviewers was solved through discussion. The information from the included articles, including author, year of publication, location of study, sample size, diagnostic method, sample type, and case number were extracted Microsoft Excel sheets (Microsoft Corp, Redmond, WA, USA).

Quality Assessment of Individual Studies

Each included study was evaluated using a simple scoring system fol-

lowing Ding *et al.* (2017) methods. Totally five questions were used to assess each of the study where a yes answer was scored 2 (Low), unclear answer was scored 1 (Some concerns), and no answer was scored 0 (High). The questions were: five questions will be used in assessing the quality of each included study. The questions include: 1) Was the research question/objective clearly described and stated?; 2) Was the sampling method described in detail?; 3) Was the period of study clearly stated?; 4) Was the diagnostic method clearly pointed out?; 5) Were the subjects categorized into different subgroups?. The risk of bias assessment is represented graphically through a traffic light plot and a summary plot, utilizing the Robvis website for visualization purposes (McGuinness & Higgins, 2020).

Statistical analysis for overall pooled prevalence and subgroup analysis of bovine tuberculosis

Random meta-analysis with logit transformation was conducted using 'meta' R package in R.4.3.0 software (Comprehensive R Archive Network, Vienna, Austria). The results were consisted of the estimated pooled prevalence, 95% confidence interval (CI), and heterogeneity of the studies (I², p-value, and Cochran's Q). The subgroup meta-analysis was conducted for subgroup diagnostic methods (molecular diagnostic and phenotypic method) and detected *Mycobacterium* species (*M. bovis* and *M. tuberculosis*). The distribution of the studies was visualized QGIS 3.32.2, with the basemap downloaded from OpenStreetMap and GADM data (Version 4.1) (https://gadm.org/download_country.html)

Risk of publication bias

Risk of publication bias was assessed by Funnel plot, trim and fill plot, Begg's test, and Egger's test with p-value <0.1 in Egger's test indicated bias potency (Egger *et al.*, 1997). Funnel plot showed the precision of the pooled estimated prevalence to the true prevalence.

Results

Literature Search

In total, 428 studies were retrieved from five databases and screened based on inclusion criteria which resulted in 8 included studies (Fig. 1). After full-text retrieval, 6 studies were used for the analysis, representing 711 samples for the bTB test. The samples were mostly collected from dairy cattle which were reported from all studies with the addition of 2 studies that used beef cattle samples. The studies were conducted in West Java (n = 3), Central Java (n = 1), Yogyakarta (n = 1), East Java (n = 2), and Bali (n = 1) provinces (Fig. 2). The methodology for bTB test was mostly done by molecular detection by polymerase chain reaction (PCR) (n = 5) and some non-molecular methods such as tuberculin test (n = 1), Ziehl-Nielsen staining (n = 2), enzyme-linked immunosorbent assay (ELISA) (n = 1). Due to limitations in the number of studies that used non-molecular tests, those tests were grouped within the same subgroup for meta-analysis. The detection of *Mycobacterium* was focused on the two most important agents, *M. bovis* (n = 5) and *M. tuberculosis* (n = 8).

Table 1. Characteristics of the included studies for meta-analysis of bovine tuberculosis in Indonesia.

Authors and year	Province	Cattle type	Sample size	Sample type	Diagnostic Method	Cases
Daulay <i>et al.</i> (2017)	West Java	Dairy	202	Skin, Feces	Tuberculin test, PCR	44
Putra <i>et al.</i> (2013)	Bali	Beef and dairy	180	Blood	ELISA	1
Putra <i>et al.</i> (2023)	Central Java, Yogyakarta, East Java	Dairy	163	Milk	PCR	0
Juwianto <i>et al.</i> (2018)	West Java	Beef and dairy	10	Swab trachea, Swab diaphragm, Lung, Trachea	PCR	1
Nugroho <i>et al.</i> (2009)	West Java	Dairy	62	Milk, Feces	Ziehl-Nielsen, PCR	15
Desire <i>et al.</i> (2024)	East Java	Dairy	94	Milk	Ziehl-Nielsen, PCR	66

Note: PCR: polymerase chain reaction; ELISA: Enzyme-linked immunosorbent assay

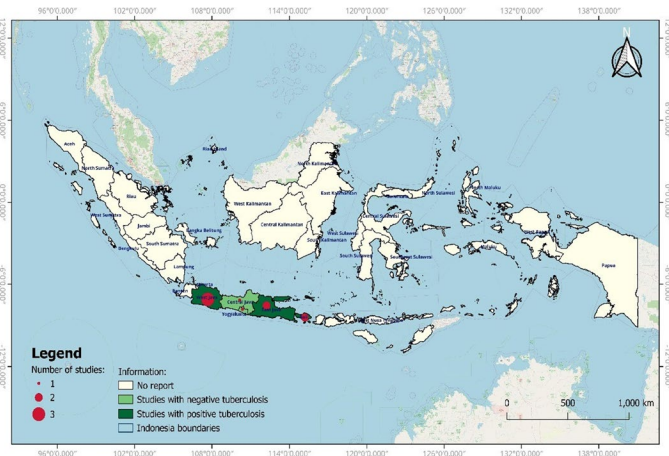


Fig 2. Distribution of bovine tuberculosis studies in Indonesia

Quality Assessment of Individual Studies

The quality assessment showed that the range of quality score is between 7-10, indicates a good quality study for all includes studies (Fig. 3). The average score of the risk assessment is 8.83 ± 1.33 .

Statistical analysis for overall pooled prevalence and subgroup analysis of bovine tuberculosis

This study assessed the estimated pooled prevalence of bTB in cattle in Indonesia with a result of 9.71% (95% CI, 1.47-43.69%). The heterogeneity tests showed a significant result among 6 included studies ($Q=96.28, I^2=95\%, p<0.01$) as can be seen in forest plot (Fig. 4). The subgroup meta-analysis on the methodology of bTB detection in Indonesia showed no significant different result between molecular and non-molecular methods (10.55% [95% CI, 0.48-42.03] vs 6.43 [95% CI, 1.64-22.11], $p = 0.92$). Similarly, based on the *Mycobacterium* detection, no significant difference was found between the estimated prevalence of *M. bovis* and *M. tuberculosis* in cattle samples (2.04% [95% CI, 0.38-10.13] vs 6.55% [95% CI, 0.15-76.17], $p= 0.57$).

Risk of publication bias

Based on funnel plot, the diagram showed a slightly asymmetry plot. Trim and fill test indicated 3 study added to make the diagram symmetry. However, the result of Egger's test ($p = 0.22$) and Begg's test ($p = 0.35$) indicates no severe publication bias in the result of this meta-analysis.

Discussion

Tuberculosis presents a significant threat to a public health concern, but there is limited information on bTB in cattle in Indonesia. This study found only six studies involving 711 cattle samples from Java and Bali

Table 2. Overall pooled prevalence of bovine tuberculosis in Indonesia and subgroup meta-analysis.

Categories	No. of studies or subgroups	Prevalence		Q	Heterogeneity		p-value for subgroup difference
		Estimates	(95 % CI)		p-value	I ²	
Overall	6	9.71	[1.47; 43.69]	92.38	< 0.01	95%	
<i>Subgroup analysis</i>							
<i>Methodology</i>							
Molecular detection	5	10.55	[0.48; 42.03]	79.28	<0.01	95%	0.92
Phenotypic	5	6.43	[1.64; 22.11]	45.85	<0.01	91%	
<i>Mycobacterium species</i>							
<i>Mycobacterium bovis</i>	5	2.04	[0.38; 10.13]	27.11	<0.01	85%	0.57
<i>Mycobacterium tuberculosis</i>	8	6.55	[0.15; 76.17]	27.2	<0.01	39%	

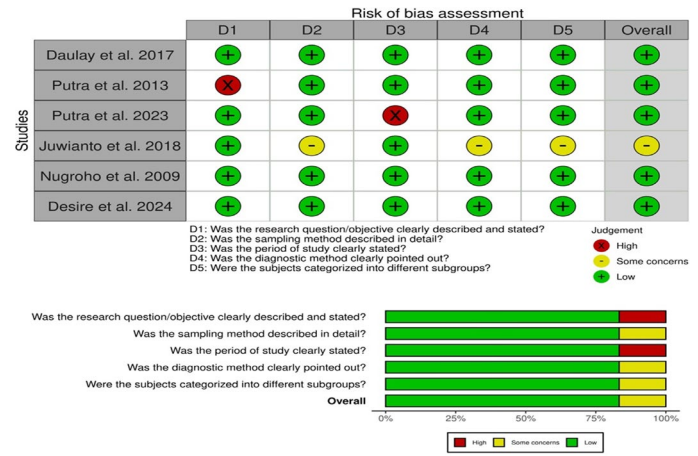


Fig 3. The quality assessment of included studies for meta-analysis of bovine tuberculosis in Indonesia

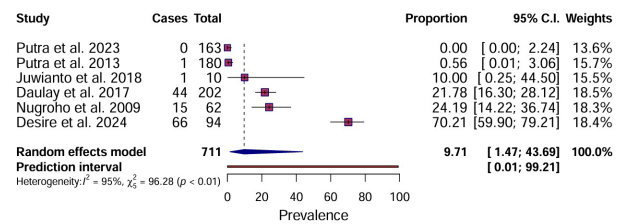


Fig. 4. The forest plot for meta-analysis of bovine tuberculosis in Indonesia.

Islands reporting the bTB in Indonesia with an estimated pooled prevalence of 9.71%. This estimated pooled prevalence is higher compared to some countries such as India (6.3%) (Srinivasan *et al.*, 2018), Ethiopia (5.8%) (Sibhat *et al.*, 2017), China (2.4%) (Gong *et al.*, 2021), and Tanzania (1.28%) (Alonso *et al.*, 2016). In Asia and Africa, the overall pooled prevalence of bTB in livestock was predicted to be lower than 10%, which align with Indonesia pooled prevalence (Ramos *et al.*, 2020). Meanwhile, other continent such as North America, South America, and Europe have higher than 30% bTB prevalence in livestock (Ramos *et al.*, 2020). However, the low prevalence of bTB in Asia, including Indonesia, is related to the insufficient report and surveillance of this disease, especially in the developing countries (Ramos *et al.*, 2020). Additionally, the burden of the disease is predicted to be higher in developing countries due to lack of animal tuberculosis control programs as also occur in Indonesia.

In Indonesia, a combination of non-molecular and molecular methods were mostly used to diagnose bTB. PCR was found in most study to identify the pathogen along with other non-molecular tests such as tuberculin test, Ziehl-Nielsen staining, and ELISA. In this study, due to the lack of number of studies, current meta-analysis combined the non-molecular tests under one group for the analysis. The results indicated that PCR and non-molecular tests resulting similar estimated prevalence which indicates any tests could be used in bTB testing. The combination

of antibody detection and PCR showed a more comprehensive information of bTB in cattle (Ramos D. F. *et al.*, 2015). Based on the subgroup meta-analysis, the cattle in Indonesia have been infected with both *M. bovis* and *M. tuberculosis* with no significant difference in estimated prevalence. This finding shows a high public health threat from zoonotic tuberculosis that can be distributed from cattle.

Globally, it is estimated that approximately 143,000 (ranging from 71,200 to 240,000) incident cases of zoonotic tuberculosis caused by *M. bovis* were reported in 2018 (World Health Organization, 2022). A meta-analysis of *M. bovis* as tuberculosis pathogen in human has revealed a prevalence of 9.7% globally with high variety, ranging from 0.4-76.7% (Taye *et al.*, 2021). A high rate of infection by *M. bovis* in human have been reported from Africa countries, such as Uganda and Nigeria with rate higher than 5% (Cadmus *et al.*, 2006; Oloya *et al.*, 2008). Meanwhile, a considerable variation of *M. bovis* infection was found South America and Asia. For instance, in South America, Brazil reported a rate of 1.6% (Silva *et al.*, 2013) while Mexico reported 30% (Torres-Gonzalez *et al.*, 2016). In Asia, infection rates ranges from 2% in Pakistan (Jabbar *et al.*, 2015) to 9% in India (Prasad *et al.*, 2005). In developed countries of North America and Europe, such as the United States, the United Kingdom, Netherland, and France the rate of tuberculosis caused by *M. bovis* is reported to be lower than 2% (Majoor *et al.*, 2011; Mignard *et al.*, 2006; Scott *et al.*, 2016; Stone *et al.*, 2012). Meanwhile, in developed countries with high meat production such as Ireland and New Zealand, a rate of approximately 3% was reported (Baker *et al.*, 2006; Ojo *et al.*, 2008).

In Indonesia, Yanti *et al.* (2019) reported that 12 out of 31 tuberculosis cases in humans were related to *M. bovis* infection. Identified risk factors for tuberculosis caused by *M. bovis* include a history of drinking raw milk and close contact with livestock (Yanti *et al.*, 2019). This indicates that *M. bovis* is circulating within the population and can be transmitted from animals to humans. Putra *et al.* (2023) used Multiple Criteria Decision Analysis (MCDA) to analyze bTB transmission between animals and humans in Indonesia, finding a moderate level of transmission. This finding should raise concerns regarding zoonotic tuberculosis in Indonesia. Control and preventive measures should be developed to address the zoonotic potential of bTB. Successful eradication of bTB in developed countries has been achieved through strategies such as active surveillance and the test-and-slaughter method, implemented in the United Kingdom, Ireland, New Zealand, and parts of the United States (Admassu *et al.*, 2015). However, in many developing countries, including Indonesia, there has been a lack of specific strategies for controlling bTB in livestock, leading to insufficient attention to the disease and a resulting public health threat (Ramos *et al.*, 2020). Active surveillance is crucial for providing the necessary data to develop appropriate control and preventive strategies. Furthermore, the World Organisation for Animal Health (WOAH) has classified bTB as a notifiable disease, underscoring the importance of active surveillance (World Organisation for Animal Health (WOAH), 2024). Therefore, an active surveillance of bTB in Indonesia should be conducted and followed by the implementation of appropriate strategies following the surveillance result.

Some limitation occurs in this meta-analysis which mainly due to small number of studies that were conducted only in several provinces in Java and Bali. Therefore, the result of this study may not fully represent the true burden of bTB in nation. This highlights the urgent need for actions such as active surveillance and epidemiological studies of bTB in Indonesia. Considering the limited previous comprehensive studies on bTB in cattle in Indonesia, the findings of this study provide a current understanding of bTB in the country, indicating an underlying zoonotic threat. More attention should be directed to this disease using a One Health approach to develop a comprehensive control strategy.

Conclusion

In Indonesia, the bTB in cattle is estimated pooled prevalence of

9.71% which analysed from six reports. The subgroup meta-analysis showed no significant different among diagnostic methods being used in the bTB detection. The identification of the agent showed no significant different of *M. bovis* and *M. tuberculosis* infection in cattle which show a zoonotic threat. Active surveillance of bTB is necessary for better knowledge of the disease and developing a strategy in mitigating the public health threat.

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Conflict of interest

The authors have no conflict of interest to declare.

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