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Research article

Prevalence and risk factors of bovine tuberculosis in slaughtered cattle, Malawi

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ABSTRACT

Bovine tuberculosis (bTB) is an infectious disease with significant socioeconomic, animal, and public health impacts. However, the prevalence of bTB remains largely unclear in Malawi due to a paucity of information. Additionally, the existence of multiple risk factors is postulated to enhance bTB transmission in animals. A cross-sectional survey to estimate the prevalence of bTB, animal characteristics and identify associated risk factors was conducted from slaughtered cattle at three major regional abattoirs (southern, central and northern regions) in Malawi. Out of a total of 1547 cattle examined, 154 (9.95%) had bTB-like lesions in various visceral organs and lymph nodes; one sample per animal was collected, processed, and cultured in the in the BACTEC Mycobacterial growth indicator tube (MGIT) 960 system. From the 154 cattle that showed tuberculous like lesions, only 112 were positive on MGIT and 87 were confirmed to have *M. bovis* based on multiplex PCR. Cattle from the southern region (odds ratio (OR) = 1.96, 95% CI: 1.03–3.85) and central region (OR = 2.00, 95% CI: 1.16–3.56) were more likely presented with bTB-like lesions at slaughter than from the northern region. The risk of having bTB-like lesions was higher in females (OR = 1.51, CI: 1.00–2.29), older cattle (OR = 2.17, CI: 1.34–3.37), and crossbreeds (OR = 1.67, 95% CI: 1.12–2.47) than in males, younger animals, and Malawi Zebu breed, respectively. The high prevalence of bTB is of critical concern and necessitates active surveillance and strengthening of the current control strategies under a One Health (OH) approach at the animal-human interface.

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1. Introduction

Bovine tuberculosis (bTB) is a chronic debilitating contagious disease characterised by respiratory complications, enlargement of lymph nodes, emaciation, and subsequent death in mammals [1]. BTB is caused by *Mycobacterium bovis*, a member of the *Mycobacterium tuberculosis* complex (MTBC) and cattle are primary hosts [1]. In cattle, bTB causes significant economic losses through condemnation of diseased organs and carcasses at slaughter, and trade restrictions [2]. The potential zoonotic transmission of bTB to humans is of public health concern. BTB is globally distributed however, developing countries bear the heaviest burden of the disease compared to developed countries [1].

In Malawi, livestock are crucial to people's livelihoods, as they are in many other developing countries, such that the interaction between people and their livestock is unavoidable [3]. The spread of bTB to and within Malawi could be exacerbated by multiple factors such as; open local borders (allowing unregulated animal movement) between Malawi and neighbouring countries where bTB is documented as 'enzootic' [4–6], traditional livestock production systems characterised by communal grazing and mixing of animals from various localities. Unfortunately, despite these factors the status of bTB in Malawi remains unclear. Several studies documented bTB prevalence [7–10]. However, these reports are from decades ago and disease dynamics and cattle population patterns may have changed significantly. The unavailability of reliable, accurate bTB epidemiological and socioeconomic data in Malawi has contributed to underestimating the disease's burden, resulting in a lack of political will, and less veterinary, and public health attention.

In our recent molecular epidemiological study, we revealed a high genetic diversity of *M. bovis* strains circulating in central Malawi [3] However, the general epidemiology and risk factors associated with bTB in cattle in Malawi are still required for a better understanding of the disease. Therefore, this study aimed to generate updated bTB epidemiological evidence in cattle and highlight the zoonotic TB threat to humans in Malawi. Further, we aimed to identify factors associated with the presence of bTB-like lesions in cattle.

1.1. The study

We conducted a cross-sectional study at three major regional abattoirs in Blantyre city (Southern region), Lilongwe city (Central region) and Mzuzu city (Northern region) in Malawi between August 2019 and February 2020 (Fig. 1). The three abattoirs are supplied by animals from the districts within each region. Hence, we projected that by sampling from these abattoirs, would provide a clear picture of bTB in cattle in Malawi. It is worth noting that not all cattle intended for human consumption pass through these abattoirs. Slaughter slabs provide butchering services within districts and major trading centers, while in remote rural areas, animals are seldom examined. In Malawi, the cattle population is estimated at 1.5 million; with over 80% being Malawi Zebu (local breed) kept under extensive traditional production systems characterised by communal grazing [11]. There are several feedlots, especially in Chikwawa

Fig. 1. Map of Malawi showing the location of the three major regional abattoirs where sampling took place (star). Tissue samples with granulomatous bTB-like lesions were collected from cattle slaughtered at an abattoir in Blantyre city (Southern region), Lilongwe city (Central region), and Mzuzu city (Northern region).

(Southern region) and Mchinji (Central region) districts, that supply cattle (predominantly Brahman crosses (Malawi Zebu/Brahman) directly to abattoirs. The study obtained ethical approval (Protocol Number September 19, 2398) from the National Health Science Research Committee (NHSRC), Ministry of Health, and the Department of Animal Health and Livestock Development (DAHLD), Lilongwe, Malawi.

A basic purposive sampling method was used to select TB-like lesioned tissues from slaughtered cattle during the period of study. Sample size was calculated using EpiTools platform found at https://epitools.ausvet.com.au/prevalencess [12]. Sample size estimation was based on 4% prevalence of bTB reported previously by Bedard et al. [10], assuming a 5% precision and 95% confidence level to ensure a minimum recovery of 20 isolates per abattoir with a recovery rate of 50% from MGIT cultures. In this platform, sensitivity and specificity values have been utilized. These gave us a sample size estimation of 985 (~1000) cattle per each regional abattoir.

In all the three abattoirs, ante- and post-mortem examination procedures were similar. Animal's descriptive information such as age (young or old), sex (male or female), and breed (zebu or crossbreed) were recorded while demographic information was retrieved from livestock movement permits accompanying the animals. Age of an animal was estimated based on dental eruption and wear profiles [13]. An animal with two or fewer permanent incisors was classified as "young", while an animal with three or more pairs of permanent incisor teeth was classified as "old" [13]. In all the three abattoirs, ante- and post-mortem procedures were similar. Routine meat inspection was carried out as described in our earlier publication [3]. Slaughtered cattle were examined and only one lesioned tissue sample with granulomatous bTB-like lesions was collected per animal and transportation to the National Tuberculosis Reference Laboratory (NTRL) in Lilongwe for storage at −20 °C until processed.

During the study period (August 2019–February 2020), a total of 1547 cattle were examined as follows: 313 from Southern region; 960 from Central region; and 274 from Northern region (Table 1). A higher proportion of Malawi Zebu slaughters was observed in all the abattoirs compared to crossbreeds (Table 1). A high proportion of crossbreeds were slaughtered in Central region compared to other regions (Table 1).

Out of the 1547 inspected cattle, 154 (9.95%) had granulomatous bTB-like lesions as follows; Southern region 32/313 (10.22%), Central region 104/960 (10.80%) and Northern region 18/274 (6.57%) (Table 2). Granulomatous bTB-like lesions were commonly found in the thoracic region (74.0%) (lung tissue, bronchial, and mediastinal lymph nodes) in all abattoirs with less observed in the prescapular lymph nodes (Fig. 2). Tissue samples were collected and submitted to NTRL.

Collected samples were processed and cultured in the BACTEC Mycobacterial growth indicator tube (MGIT) as described previously [3]. Briefly, all the fat and connective tissue were removed from the sample using a pair of sterile scissors and forceps. About 5 gm of the sample was crushed to a paste in a sterilized glass homogenizer, and 1 mL of phosphate buffer saline (PBS; pH 6.8) was added. The mixture was decontaminated by adding an equivalent volume of 4% NaOH and incubated for 15 min at room temperature. Then, 10 mL of PBS was added and centrifuged the contents at 3200 g (18 °C) for 20 min. The supernatant was carefully removed, 500 μl PBS added to the pellets and slightly vortexed to mix. This was inoculated in the BACTEC Mycobacterial growth indicator tube (MGIT) [14]. Out of the collected 154 samples with bTB-like lesions, two samples from southern region were not cultured due to logistical constraints, hence the denominator for cultures is 152 (Table 2). Growth was observed in 73.7% (12/152) of the culture samples.

Genomic DNA was extracted by boiling method involving subsequent heating (at 95 °C for 15 min) and immediate cooling (at −20 °C for 30 min). Identification of MTBC species, was done by a PCR targeting the insertion sequence IS6110 [15]. *M. tuberculosis* H37Rv (laboratory strain) (amplicons size was 123bp) and double distilled water (DDW) were used as positive and negative control, respectively. Out of 112 DNA samples, 87 (77.67%) were detected as MTBC (Table 2). Then, we performed a multiplex PCR [16] as described previously [3] for differential detection of *M. bovis* and *M. tuberculosis*. The confirmation was based on the amplicon sizes i.e., 168 bp for *M. bovis* and 337 bp for *M. tuberculosis*. *M. bovis* BCG Tokyo-172 and *M. tuberculosis* H37Rv laboratory strains were used as positive controls while DDW was negative control. It is worth noting that we did not target other MTBC species other than *M. bovis* and *M. tuberculosis* because of logistical constraints. The 87 DNA samples detected as MTBC by IS6110 PCR [15] were all detected as *M. bovis*. Cattle positive with *M. bovis* was 4.47% (2.18–6.76), 6.56% (5.00–8.13) and 3.79% (1.49–6.09) in Southern, Central and Northern region respectively. The overall estimated prevalence of *M. bovis* was 5.62% (CI 95%: 4.48–6.77) (Table 2). For the purpose of this study, the case definition of cattle positive with *M. bovis* refers to cattle whose lesions were collected, cultured and confirmed having *M. bovis* by multiplex PCR [16].

We performed statistical analysis using the R software version 4.0.5 [17]. Using binary logistic regression, we modelled the dependence of TB lesions on various variables. The variables included in the multiple logistic regression model were selected through a univariate screen, with variables having a p-value less than 0.2 qualifying. The multivariate model was built using R, binomial regression with logit link function. Univariate regression analysis was performed to investigate the risk factors associated with bTB-like

Table 1
The population structure of cattle slaughtered in three regions in Malawi.

Abattoir location	Inspected	Animal characteristics					
		Sex		Age		Breed	
		Male	Female	Young	Old	Zebu	Cross
Southern region	313	292 (93.29)	21 (6.71)	244 (77.06)	69 (22.04)	238 (76.04)	75 (23.96)
Central region	960	653 (68.02)	307 (31.98)	845 (88.02)	115 (11.98)	711 (74.06)	249 (25.94)
Northern region	274	114 (41.61)	160 (58.39)	194 (70.8)	80 (29.2)	269 (98.28)	5 (1.82)
Total	1547	1059	488	1283	264	1218	329

Note: In the parenthesis are the proportions of inspected animals as a percentage.

Table 2
Summary of the lesions and *M. bovis* isolated from MGIT culture from slaughtered cattle at three regional abattoirs in Malawi.

Variables	Levels	No. inspected animals	No. bTB - like lesioned	MGIT (Positive)	MTBC (Positive[a])	mPCR *M. bovis* (Positive[b])	Percentage of cattle with *M. bovis*[d]
Abattoir location	Southern	313	32[c]	18	14	14	4.47 (2.18–6.76)
	Central	960	104	81	63	63	6.56 (5.00–8.13)
	Northern	274	18	13	10	10	3.79 (1.49–6.09)
Sex	Male	1059	89	65	50	50	4.72 (3.44–6.00)
	Female	488	65	47	37	37	7.58 (5.23–9.93)
Age	Old	264	50	42	33	33	12.5 (8.51–16.49)
	Young	1283	104	70	54	54	4.21 (3.11–5.31)
Breed	Crossbreed	329	53	40	31	31	9.42 (6.27–12.58)
	Zebu	1218	99	72	56	56	4.60 (3.42–5.77)
Total[e]		1547	154	112	87	87	5.62 (4.48–6.77)

[a] IS6110 MTBC PCR (Eisenach et al., 1990).
[b] Multiplex PCR (mPCR) for differentiating *M. bovis* and *M. tuberculosis* (Bakshi et al., 2005).
[c] Two samples with bTB-like lesions from Southern region were not cultured due to logistical constraints.
[d] 95% confidence interval.
[e] Totals are calculated based on the variable abattoir location.

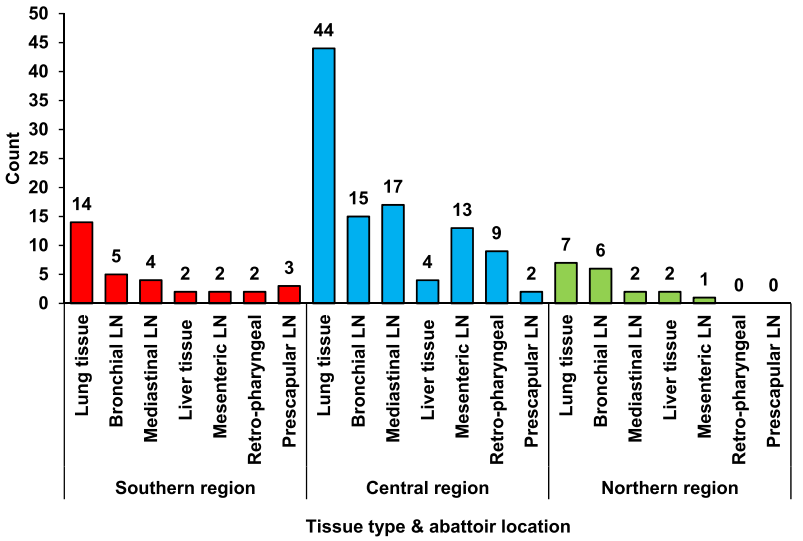

Fig. 2. Distribution of the raw counts of bTB-like lesions from cattle slaughtered in the three regions of Malawi: Southern region – 32 lesions, Central region – 104 lesions, and Northern region – 18 lesions.

Table 3
Univariate and Multivariate logistic regression analysis of factors associated with the presence of bTB-like lesions in cattle at slaughter at the three major regional abattoirs in Malawi (n=1547).

Variables	Levels	bTB-like lesions		Univariate analysis			Multivariate analysis		
		Positive	Negative	OR	95% CI	p-value	OR	95% CI	p-value
Abattoir Location	Northern	18	256	1			1		
	Central	104	856	1.73	1.05–2.99	0.0391	2.00	1.16–3.56	0.0162
	Southern	32	281	1.62	0.90–3.01	0.1163	1.96	1.03–3.85	0.0446
Sex	Male	89	970	1			1		
	Female	65	423	1.67	1.19–2.35	0.0029	1.51	1.00–2.29	0.0504
Age	Young	104	1179	1			1		
	Old	50	214	2.65	1.82–3.81	<0.0001	2.17	1.38–3.37	0.0006
Breed	Zebu	101	1117	1			1		
	Crossbreed	53	276	2.12	1.48–3.02	<0.0001	1.67	1.12–2.47	0.0105

OR = Odds ratio.
CI = Confidence Interval.

lesions in cattle slaughtered at major regional abattoirs. Among the abattoirs, the odds of cattle presenting with bTB-like lesions were higher for Central region (OR = 1.73) and Southern region (1.62) compared to Northern region (Table 3). This difference could be assumed to be real or a result of cattle population structure differences within the abattoirs. Further, the crossbred cattle were found to be 2.12 times more likely with bTB like lesions than zebu cattle. The analysis also showed higher odds of bTB-like lesions in females compared to males (OR = 1.67 (1.19–2.35)) (Table 3). All variables (i.e., abattoir location, sex, age, and breed) had a p-value less than the set threshold of 0.2 and were included in the multivariate regression analysis. Multivariate regression analysis was performed to examine the dependence of bTB-like lesions on various risk factors after controlling for confounding variables (Table 3). The results revealed that the Central and Southern regions showed had higher odds (OR = 2.00 and OR = 1.96, respectively) than Northern region, providing evidence that bTB-like lesions were significantly more prevalent in these areas and not necessarily influenced by the population structure. The odds for the presentation of bTB-like lesions were higher for old than young animals, and for crossbreed than Malawi Zebu (Table 3). Our dataset included only one abattoir per major city in the three regions, with government standardized meat inspection protocols. Diagnostic tests were conducted at a single point in time, with no repeated measurements. Thus, abattoir-level variations were assumed to be negligible, making multilevel/mixed effects analysis unnecessary.

To assess the predictive/classification ability of the model, the R package caTools was used to split the dataset into "train data" (80%) and "test data" (20%) by specifying "SplitRatio = 0.8". Next, the model was trained using the "train data", and its ability to classify animals with respect to bTB-like lesions was determined from the area under the receiver operating characteristic (AUROC). The percentage misclassification error was 17.8%, while the AUROC was 0.6.

2. Discussion

In the present study, the prevalence of bTB was estimated among cattle slaughtered at three major regional abattoirs in Malawi. Our findings revealed that 9.95% (154/1547) of slaughtered cattle had gross pathological lesions suggestive of bTB. The current elucidated bTB prevalence is higher than the 3.85% previously reported by Bedard et al. [10]. Thus, supporting our earlier hypothesis that disease dynamics and cattle population pattern changes over the years might significantly influence the prevalence of bTB. Further, highlighting the importance of current data in understanding the bTB burden in Malawi. Our findings indicate a high bTB prevalence in cattle hence a threat to public health in Malawi.

The prevalence of bTB varies between regions in Malawi. The Southern and Central regions recorded a higher prevalence compared to the Northern region (Table 2). The higher odds of bTB-like lesions from the Southern region (OR = 1.96) and Central region (OR = 2.0) statistically substantiated this observation compared to Northern region (Table 3). Thus, our findings support previous findings by Bedard et al. [10] (1993) that bTB distribution varies across Malawi. The current study, however, differs from the findings of the Bedard and colleagues' investigation, which indicated that the Northern part of Malawi carried the largest burden of bTB. The disparity between our study and earlier work might be attributed to differences in methodology, changes in cattle population structure, and bTB dynamics considering that the two studies were carried out over 25 years apart.

We were able to identify districts with high bTB incidence based on demographic data obtained from cattle movement permits, such as Chikwawa in the southern region, Lilongwe in the central region, and Mzimba in the northern region. Our findings agree with reports by the Department of Animal Health and Livestock (DAHLD) that listed Chikwawa, Lilongwe, and Mzimba districts as hotspots for bTB outbreaks between 2005 and 2015 [18]. Previous studies [3,18] linked bTB outbreaks in these areas, especially Lilongwe and Mzimba, to the introduction of exotic dairy breeds. Additionally, there are unregulated livestock movement and communal grazing along the local borders between Malawi and neighbouring countries, for example, between the northern part of Mozambique and Chikwawa district. Unfortunately, this area is buffered by Lengwe National Park thus wildlife may play a role, albeit this remains to be determined. Furthermore, there could be sampling biases because we were only able to sample for a much shorter period, especially in the northern region. Therefore, further studies such as systematic sampling for extended period are required to expand our findings. The prevalence estimates based on confirmed *M. bovis* cultures were lower than those for bTB-like lesions (Table 2). We speculate that the lower recoveries of *M. bovis* might be due to the difficulty in culturing *M. bovis* because of its slow replication rate and fastidiousness [19]. Another reason could be due to intermittent electricity supply at the storage centre that could result to sample degradation. Based on MGIT culture positives, the overall minimum percentage of cattle with *M. bovis* was 5.62% (95% CI = 4.48–6.77).

Older animals were more likely to present with bTB-like lesions at slaughter than young animals (Table 3). It is postulated that the risk of bTB infection positively correlates with the age of an animal, with older animals being at higher risk due to prolonged exposure to the pathogen in the environment, reactivation of latent infections, and physiological decline of immunity [20–22]. Cross breeds were 1.67 times more likely having bTB-like lesions at slaughter than Malawi Zebu. Similar associations were observed in other parts of Africa [23,24]. Thus, despite their improved productivity, crossbreeds seem to be more susceptible to bTB. The risk factors identified in our multivariate model may have important implications in designing screening and prevention strategies regarding bTB. However, a potential shortcoming of our approach is that the computed odds ratios cannot be used for discriminating or predicting risks among animals [25]. In fact, this limitation is shown by the AUROC value of 0.6 obtained in our study, consistent with a low prediction ability [26].

The distribution of bTB-like lesions and recovery of *M. bovis* showed anatomical localization in the thoracic region (lung tissue, bronchial and mediastinal lymph nodes) (Fig. 2). This suggests inhalation as the route of infection, as would be expected for an airborne pathogen. In both feedlot and communal grazing of animals, head-to-head contact increases the risk of inhalation. The overall bTB prevalence elucidated in this study is lower compared to those reported in neighbouring countries e.g., Zambia at 14.1% [5], Mozambique at 39.6% [27], Tanzania at 13.2% [4]. Our findings, and indeed of the other studies mentioned above, substantiate the fact that bTB is endemic in livestock in most African countries [28].

2.1. Limitations of this study

We could not validate positive MGIT cultures using methods such as smear microscopy and further identify MGIT positive cultures (those that tested MTBC negative) because of logistical challenges. However, we speculate that the positive MGIT cultures could belong to other mycobacterial or bacterial species hence further studies are required to elucidate the overall population of mycobacterial infections in these abattoirs.

3. Conclusion

The present study has highlighted the prevalence of bTB in cattle slaughtered at the three major regional abattoirs in Malawi. The presence of bTB in cattle poses significant socioeconomic consequences to livestock farmers and potential zoonotic transmission to the public. Our study findings emphasize the importance of active surveillance and strengthening current control strategies or formulating new policies to cut off the transmission of *M. bovis* infection within animals and to humans.

Author contribution statement

Thoko Flav Kapalamula: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Francis Kawonga: Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Misheck Shawa: Analyzed and interpreted the data.

Jeewan Thapa; Mirriam Ethel Nyenje; Rajhab Sawasawa Mkakosya: Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Joseph Yamweka Chizimu; Kyoko Hayashida: Performed the experiments; Analyzed and interpreted the data.

Stephen Gordon; Chie Nakajima: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

Musso Munyeme; Bernard Mudenda Hang'ombe: Analyzed and interpreted the data; Wrote the paper

Yasuhiko Suzuki: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Data availability statement

Data will be made available on request.

Declaration of interest's statement

The authors declare no competing interests.

Additional information

No additional information is available for this paper.

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