



Prevalence of Tuberculosis Among Animals Selected from Slaughterhouses in Mbarara district, Uganda

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Abstract

Tuberculosis (TB) is considered one of the most widespread infectious diseases and a leading cause of death and morbidity in both human and animal populations worldwide. The 2022 WHO global report ranked Uganda as a high burden TB country, with an estimated incidence of 198/100,000. The disease is caused by *Mycobacterium tuberculosis complex*, a group of seven species of the bacteria including *Mycobacterium bovis* the cause of bovine-type tuberculosis (bovine TB). *Mycobacterium bovis* has a wide host range infecting cattle, goats, cats, dogs, buffalo, sheep, humans and wildlife. Slaughterhouses have been reported to be potential focus areas for public health monitoring. The aims of this study were to: 1) determine the effectiveness of slaughterhouses serving as active bio-surveillance centers for bovine TB and 2) determine the prevalence of TB among cattle, sheep and goats slaughtered at selected facilities. The sampling design was purposive, targeting Mbarara, a major cattle-producing district in Uganda. Also, Mbarara was accessible, as most of the country had restricted movement of people and animals due to the COVID-19 pandemic. Blood, lymph nodes and lung tissue samples were obtained from 29 cattle, 17 goats and 10 sheep from three slaughter facilities and tested using gross pathology and Ziehl-Neelsen (ZN) staining. The overall prevalence of Bovine TB, based on a positive lymph node or lung, was 8.9% (5/56) and was higher in goats and sheep than cattle; In lymph nodes specifically, the TB prevalence was 3.4% (1/29) in cattle, 11.8% (2/17) in goats, and 10% (1/10) in sheep, whereas for lung tissue, it was 3.4% (1/29) in cattle and 17.6% (3/17) in goats; however, the difference was not statistically significant ($p>0.05$). Slaughterhouses could serve as active surveillance centers for important diseases such as bovine TB. However, additional control protocols may be warranted to reduce TB prevalence in the country.

Keywords: Tuberculosis, *Mycobacterium bovis*, Slaughterhouses, Abattoirs

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Introduction

Tuberculosis (TB) is considered one of the most widespread deadliest infectious diseases worldwide (Ayalew *et al.*, 2023; Litvinjenko *et al.*, 2023; Reta *et al.*, 2024; WHO, 2024a). Globally, tuberculosis has likely regained its status as the foremost cause of death from a singular infectious agent after three years during which it was supplanted by coronavirus disease (COVID-19). It was the primary cause of mortality among those with HIV and a significant contributor to deaths associated with antimicrobial resistance (Chanda-Kapata *et al.*, 2022; Lungu *et al.*, 2022; WHO, 2022a; 2024b). Notwithstanding its elevated mortality rate, tuberculosis (TB) seldom receives media attention. The disease is the primary source of mortality and morbidity in both human and animal populations, making it a significant global public health concern (Litvinjenko *et al.*, 2023). The problem is exacerbated by the rising incidence of drug-resistant tuberculosis (DR-TB) (Günther *et al.*, 2022; Tiberi *et al.*, 2022; Toft *et al.*, 2022).

Low- and middle-income nations represented 80% of cases and fatalities, with the African Region comprising 23% of new cases and 31% of tuberculosis-related deaths, although being under 15% of the global population (Valença *et al.*, 2015; Kalonji *et al.*, 2016; Toft *et al.*, 2022; Asgedom *et al.*, 2023). According to the 2021 WHO global list of high-burden countries for TB, HIV-associated TB and drug-resistant TB, Uganda is one of the 30 countries with the highest tuberculosis (TB) burden globally, with an estimated TB incidence of 200 cases per 100,000 people, and a high co-infection rate with HIV (WHO, 2021b). The 2022 WHO global report also ranked Uganda as a high burden TB country, with an estimated incidence of 198/100,000 (WHO, 2022b).

Zoonotic TB is caused by *Mycobacterium tuberculosis* complex, a group of seven species of bacteria (Damene *et al.*, 2020; Duffy *et al.*, 2020; Ayalew *et al.*, 2023). Zoonotic transmission of TB (zTB) to humans is frequent particularly where TB prevalence is high in livestock (Damene *et al.*, 2020; Duffy *et al.*, 2020; Luciano and Roess, 2020; Ayalew *et al.*, 2023). *Mycobacterium bovis* is one of these groups

and is the causative agent of bovine tuberculosis, exhibiting an exceptionally broad host range. The bacterium infects cattle, goats, felines, canines, buffalo, sheep, and humans. While humans and other species serve as spillover hosts, the bacterium is not maintained within those species (Miller and Sweeney, 2013; Ghebremariam *et al.*, 2018).

Mycobacterium bovis, a Gram-positive zoonotic bacterium, the aetiologic agent of Bovine TB is disseminated by the consumption or inhalation of animal bio-products such as meat and milk. Bovine tuberculosis is a public health risk, especially in developing nations (Katale *et al.*, 2013; Terefe, 2014; Mohamed, 2020; Borham *et al.*, 2022). There are an estimated 140 000 cases of zoonotic tuberculosis each year that result in approximately 11 400 deaths (Duffey, 2024).

In Uganda, bovine TB in cattle has been a challenge to the Ministry of Agriculture, Animal Industry and Fisheries (MAAIF) for decades (Nalapa *et al.*, 2017). Approximately 7% of the bovine TB cases in Uganda are attributed to zoonotic TB from animal bioproducts (Kazoora *et al.*, 2014; Gortázar *et al.*, 2023), with prevalence in the cattle population reaching up to 28% (Gortázar *et al.*, 2023). The recent WHO global report in 2022, places Uganda as a high burden TB country, with an estimated incidence of 198/100,000 (WHO, 2022b; Henry *et al.*, 2024).

Slaughterhouses in developing nations have been reported to be potential focus areas for public health concern (Nasaka, 2014; Cook *et al.*, 2017; Nalapa *et al.*, 2017; Ovuru *et al.*, 2024). Therefore, researchers, public health authorities, policymakers, and abattoir managers can use slaughterhouses as active disease monitoring points to gather data for better control strategies to reduce the spread of zoonotic diseases like TB.

The objectives of this study were: 1) to determine the effectiveness of slaughterhouses in serving as active bio-surveillance centres for bovine TB and 2) to determine the prevalence of TB among cattle sheep and goats slaughtered based on gross pathology and Ziehl-Neelsen (ZN) staining.

Materials and methods

Ethics approval and consent to participate. This was a case study made as part of the recommended Ministry of Agriculture, Animal Industry and Fisheries (MAAIF) routine district veterinary officer's surveillance for major communicable diseases. So, Institutional Animal Care and Use Committee (IACUC) approval was not applicable for sample collection. Individual sample identifiers (i.e., animal identification number) were removed during data analysis, so IACUC approval was determined to be unnecessary for the study. The samples were collected as part of a routine herd health assessment, therefore an IACUC wasn't needed. The Ministry of Agriculture, Animal Industry and Fisheries (MAAIF) gives authority to the field veterinarians to conduct routine herd health checks and publish findings.

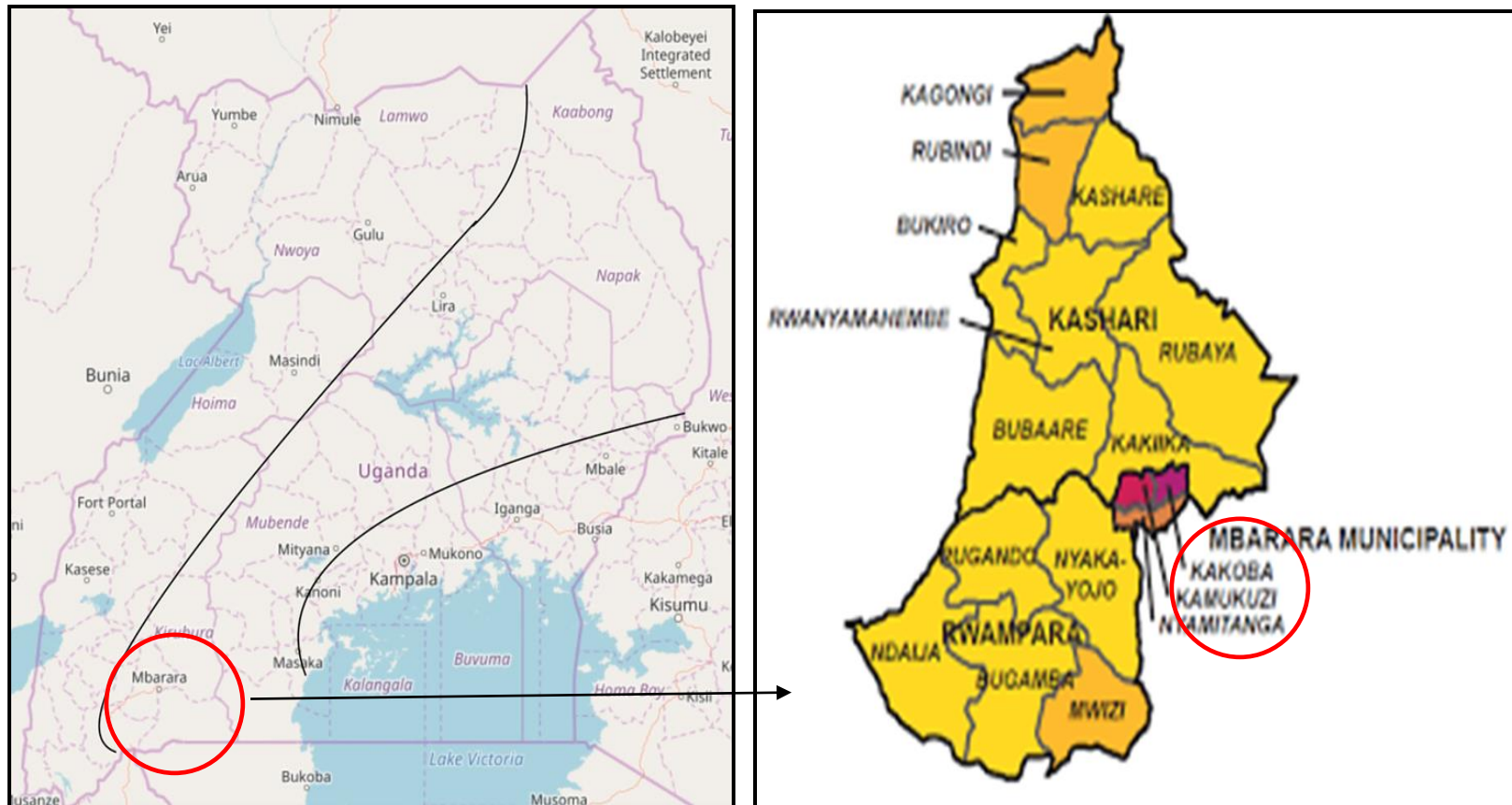
Study Area. The study area was Mbarara district, the administrative capital of southwestern Uganda located at Latitude 0.6121° S, Longitude 30.6373° E. The district borders Ibanda and Kiruhura

districts to the north; Mbarara City to the east; Mbarara City to the south; Sheema and Buhweju Districts to the west. The district headquarters is located 270 kilometers (170 miles), by road, southwest of the capital city, Kampala. Mbarara district is part of the Southwestern Rangelands Agro-ecological Zone (SWRAEZ) which covers part of the cattle corridor along with 12 other districts. The zone has a total surface area of 35,432 km², which is about 14.7% of the national surface area (241,550.7 km²) and has a total human population of 3,847,300. The main economic activities in the zone include crop and livestock farming. The livestock sector includes sheep, goat, and cattle.

Study site selection. The study area (Mbarara district) was selected based on previous reports of TB cases in food animals in this region (Nalapa *et al.*, 2017; Pullen *et al.*, 2019). Additionally, Mbarara district is one of the major cattle producing districts located within the so-called *cattle corridor* of Uganda (Kisaalita and Sempiira, 2017) (Figure 1).

Figure 1

Map of Mbarara district showing cattle corridor and the location of slaughter facilities sampled. Source (Pullen et al., 2019)



Study design and sampling strategy

This was a cross-sectional study conducted in 2021 as part of the Summer Research Experience (SRE) under the Tropical Veterinary Medicine and One Health (TVM&OH) study Abroad program in Uganda. The samples were collected from different slaughterhouses in Mbarara municipality (Figure 1). The study sites were selected purposively and conveniently based on access and where slaughter activity was ongoing as there was restricted movement of animals and animal products at the time (June 2021) due to the COVID-19 pandemic. A total of 5 to 10 animals were slaughtered daily at two of the three locations sampled so all animals slaughtered at the time of sampling were included in the study. The research team sampled animals over three days (June 23rd to June 25th, 2021). Each slaughter place was visited once a day except for Bwizibwera which was sampled over two days. The sampling was conducted very early mornings during slaughter as follows 1) Bwizibwera slaughter slab located in Rugarama parish, Bubaare subcounty, Mbarara district – June 23rd and June 24th, 2025) 2) Rubindi in Mbarara town council (June 24th, 2025) and 3) Mbarara municipality (June 25th, 2021).

Sample collection. Tissue samples (lymph nodes and lung tissue) were collected and put in zip bags. All samples were labelled appropriately and immediately transported to the National Agricultural Research Organisation (NARO) diagnostic laboratory in the Kampala metropolitan area for processing under the standard protocol for sample transportation and preservation (WHO, 2021) (Figure 2-A, B).

Gross examination and smear preparation. Lymph nodes and lung tissue were examined

grossly for tuberculous lesions. Deep incisions were made into the tissues and examined as described by Gupta *et al.* (2016) and Ganchua *et al.* (2020) and; smears were made for each tissue sample. (Figure 2 –C, D).

Ziehl-Neelsen (ZN) stain. This is the standard diagnostic tool for rapid TB diagnosis. Microscopic examination of clinical samples for acid-fast bacilli using the ZN stain can detect 60% to 70% of culture-positive samples with a lower limit of detection of 5×10^3 organisms/mL. (Mohan *et al.*, 2022). A modified Ziehl-Neelsen's (ZN) carbol-fuchsin stain (Chen *et al.*, 2012; Mohan *et al.*, 2023) was used to identify *Mycobacterium* spp. The prepared smear on a slide was stained with ZN-carbol fuchsin stain, and heated beneath it, for 5 min. The carbol fuchsin was kept for 15 minutes, then rinsed with tap water, followed by rinsing with acid alcohol mixture (3 % hydrochloric acid in 70 % ethanol) and subsequently with tap water again. The product was counterstained with 1% methylene blue for 10 seconds before rinsing it with tap water. The slide with the smear was examined using immersion oil (100x) Objective. Acid-fast bacteria appear red, while the background of debris stains blue. (Figure 2- E, F)

Data Analysis. Data for other variables such as district of origin of the animals, breed sex and age were collected at antemortem inspection. Data generated were compiled in an excel spreadsheet and descriptive analyses summarized using SAS and EpiInfo software.

Figure 2

Sample Collection (A-goats, B-Cattle, C-Lymph node), D-packaging of samples, E, F- Ziehl-Neelsen (ZN) staining



Results

A total of 56 samples from 29 cattle (52%), 17 goats (30%) and 10 sheep (18%) were obtained from the three slaughter facilities (two rural

slaughter slabs and one city abattoir) in Mbarara district (Figure 3) and Table 1. The overall TB prevalence was too low (5/56, 8.9%) to run any advanced analyses such as chi-square, Student's t-test or Wilcoxon signed-rank test.

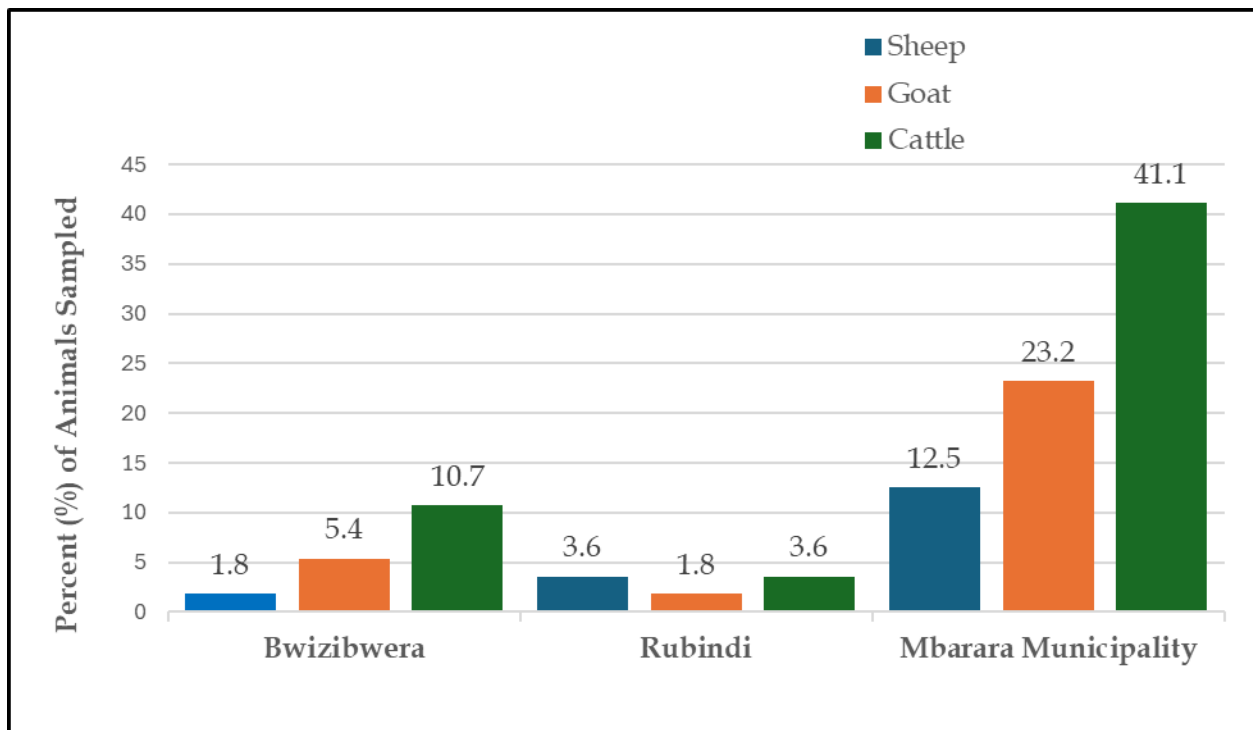
Movement Permits

The research team was able to verify permits for only 9 out of a total of 56 (16%) animals sampled. Four of 9 (44.4%) and 5 of 9 (55.6%)

were from Bwizibwera and Rubindi slaughter places, respectively. We did not have the opportunity to access movement permits from Mbarara Municipal (city) slaughterhouse.

Figure 3

Distribution of Samples by Species and Slaughterhouse (N=56) in Mbarara district, Uganda



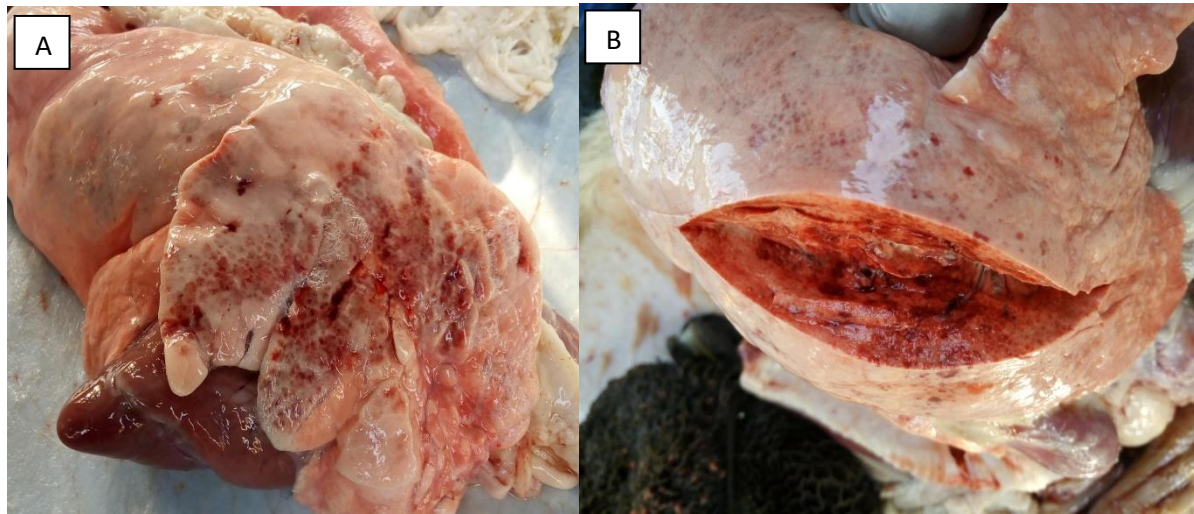
Gross Pathology

Two lung tissue samples out of 56 (3.6%)—one from a goat and another from a sheep—at one of

the rural slaughter slabs (Rubindi) showed gross pathological lesions suggestive of TB. Both lungs had significant caseating granuloma lesions, as depicted in Figure 4.

Figure 4

Gross Pathology Results suggestive of TB lesions-A-Goat, B-Sheep in Mbarara district, Uganda



Overall prevalence of TB

Prevalence of bovine TB in lymph node tissue, rates of 1/29, 3.4% (95% CI; 0.01-0.17), 2/17, 11.8% (95% CI; 0.03-0.34) and 1/10, 10% (95% CI; 0.02-0.40) were detected from cattle, goats and sheep in Mbarara district slaughterhouses, respectively. Prevalence of

bovine TB in lung tissue, rates of 1/29, 3.4% (95% CI; 0.01-0.17) and 3/17, 17.6% (95% CI; 0.06-0.41) were detected from cattle and goats in Mbarara district slaughterhouses, respectively. None of the sheep lung tissues tested presented evidence of Acid-Fast Bacillus Test positive results for the bacteria. (Figure 5). A data summary table is included (Table 1).

Table 1

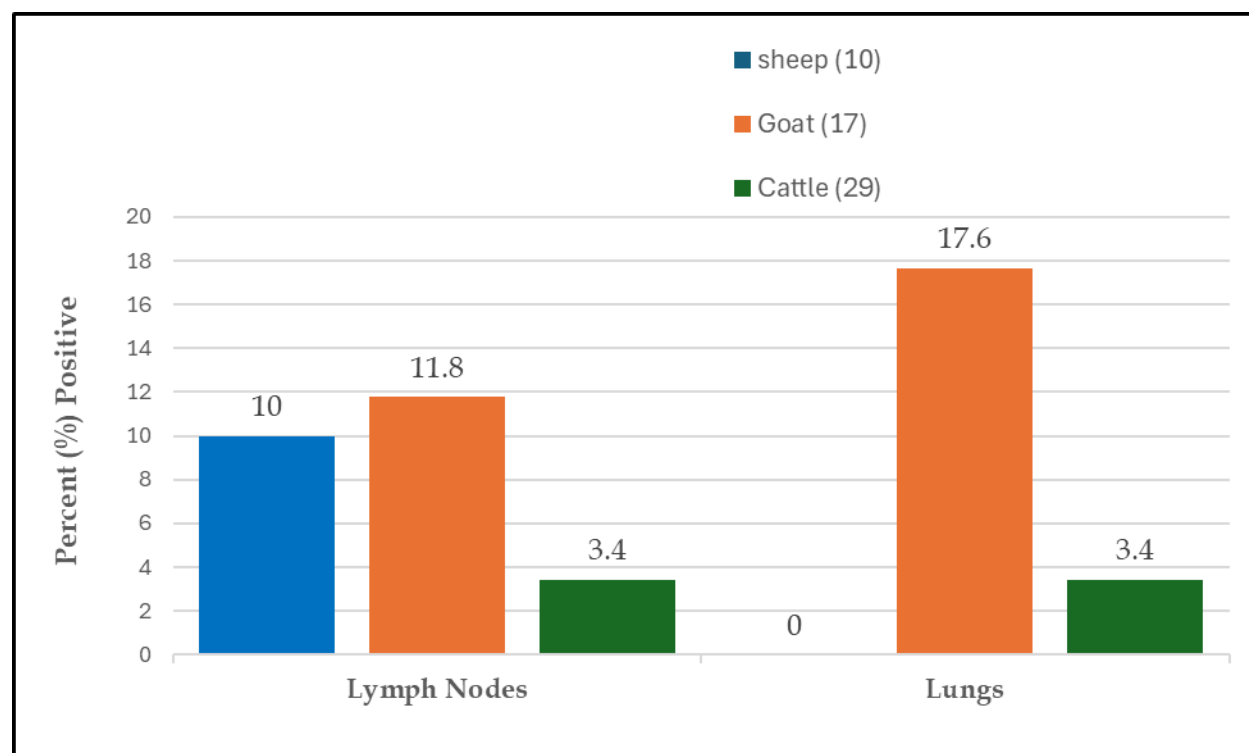
Characteristics of animals sampled from Mbarara district by species, age, sex, and slaughterhouse

Variable	Frequency	Percent
Animal Species		
Cattle	29	52
Goat	17	30
Sheep	10	18
Total	56	
Sex		
Female	7	44
Male	9	56
Total	16	
TB Status - Lymph		

Node		
Negative	52	93
Positive	4	7
Total	56	
TB Status - Lung		
Negative	52	93
Positive	4	7
Total	56	
Animal Origin		
Bwizibwera	10	18
Rubindi	5	9
Mbarara Municipal	41	73
Total	56	

Figure 5

Ziehl-Neelsen stain results for lymph node and lung samples from Mbarara district, Uganda



Discussion

The socioeconomic status of Ugandans, their coexistence with domestic animals and wildlife, the relatively high prevalence of HIV/AIDS, and the lack of public awareness about zoonotic diseases can easily predispose them to bovine TB. The primary objective of this study was to examine the feasibility of utilising slaughterhouses in Uganda as biosurveillance sites and to determine the prevalence of bovine tuberculosis in Mbarara municipality.

In the present study, gross anatomy analysis showed pathological lesions that were suggestive of bovine TB. A similar study in Ethiopia at the Adama municipal abattoir (Terefe, 2014) involving routine, detailed inspections at the slaughterhouse, reported gross pathological lesions of bovine tuberculosis. Also, Garcia-Diez *et al.* (2023) reported how TB generates the largest volume of condemnations at slaughterhouses. Although the primary role of slaughterhouses was to provide meat inspection and safe meat, they routinely generate large amounts of data (antemortem and postmortem) that can contribute to surveillance of broader areas including animal health, public health, One Health, food safety, animal welfare, antimicrobial resistance, and/or prevalence of foodborne and zoonotic diseases (Garcia-Diez *et al.*, 2023).

This study documents the prevalence of bovine TB in lymph nodes of cattle (3.4%), goats (11.8%), and sheep (10%) at the slaughterhouses in Mbarara district. This is consistent with previous studies (Terefe, 2014; Ghebremariam *et al.*, 2018) that indicated the involvement of lymph node enlargement in cattle, goats, and sheep that have bovine TB. In the case of lungs, the prevalence was 3.4% in cattle and 17.6% in goats, as has been found by previous researchers (Gupta *et al.*, 2016; Kalonji *et al.*, 2016; Nalapa *et al.*, 2017) that lungs are key sites for bovine TB. A previous study conducted in Uganda to determine the prevalence of bovine tuberculosis in slaughtered cattle along the Lyantonde-Mbarara Highway, reported a prevalence of *Mycobacterium bovis* of 4.12% (Nasaka, 2014).

This prevalence was similar to the 3.4% prevalence reported by our study though slightly higher. It is possible that the difference in results was due to the small sample size (56) of our study compared to that of Nasaka *et al* (2014). Furthermore, sample analysis techniques differed slightly. While our study used gross pathology, and ZN staining, Nasaka *et al* used liquid culture for 8 weeks and speciation was done using PCR genomic deletion (Regions of difference) analysis and the HAIN Life science Genotype Mycobacterium CM and AS kits. Although the ZN staining for *Mycobacterium* is a time-tested technique (Mohan *et al.*, 2022), it has a sensitivity that typically ranges from 60% to 70%. This is a limitation of this method as it may not detect all cases of tuberculosis. A positive smear does not always confirm a diagnosis of TB, as some stained mycobacteria are not *M. tuberculosis* (nontuberculous mycobacteria). Furthermore, smear-negative results do not exclude the possibility of TB.

All animals moving across jurisdictions (villages, parishes, sub counties, and districts) for various reasons (religious, cultural, breeding or slaughter) are supposed to be issued a certificate and a permit from authorized personnel such as the district veterinary officer to transport the animals safely. The research team was able to verify permits for only 9 out of a total of 29 (31%) animals sampled. Four of 9 (44.4%) and 5 of 9 (55.6%) were from Bwizibwera and Rubindi slaughter facilities, respectively. The fact that not all animals were transported with full and proper documentation was troubling given that restriction of livestock movement was a major mode of control of many animal diseases. Also, we were only able to sex the animals from the two smaller slaughterhouses. Mbarara Municipality (city) processed many more animals (>100/day) at a faster pace which might explain why we were unable to track the sex of all the animals sampled. Also, documentation is secured on arrival, so it is possible that documents had already been secured & stored away by the time we arrived at the slaughterhouse.

Due to access limitations related to COVID-19, the research team was unable to review and verify the existence of antemortem and

postmortem data records and the list of diseases responsible for meat condemnations, nor did we establish existence of and conduct a tour of a functional microbiology lab on the premises of Mbarara Municipal slaughterhouse. Slaughterhouses can be used to conduct surveillance on a variety of factors (e.g. animal welfare, antimicrobial resistance) that impact animal and human health. This was a missed opportunity.

The presence of TB at slaughterhouses, including rural slaughter slabs, was alarming given that most meat inspection services are clustered in large cities and not widely present in rural areas. This underscores the use of slaughterhouses as active surveillance centers to monitor the occurrence of important diseases such as bovine TB. This would complement existing control systems on the farm (for livestock diseases) or alert public health officials to launch rigorous control protocols. Ultimately, improved TB control in the country could possibly lead to transitioning off the list of the 30 high TB burden countries in the world.

Conclusion

The presence of TB positive cases at the slaughterhouses in Mbarara therefore confirms their possible use as biosurveillance points. The *Slaughterhouses in Uganda can serve as active bio-surveillance points by making relevant changes to reduce zoonotic disease spread*. Data from our study showed that slaughterhouses could serve as big centers for testing and controlling important economic and public health diseases such as bovine TB and other zoonoses. The implementation of more rigorous protocols, enforcements and surveys may be key to decreasing the cases in this district and overall, the country. Although the presence of TB positive animals from lymph nodes and lung tissues were higher in goats and sheep than cattle, the results were not statistically significantly different. This underscores the need to conduct larger studies in the future using analytical methods such as PCR with higher sensitivity and specificity.

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