

# Pathological characterization of lesions and bacteriological isolation of causative agents of swine tuberculosis at Bishoftu and Addis Ababa Abattoirs, Bishoftu, Ethiopia

## Abstract

**Background:** TB in swine is natural and virtually common in developed countries where swine is farmed. Although this re-emerging disease is very common in developing country as well; there is scanty information on the prevalence of swine tuberculosis in Ethiopia. Therefore, this study was developed to assess the prevalence of swine tuberculosis by using bacteriological and histopathological techniques.

**Methods/Principal Findings:** A cross sectional study was conducted at Bishoftu and Addis Ababa Abattoirs from September 2017 to May 2018 to estimate abattoir based prevalence of tuberculosis in swine, to isolate *Mycobacterium* species involved and to characterize the lesions. Five hundred and fifty six (556) swine were examined. Tubercle like granulomatous lesion were detected in 19.6% (109/556) of different organs of which 12% (69/556) was in lymph nodes, 5.7% (32/556) in the lungs, 1% (6/556) in the liver and 0.36% (2/556) in the spleen. Based on microscopic alteration 4.7% (26/556) of lesions were tuberculous granulomatous type with central necrosis, calcified foci, epitheloid cells admixed with lymphocyte and connective tissue boundaries, 3.6% (20/556) pyogranulomatous and 2.5% (14/556) non necrotic granulomatous lesion without epitheloid cells and connective tissue capsules. The tuberculous lesions found in lymph node were statistically significant ( $P < 0.05$ ) than in other organs. The multivariable logistic regression analysis showed that old aged swine were more likely to have characteristic tuberculous lesion (OR = 3.14, 95% CI, 1.62-6.09) than younger ones. From the tissue cultured, 7.5% (3/40) yield growth on primary culture media. The observed colony morphology was smooth whitish or yellowish color, sticky, off-white and breaks apart easily and two (5% (2/40)) of these growth were acid fast positive by Ziehl-Neelsen staining technique.

**Conclusion/Significance:** This study highlights that TB is prevalent in swine and pork can be source of tuberculosis to human when consumed under cooked. Therefore, routine abattoir inspections should be conducted, further molecular and biochemical research to isolate the species of microbacteria is highly recommended to elucidate the type of lesion and its magnitude in different body organs by considering large number of swine.

**Keywords:** *mycobacterium tuberculosis*, bacilli, Bishoftu, TB

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**Abbreviations:** SLN, submandibular lymph node; PLN, parotid lymph node; MLN, mesenteric lymph node; RLN, retropharyngeal lymph node; LCR, left cranial; LCA, left caudal; RCR, right cranial; RML, right middle; RCA, right caudal; RAC, right accessory lobes of lungs; LR, liver; SP, spleen; TN, tuberculosis

## Introduction

Tuberculosis (TB) is considered as a re-emerging, infectious granulomatous disease in animals and people caused by acid-fast bacilli of the genus *Mycobacterium*. Tuberculosis occurs frequently in man, domestic and wild animals. The tubercle bacilli are *Mycobacterium tuberculosis*, the agent of the disease in primates, *M. bovis* in other mammals and *M. avium* in birds. Swine are susceptible to all the three types of tubercle bacilli. Swine and dogs are susceptible to both *M. bovis* and *M. tuberculosis*. Cats are quite resistant to *M. tuberculosis*.<sup>1</sup> Tuberculosis continues to be an important disease both in humans and animals. It causes morbidity, mortality and economic

loss worldwide. The occurrence of *Mycobacterium bovis* disease in humans, domesticated and wild animals confirms the relevance of this zoonosis.<sup>2-18</sup>

Swine are natural hosts for mycobacterial infections including those due to *M. bovis*.<sup>19</sup> The most common cause of swine tuberculosis is *Mycobacterium avium*, but infection with mammalian tubercle bacilli, including *M. tuberculosis*, *M. bovis*, and *M. africanum* occur coincident with infections of cattle, wildlife, and human beings. The finding of severe, diffuse tuberculous lesions strongly suggests that Nebrodi black pigs are susceptible for *Mycobacterium* spp. and that they might act as a distributor for these microorganisms.<sup>3</sup> Lesions occurring in swine naturally infected with *M. bovis* and *M. tuberculosis* are indistinguishable. Granulomatous lesions are most often found in the cervical, submandibular, and mesenteric lymph nodes, but in advanced disease lesions may also be found in the liver and spleen. Typically, enlarged nodes contain small, white or yellow, caseous foci, usually without any evidence of calcification. Swine

with disease due to *M. tuberculosis* may have similar regionalized lesions with human beings. Swine are particularly susceptible to *M. bovis*, which is usually acquired from shared grazing or ingestion of contaminated dairy products. This can cause a rapidly progressive, disseminated disease with caseation and liquefaction of lesions. In industrialized countries most tuberculous lesions in swine are caused by bacteria of the *M. avium* complex (*M. avium ss hominis*, *M. avium ss avium*) and *M. intracellulare*. Lesions are most often observed in lymph nodes associated with gastrointestinal tract. However, it is important to emphasize some swine may develop progressive disease involving the liver, spleen and other organs of the abdominal and thoracic cavities.<sup>4,20</sup>

The lesions in swine infected with *M. tuberculosis* are often associated with lymph nodes of the gastrointestinal tracts. Caseous lesions are most commonly found in the mesenteric or submaxillary lymph nodes; however, microscopic lesions have been observed in the portal and thoracic nodes and in the parenchyma of the lungs.<sup>2</sup> The De initiative diagnosis to check whether a certain herd is infected or not requires a clear evidence of the agent based on bacteriological culturing, histopathology and molecular methods.<sup>21,22</sup> The main bottleneck for transmission of TB in many country is from wild species and vice versa. According to Pederson et al.,<sup>5</sup> infection in white-tailed deer (*Odocoileus virginianus*) has been followed by infection in cattle in some Midwestern states. Infection has also been documented in feral swine (*Sus scrofa*) on the Hawaiian island of Molokai and in various European countries, but no large-scale survey of antibody exposure to the bacteria has been conducted in feral swine in the US.<sup>6</sup> A major effort to eradicate tuberculosis in swine and in people markedly reduced the incidence of the types of tuberculosis usually seen in swine and people. The serovars of avian type TB found in confinement-raised swine have been found in people may translate into increased concern for TB in both swine and poultry. Shared research in human and animal health can efficaciously speed the development of new diagnostic tests for humans and livestock, and improve TB surveillance, control, and eradication programs.<sup>1,20</sup>

The main objective of the current study was therefore is the first of its kind in providing baseline information with regard to pathological

and histopathological study. The ultimate aim was pathological and bacteriological methods; were used for the diagnosis of swine tuberculosis. Correlation of gross, microscopic and bacteriological findings to increase the sensitivity of TB detection provided the true epidemiological status of the disease and useful information in the swine population in Ethiopia that can be utilized for future research and disease control purpose at national and global level TB control efforts and reducing the risk of its zoonotic significance.

## Methods

### Study area and study population

The study was conducted from September 2017 to May 2018 in Addis Ababa and Bishoftu abattoirs. These two abattoirs were selected purposively because they were the only abattoirs slaughtered swine. This animal species has been brought to the abattoirs from different towns of the country.<sup>7</sup> The geographical location of Bishoftu is at a latitude and longitude of 8°45' N and 38°59' E, respectively, with an elevation of 1920 meters above sea level. The reliable seasonal rainfall and temperature prediction would have several advantages for record of disease prevalence and increased agricultural productivity in the country.<sup>8</sup> Apart from Addis Ababa and Bishoftu, swine were brought for slaughter to these abattoirs from surrounding Oromia Zone, Sabata and Burayyu which are at most within 60km radii from Addis Ababa. Swine brought from each area were kept under intensive management system, feed poultry manure and left over (disposed carcass of other animals) from abattoirs.

Swine brought to Addis Ababa and Bishoftu abattoirs during the study periods for slaughter were study animals without discrimination of their age, sex, breed and body condition scores. Accordingly, 335 swine, from Addis Ababa and its surrounding and Bishoftu town, brought to Addis Ababa and 221 to Bishoftu abattoirs in total 556 swine were included in the current study. The average number of swine presented to slaughter per day was about 15 to 25 swine in Addis Ababa abattoir and about 5 to 10 swine in Bishoftu abattoir. Slaughtering of swine was done once in a week, commonly, on Monday. (Figure 1).

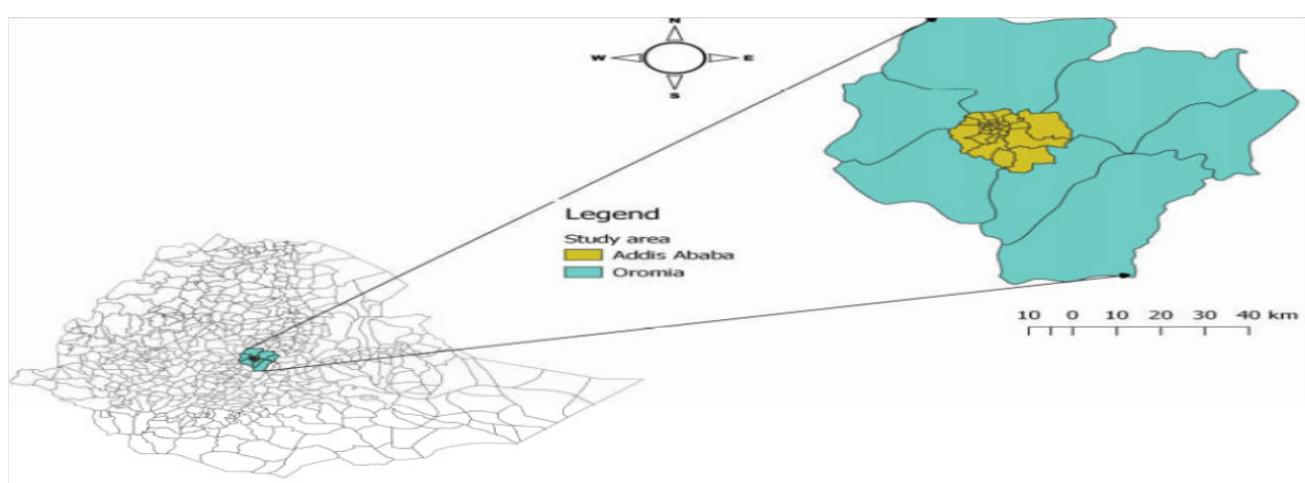


Figure 1 Map of study area- Addis Ababa and its surrounding Oromia Zone.

## Study design and sample size

A cross-sectional study was employed for the current study. Previous studies on the abattoir gross lesions prevalence of swine TB were reported to be 3.5% (34/984)<sup>9</sup> and 5.8% (49/841)<sup>10</sup> in central Ethiopia. However, there was no a study report based on histopathological diagnostic techniques. Therefore, for the current study, the required sample size were calculated by considering 50% expected prevalence (p), 95% confidence interval (z = 1.96) and 5% desired absolute precision (d). The sample size was determined by making use of the formula after Thrushfield and Christley.<sup>23</sup>

$$\text{Hence, } n = \frac{(z)^2 p(1-p)}{d^2}$$

Where n = is the required sample size, p = is the expected prevalence, z = multiplier of the 95% confidence interval and d = desired absolute precision. By making use of the above formula the calculated sample size (n) was 384; however, to increase precision 556 swine were chosen from the two abattoirs.

## Sample collection and sample processing

Non probability sampling method was employed to all slaughtered swine at Addis Ababa and Bishoftu abattoir. Hence, antemortem examination was carried out on all live swine. These included any abnormality in movement (gait), reaction to touch and sound, any visible discharges from natural orifices, coughing, lesions on body and body conditions. Data such as age, sex, origin and body condition scores were collected during antemortem inspection by discussion with the owners and abattoir swine care providers. Postmortem inspections were done and all collected tissues were cut in thin slices (roughly 3 mm wide), and the presence of macroscopic TBL was recorded<sup>11</sup> and Di Marco et al.,<sup>24</sup> by observation of any visible gross lesions and by palpation of organs and lymph nodes during and/or after the animals were slaughtered. The lymph nodes inspected include submandibular, mesenteric, retropharyngeal, parotid, mediastinal, tracheobronchial lymph nodes as well as lungs, liver and spleen were inspected.

Pathology scoring was conducted on tissues with tuberculous lesions to determine the severity of the lesions. The lungs and lymph nodes were removed for the investigation of tuberculous lesions based on semi-quantitative procedure developed previously by Amen et al.<sup>12</sup> Briefly, lesions in the lobes of the lungs were scored separately as follows: 0 = no visible lesions; 1 = no gross lesions but lesions apparent on slicing of the lobe; 2 = fewer than five gross lesions; 3 = more than five gross lesions; 4 = gross coalescing lesions. The scores for the individual lobe were added to generate lung score. Similarly, the severity of gross lesions in individual lymph node was scored as follows: 0 = no gross lesions; 1 = small lesion at one focus; 2 = small lesions at more than one focus; 3 = extensive necrosis. Scores of individual lymph node were added and generated the lymph node score. The total pathology score per swine was obtained from the sum of lung and lymph node scores.

Gross lesions indicative of TB were observed on lung and/or lymph nodes to collect tissue samples. Briefly, approximately 2mm thick tissue specimens from active lesions were collected into sterile universal bottles filled with 5ml of 0.9% saline solution and transported on ice box packed with ice packs to keep the cold chain until they reached Aklilu Lama Institute of Pathobiology (ALIPB) TB laboratory for *Mycobacterium* culture. Whenever immediate

culturing was not done then samples were kept at 4°C for two days and refrigerated at -20°C when to stay for longer time to detect mycobacteria.<sup>13</sup> Mycobacterial culturing was conducted under biocontainment safety cabinet level two (BSC-II) facilitated with HEPA filter (negative pressure). A presumptive diagnosis was made by histopathology by collecting tissue samples of 3-5mm in 10% buffered formalin from lung, liver, spleen and lymph nodes including active part of the lesion and some surrounding apparently normal tissue<sup>14</sup> and taken to pathology laboratory of the National Animal Health Diagnosis and Investigation Center (NAHDIC).

## Histopathology

Briefly, the formalin fixed tissues were trimmed to 2-3mm, dehydrated in increasing alcohol concentration, cleared in three passes of xylene and impregnated with molten paraffin. Tissue block were made and then, the tissues were sectioned at 5µm thickness and the sectioned tissue (ribbon) was straighten on water bath. Then, the ribbon was adhered on frost ended and clear slide which is labeled and put in an incubator (annex I). The slides were deparaffinized in 3 changes of xylene, hydrated in decreasing alcohol concentration, placed in hematoxylin, rinsed in tap water, decolorized in acid and checked for differentiation of nucleus and cytoplasm. Then, rinsed again in tap water and stained in Eosin. Dehydrate again in increased alcohol concentration, clear in three changes of xylene and mount cover slide with Canada Balsum. Finally, the slides were read under the microscopic.<sup>14</sup> Samples from lymph nodes, liver and spleen were categorized according to Varelo et al.,<sup>25</sup> as follows: Positive: tubercular granuloma displaying central necrosis with or without mineralization surrounded by macrophages, lymphocytes, plasma cells, neutrophils, epithelioid cells, and Langerhan's giant cells, and enclosed partly or completely by a thin capsule. Inconclusive: lesion characterized by irregular with no capsulated clusters of epithelioid macrophages; associated with a not Langerhan's-type multinucleated giant cells and necrosis. Negative: features not consistent with tubercular granuloma, including significant eosinophilic infiltrates and lymphoid hyperplasia.

## Mycobacterial isolation

The 40 granulomatous samples were collected from lungs and lymph nodes and processed for isolation of mycobacteria in accordance with Amen et al.,<sup>15</sup> and OIE.<sup>14</sup> The specimens were sectioned using sterile blades, minced with scissors and homogenized with a sterile mortar and pestle under a biological safety cabinet level two (BSC-II). The homogenates were decontaminated by adding an equal volume of 4% NaOH on the sample in order to remove contaminants and 3 ml distilled water or PBS. Then, the processed specimens were centrifuged at 3,000 rpm for 15 minutes to concentrate the mycobacteria. The supernatant was discarded and the sediment was neutralized by 1% (0.1 N) HCl using phenol red as an indicator. Neutralization was achieved when the color of the solution changed from purple to yellow.<sup>14</sup> Next, 0.1 ml of suspension from each sample was spread onto a slant of Lowenstein Jensen (L-J) medium. Duplicate slants were used in which one was seeded on L-J enriched with sodium pyruvate and the other enriched with glycerol.<sup>14</sup> The cultures were incubated aerobically at 37°C and 5% CO<sub>2</sub> in slant position for one week with daily observation and 8–12 weeks in an upright position with weekly observation for growth of colonies. Positive cultures were confirmed with Ziehl Nelseen staining and preserved with freezing media while at the same time heat killed

in water bath at 80°C for 45-60 minutes. The frozen and heat killed samples were stored at -20°C for further molecular typing.

## Statistical analysis

The field collected data was entered, classified, filtered and coded using Microsoft Excel Spread Sheet (2010) and analyzed using the software package STATA 13<sup>26</sup> and IBM SPSS version 20. Prevalence was calculated as the proportion of suspected lesion positive animals from the total number of animals sampled.<sup>23</sup> Descriptive statistics was used for statistical summary. Univariable analysis and Chi-square ( $\chi^2$ ) test was performed to evaluate the statistical association of different risk factors with the result of gross and histopathology. Multivariable logistic regression analyses were used to analyze strength of association of the potential risk factors after their p-value was identified to be  $< 0.05$  in univariable analysis. Effects were reported as statistically significant for the p-values less than 0.05. In cases of estimating the effect of different risk factors in terms of OR

with corresponding 95% confidence interval, statistical significance was assumed.

## Results

### Post-mortem and gross pathology

On the basis of gross pathology, in 556 slaughtered swine showed that 19.6% (109/556) were found to have tuberculous like lesions in parenchymatous organs of which 12% (69/556) were from lymph nodes, 5.7% (32/556) of the lungs, 1% (6/556) of the liver and 0.36% (2/556) of the spleen (Figure 2). The tuberculous like lesions found in lymph nodes, particularly those of mesenteric and retropharyngeal, were statistically significant ( $P < 0.05$ ) than in lungs and associated lymph node. The majority of the lesions were considered to be of typical tuberculous lesions characterized by central round, oval, or irregular, often coalescing areas of caseous necrosis and calcification. The calcified lesions were exceedingly distributed in different lymph nodes and parts of the lungs (Figure 3).

Pathological lesions were commonly seen in mesenteric lymph node 3.4% (19/556) and retropharyngeal lymph node 3.4% (19/556) (Table 1). Among lung lobes found with TB like lesion right cranial, left caudal and left cranial lobes had 5 pathological lesion score with frequency 1.3% (7/556), 1.1% (6/556) and 1.1% (6/556) respectively (Table 2).

**Table 1** Frequency of lesions in lymph nodes, liver and spleen based on pathological score

Lymph nodes	Total pathological score	Frequency	Percent (%)
SLN	3	17	3.1
PLN	2	14	2.5
MLN	4	19	3.4
RLN	4	19	3.4
Lr	-	6	1.1
Sp	-	2	0.4

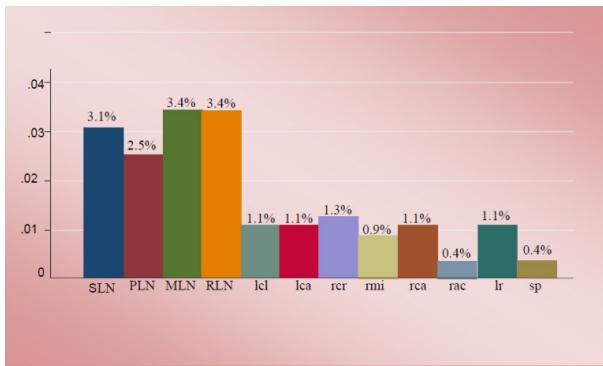
\* SLN, submandibular lymph node; PLN, parotid lymph node; MLN, mesenteric lymph node; RLN, retropharyngeal lymph node; LR, liver; SP, spleen

**Table 2** Frequency of lesion in different lobes of the lung based on pathological score

Lobes of lung	Total pathological score	Frequency	Percent (%)
Lcr	5	6	1.1
Lca	5	6	1.1
Rcr	4	7	1.3
Rml	4	5	0.9
Rca	5	6	1.1
Rac	4	2	0.4

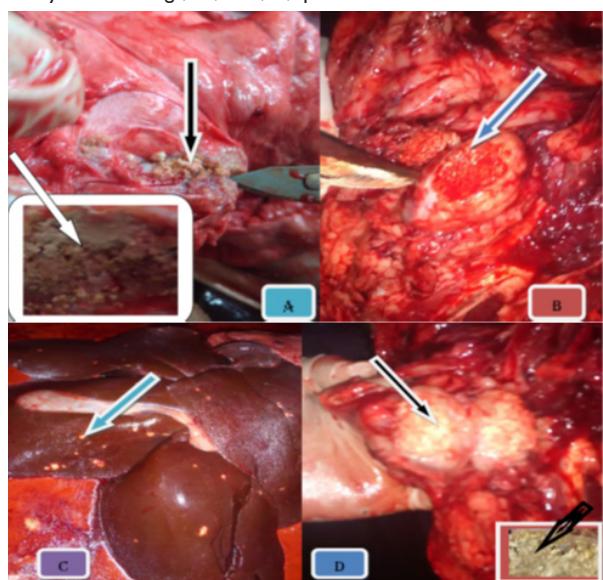
\* LCR, left cranial; LCA, left caudal; RCR, right cranial; RML, right middle; RCA, right caudal; RAC, right accessory lobes of lungs

Based on the stages of granuloma, 4.7% (26/556) lesions were calcified granulomas, 4.1% (23/556) lesions were necrotized, 3.6% (20/556) lesions were pyogranulomatous and 2.5% (14/556) lesions were resulted granulomas without calcification (Table 3).



**Figure 2** The mean prevalence of tuberculous like lesions in lymph nodes, lungs, liver and spleen.

\*\*SLN, submandibular lymph node; PLN, parotid lymph node; MLN, mesenteric lymph node; RLN, retropharyngeal lymph node; LCR, left cranial; LCA, left caudal; RCR, right cranial; RML, right middle; RRA, right caudal; RAC, right accessory lobes of lungs; LR, liver; SP, spleen.



**Figure 3** Gross TB like lesion in different organs. Note: The typical caseous calcified on left caudal lung (A), caseous exudates lesions in retropharyngeal lymph node (B), multifocal hepatic nodules (C), and typical caseous exudates in submandibular lymph node; after paraffinized (black arrow) (D).

**Table 3** Frequency of different types of lesions in lymph nodes, lungs, liver and spleen

Types of lesions	Frequency	Percent (%)
CG	26	4.7
N	23	4.1
PG	20	3.6
G	14	2.5

\*Note: CF, calcified granuloma; G, granulomas without calcification; N, necrotized lesions; PG, pyogranulomatous

**Table 4** Univariable logistic regression of different risk factors to postmortem lesion findings

Variables	No. Swine	No. Positive	Prevalence of TB (%)	OR	$\chi^2$	P-value
Sex						
Female	297	43	14.47		0.1	0.75
Male	259	40	15.44		1.07	
BCS						
Poor	264	21	7.9		26.81	0.000*
Moderate	174	28	16.09		0.47	0.010*
Good	118	34	28.81		0.21	0.000*
Age (in year)						
1	254	26	10.23		27.58	
2	212	28	13.2		1.33	0.319
3	72	19	26.38		3.14	0.001*
4	18	10	55.56		10.96	0.000*

BSC, body condition score;  $\chi^2$ , Chi-square; OR, odds ratio(crude); \* = statistically significant

**Table 5** Multivariate analysis of risk factors associated with postmortem lesion findings

Variables	No. Swine	$\chi^2$	OR	95% CI for OR	P-value
BCS		26.81		Lower	Upper
Poor	264				0.000*
Medium	174		0.47	0.26	0.83
Good	118		0.21	0.11	0.38
Age (in year)		27.58			0.000*
1	254		1		
2	212		1.3	0.75	2.23
3	72		3.14	1.62	6.09
4	18		10.95	3.97	30.22

BSC = body condition score; CI = Confidence interval;  $\chi^2$  = Chi-square; OR = odds ratio (adjusted); \* = statistically significant

### Histopathological lesions

The most characteristic microscopic lesion was tubercle granuloma with central necrosis and calcification. The central area is made of necrotic cellular debris, calcium deposits, and connective tissue capsule walled off the granuloma from the surrounding tissue.

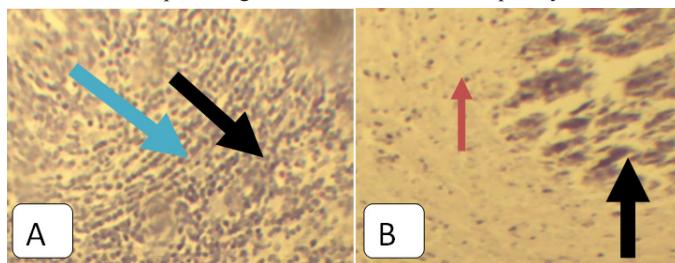
### Association of different risk factors considered with gross lesions

The univariable and multivariable logistic regression analyses of the different putative risk factors considered for tuberculous like lesion positivity were indicated in Tables 4&5 respectively. Swine with poor body condition had higher odd (likelihood) to develop TB like lesions (OR=0.21, 95% CI, 0.11-0.38) than good body conditioned swine. Old aged swine were positive to have tuberculous like lesions (OR=3.14, 95% CI, 1.62-6.09) than younger ones.

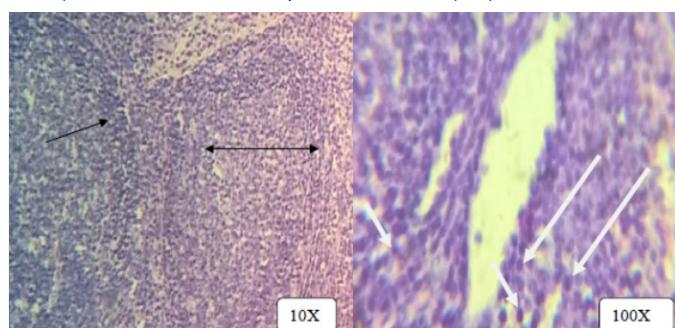
The next layers from center to outer were made up of lymphocytes, macrophages, epitheloid macrophages distributed under connective tissue layers (Figure 4A). The frequency of characteristic tubercle granuloma was 4.7% (26/556) (Figure 4B). The presence of concomitant pyogranulomatous and granulomatous lesions in

different organs was observed in 7.1% (40/556). Some granulomas were characterized by necrotic foci and intense calcification and fibrosis with absence of epithelioid cells. Multiple small granulomas in the lymph node (Figure 5) with less dense lymphocyte at periphery and epithelioid cells surrounding the deep outer lymphatic layer of the granuloma.

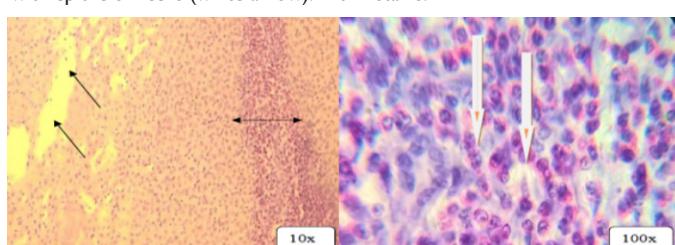
Multifocal hepatonecrosis with total removal of hepatocytes and infiltration of inflammatory cells (mixed mononuclear and polymorphonuclear leukocytes) (Figure 6). At the periphery of this central necrosis are huge infiltrations of inflammatory cells (eosinophils, neutrophils and lymphocytes) in the portal triad region. Some foci of hepatic degeneration with swollen hepatocytes.



**Figure 4** A) Characteristic epithelioid macrophages in the lymph nodes. Note: The epithelioid macrophages shown as elongated (Blue arrow) and the lymphocytes in the next layers (black arrows). H & E stains (10x). B) The characteristic granulomas in the lymph node with calcium deposits (red arrow) at the center, followed by severe necrosis and cellular debris (black arrow) immediate to calcium deposits. H & E stain (10x).



**Figure 5** Multiple small granulomas in the lymph node with central necrosis, layer of dense lymphocyte at periphery (double headed black arrows) admixed with epithelioid cells (white arrow). H & E stains.

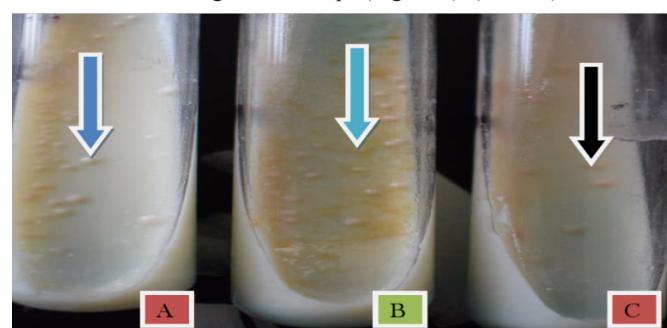


**Figure 6** Multifocal hepatonecrosis with total removal of hepatocytes (black arrows), infiltration of inflammatory cells into portal triads (double head arrows) and infiltration of inflammatory cells (white arrows). H & E stains.

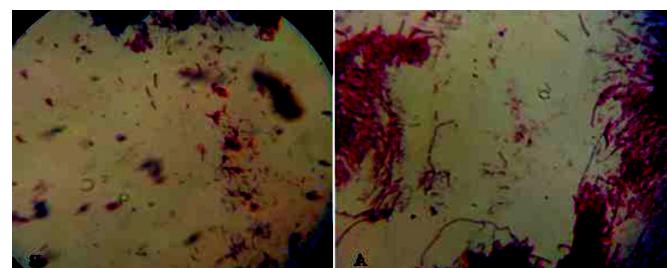
### Mycobacterial isolation and characterization

Mycobacterial culturing was conducted from characteristic tubercle lesions of which 7.5% (3/40) showed growth on primary culture media. Of those cultures which showed visible colony, 5% (2/40) were seen on Lowenstein-Jensen (L-J) media enriched with

glycerol and the rest 2.5% (1/40) were grown on L-J media enriched with pyruvate. Three grown cultures were subjected to Ziehl-Neelsen staining technique in order to check the presence of acid fast bacilli. 5% (2/40) of the cultures were found to be acid fast positive. The observed colonial morphology was smooth whitish or yellowish color, sticky, off-white and breaks apart easily (Figure 7). The results attained of Z-N staining were cocci, short and some long rod shaped and also found in single and clumps (Figure 8), (Table 6).



**Figure 7** Grown bacilli on L-J culture media. Note: (A & B) Colonies growth on L-J medium supplemented with glycerol; (C) Colony growth on L-J medium supplemented with pyruvate (Arrows indicate colony growth which is characterized as smooth, whitish or yellowish in color).



**Figure 8** AFB staining positive isolates from mycobacteriological culture colony grows. From lymph nodes (Mesenteric and retropharyngeal) TB lesion (arrows indicate acid fast bacilli which a short and long, in Single or clump).

### Discussions

There is little information on TB in swine and no report on histopathologic diagnosis with few published on swine TB in Ethiopia. The recognition of lesions of swine tuberculosis during meat inspection is of paramount importance for the surveillance and control of TB infection. Domingo et al.<sup>27</sup> indicated that failure to detect a lesion during abattoir meat inspection has the greatest significance in cattle with single lesion. As the same time; once the lesion is missed there is no further chance of detecting the disease in the animal. This idea also supports the chance of missing small sized lesions while inspecting swine carcasses at abattoir. Furthermore, the early slaughtering of swine before development of tuberculosis lesions in different organs and inadequate illumination in an inspection room of the abattoirs are the major factors determining the detection of tuberculous lesion/s. The slaughtered swine at an early age without sufficient time for lesion development and progression in the present study (45.6% (254/556)), was in agreement with the findings of the Domingo et al.<sup>27</sup>

The present study revealed tuberculous like lesions in 19.6% (n = 109) of swine carcasses inspected which was comparable to 19.8% reported by Cleaveland et al.<sup>28</sup> in cattle slaughtered in rural Tanzania. The current result was higher than 5.8% reported by

Arega et al.,<sup>10</sup> in Addis Ababa and Bishoftu abattoir in swine, 7.96% reported by Regassa<sup>29</sup> in Wolaita abattoir in cattle, 8.8% reported by Biffa et al.,<sup>30</sup> in Hawassa municipal abattoir in cattle, 9% reported by Nemomsa et al.,<sup>16</sup> in Butajira abattoir in cattle and 11.50% reported by Abdurohaman<sup>31</sup> in Butajira abattoir in cattle. On the other hand, the finding of this study was lower than 24.7% reported by Biffa et al.,<sup>30</sup> and 24% by Mamo<sup>32</sup> in cattle at Adama municipal abattoir. The higher prevalence recorded in the present study could be due to the fact that swine feed mainly poultry meal and their offal's. Furthermore, the

variations in prevalence could be attributed to the possible differences in the epidemiology of the disease in different species of animals, origin, housing, age, body condition scores of the animals and types of production system. The majority of swine under this study were originated from intensive poor management production system. According to Ameni et al.,<sup>15</sup> Radostits et al.,<sup>42</sup> and Mamo et al.,<sup>17</sup> the intensive livestock management system could contribute to the development of mycobacterial infections than the extensive livestock management system which is in line with the current finding.

**Table 6** Bacterial culture result from tissues suspected tuberculosis lesions

Sample type	Growth		Z-N stain Positive (%)	
	L-J media with pyruvate		L-J media with glycerol	
	Total	Positive	Total	Positive
SLN	10	1(2.5)	10	-
PLN	7	-	7	-
MLN	10	-	10	1(2.5)
RLN	8	-	8	-
Lung	5	-	5	1(2.5)
Total	40	1(2.5)	40	2(5)

\*SLN, sub-mandibular lymph node; PLN, parotid lymph node; MLN, mesenteric lymph node; RLN, retropharyngeal lymph node

The difference in the prevalence of gross lesions between sexes was statistically insignificant ( $\chi^2 = 0.1$  and  $P > 0.05$ ). The overall gross lesion prevalence in different organs of the both sex was 19.6% (109/556). This study result is higher than the previous studies by Teklu et al.,<sup>33</sup> who reported 4.53% in cattle in Hossana, Shitaye et al.,<sup>9</sup> who reported 3.5% in swine in Addis Ababa, Arega et al.,<sup>10</sup> who reported 5.8% in swine in Addis Ababa, and to some extent consistent with the report by Cleaveland et al.,<sup>28</sup> who reported 19.8% in cattle in Tanzania. The possible reason for this discrepancy might be due to the difference in lesions distribution of female and male animals, in which it was higher in females, sample size and study methodology. Less number of male swine and relatively more number of adult female swine as a means of culling from the breeding stock were presented for slaughter to the study abattoirs during the current study duration.

There was statistically significant difference in this study ( $\chi^2 = 27.58$  and  $P < 0.05$ ) between age and lesions in different organs (lungs, lymph node, spleen and liver). This result is in consistent with the reports of Gebremedhin et al.,<sup>34</sup> who reported 2.6% in Dilla Municipal Abattoir in cattle and Nemomsa et al.,<sup>16</sup> who reported 9% ( $p < 0.05$ ) in Butajira abattoir in cattle. The older swine (OR = 10.95) were nearly eleven times positive to have the gross pathological lesions than the younger ones. The results of the current finding also agreed with the findings of Barwinnek and Taylor,<sup>35</sup> Ameni et al.,<sup>15</sup> Regassa et al.,<sup>36</sup> and Biffa et al.,<sup>37</sup> in cattle who indicated as the age of the animal increases the probability of acquiring TB infection also increases. O'Reilly et al.,<sup>38</sup> pointed out that; the protective capability is declining in aging animals due to a weaker immune system. Furthermore, Humblet et al.,<sup>39</sup> indicated stresses, malnutrition and immunosuppression increase with age. The findings of all these authors supported the current result. The possible reason of difference between ages in current study will be the number of animals considered in each category is not proportional.

The effect of difference in the prevalence of swine TB among swine having different body condition scores was statistically significant ( $\chi^2 = 26.81$ ;  $P < 0.05$ ). The prevalence was highest in swine with poor body condition (47%) as compared to swine with medium body condition (21%) and good (8%) body conditions. This finding is in agreement with study reported by Nemomsa et al.,<sup>16</sup> who reported the higher prevalence of tuberculosis lesions in poor body condition than good body condition animals ( $\chi^2 = 10.38$ ;  $p < 0.05$ ) in Butajira abattoir in cattle. This could be due to the weak protective immune response in poor body conditioned swine when compared to swine with good body condition. Moreover, TB in poor body conditioned swine may result in extensive lesions and wasting of the body due to its chronic nature. The present result is in consistent with previous reports of Collins et al.,<sup>40</sup> Radostits et al.,<sup>41</sup> and Radostits et al.,<sup>42</sup> in those animals with good body condition have relatively good immunological response to the infectious agent than animals with medium and poor body condition scores. On the other hand, the animals with poor body conditions and in nutritional deficiency have reduced resistance to TB which is also in consistence with the report by Doherty et al.<sup>43</sup>

A significant difference between the lungs and lymph node ( $P < 0.05$ ) with lesions was associated with the route of infection. In addition, observation of tuberculous lesions in 3.4% (19/556) of the mesenteric and retropharyngeal lymph nodes may also imply that, the occurrence of intestinal tuberculosis was found to be more frequent than respiratory form. This finding is in agreement with the report described by Shitaye et al.,<sup>9</sup> who observed tuberculous lesions in 33% (5/15) in mesenteric lymph node of swine that dictated that the occurrence of intestinal tuberculosis is more frequent than respiratory tuberculosis. However, the current finding is in disagreement with Corner [44], Neill et al.,<sup>45</sup> Collins,<sup>46</sup> Whipple et al.,<sup>47</sup> and Teklu et

al.,<sup>33</sup> who reported that greater than 84% TB lesions due to *M. bovis* occurred in the respiratory system indicating inhalation/aerosolization in cattle to be the predominant route of transmission. Thoen et al.,<sup>1</sup> described on the similar that ingestion of contaminated poultry faeces and offals and shared grazing with infected animals and/or ingestion of contaminated dairy products are the major sources of TB infection in swine.

Granuloma with calcification was the lesion most frequently detected in the examined organs and occurred in 4.7% of the cases (Figure 5) in the current histopathological finding. In consistence to this finding, Shitaye et al.,<sup>9</sup> reported 3.4 % granulomatous lesions typical to swine TB. Granulomatous lesions typical to swine tuberculosis, manifesting granulomas with central necrosis surrounded by epitheloid cells distributed under connective tissue layers, were observed in 7.1%. Consistent to this finding, Whipple et al.,<sup>47</sup> described that manifestation of typical granulomatous lesions in tissues with gross lesions was evident histologically.

The growth rate of mycobacteria on culture media in the current study was 7.5% (3/40). Amanfu<sup>48</sup> and Cleaveland et al.,<sup>28</sup> pointed out the poor growth rate of *M. bovis* on standard L-J medium. However, Teklu et al.,<sup>33</sup> explained *M. avium* and *M. tuberculosis* to be well grown on standard L-J medium. Furthermore, Teklu et al.,<sup>33</sup> indicated the presence of caseous and/or calcified lesions may not be truly tuberculous lesions; Pritchard<sup>49</sup> and Diguimbaye Djaiibe et al.,<sup>50</sup> also implied that viable mycobacteria may not be present in calcified lesions. The 7.5% culture positivity from the lesion current finding, was lower than 47% reported by Amen et al.,<sup>51</sup> 35% reported by Müller et al.,<sup>52</sup> 32% reported by Shimeles,<sup>53</sup> 31.4% reported by Woyessa et al.,<sup>54</sup> and 23.6% reported by Araujo et al.,<sup>55</sup> in slaughtered cattle, and 30.6% by Arega et al.,<sup>10</sup> in swine. However, the present finding was higher than that of Aknaw et al.,<sup>56</sup> who reported 2.9% in slaughtered cattle at Bishoftu abattoir. This findings justify that the types of culture media and weakly developed and/or trace number of tubercle bacilli in swine tissue may result in lower growth rate.

Mycobacteria in this study were detected by Z-N staining in culture positive samples. This finding was consistent with a previous report by Gracey.<sup>57</sup> *M. bovis* are often low in bovine specimens and they can be visualized by Z-N only if a limited quantity (at least  $5 \times 10^4$  mycobacteria/ml) of mycobacteria are present.<sup>58</sup>

## Conclusion

In conclusion, the results of this study show that, histopathological analysis followed by microbacterial isolation and Z-N staining indicated that swine can serve as a source of infection of tuberculosis to swine, human and/or other animal species. However, comparison of cultural isolation of *Mycobacterium* to pathological lesions indicated culture positivity to be smaller than lesions. This is due to the fact that lesions may be caused by other infectious agents rather than *Mycobacterium* species. Therefore, the diagnosis of mycobacteriosis in swine on a herd basis is important and usually directed to detect lymph nodes on the first hand and lungs as the second option from swine at slaughter. These results should be considered to prevent misdiagnosis of TB based on gross lesions and to establish specific control measures against these pathogens in pigs and related domestic animal species.

## Author contributions

Conceived and designed the experiments: JS, KD, TD. Performed the experiments: JS, KD. Analyzed the data: KD, TD, SL. Contributed

reagents/ materials/analysis tools: GM, GA. Wrote the paper: JS, KD, TD. Revised the final version of the paper for publication: TD, KD, GM, JS.

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## Ethics statement

Ethical approval was obtained from Addis Ababa University College of Veterinary Medicine dated 14/11/2107 (VM/ERC/05/10/018, 03/01/2018), Bishoftu, Ethiopia.

## Conflicts of interest

The author declares there are no conflicts of interest.

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