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Original Article

A Survey for Mycobacterium spp. in Post slaughter Matrix of Donkeys

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ABSTRACT

The presence of Mycobacterium in the post-slaughter matrix of animals is a public health concern. The issue is compounded during postmortem meat inspection at the abattoir when judgments on suspected cases of Tuberculosis (TB) are based solely on gross morphological lesions, without complementary laboratory tests. As a result, carcasses from animals with latent TB infection may be approved for human consumption. The aim of this study was to determine the prevalence of TB in the carcasses of donkeys slaughtered at an abattoir. A survey was conducted from January 1st, 2024 to March 31st, 2024 involving 4200 animals slaughtered within the period. Tissue samples (Lung, spleen, liver) were purposively obtained from carcasses with lesions consistent with suspected TB cases. Tissues were collected in sterile Universal bottles and transported to the laboratory in a Giostyle maintained at a temperature of 4-5 degrees Celsius. Acid-fast bacilli were identified using Ziehl-Neelsen stain. In all 26 organs obtained from individual animals that met the case definition for suspected TB, 8 (31%) tested positive for Acid-fast bacilli, the spleen accounts for 2 (25%), liver 2 (25%), and lungs 4 (50%). The prevalence of tuberculosis in the carcasses of slaughtered donkeys during this period was 0.2%. Given the absence of laboratory screening equipment and facilities to screen for suspected TB in carcasses, the risk of TB zoonosis remains high. To address this issue, we recommend the immediate establishment of dedicated tuberculosis screening facilities in all abattoirs within the state to enhance the detection and confirmation of suspected cases of tuberculosis in meat.

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INTRODUCTION

Tuberculosis is caused by the bacterium Mycobacterium Tuberculosis which causes granulomatous lesions in the tissue of affected individuals (Center for Disease Control, 2016; Gong et al., 2021; Sibhat et al., 2017). Tuberculosis affects a wide range of animals and Humans (Hlokwe et al., 2019; O'Reilly & Daborn, 1995). Transmission of Organism occurs mainly through aerosol from infected individuals and consumption of contaminated unpasteurised milk (Center for Disease Control, 2016; CFSPH, OIE, 2024), infection may also occur by consumption of contaminated meat from an improperly processed carcass. Clinical manifestation of TB infection occurs in two forms: Latent Tuberculosis and symptomatic Tuberculosis. The former affected individuals are asymptomatic with a positive tuberculin test however, the condition may progress to the symptomatic form with characteristic clinical and gross morphologic lesions when left untreated (Centers for Disease Control, 2016). Tuberculosis (TB) was the third leading infectious cause of death in Man behind COVID-19, HIV, and AIDS in 2022(Africa CDC, 2023).

The World Health Organisation (WHO) in its 2018 report on the global burden of tuberculosis reported 240,000 tuberculosis cases of which 143,000 were caused by Mycobacterium bovis (World Health Organization, 2019). In a three-year review on the global burden of tuberculosis published in 2023, WHO reported an increase in the burden of Tuberculosis from 10.0 million in 2020 to 10.3 million in 2021 and 10.6 million in 2022, eight countries (Bangladesh, China, the Democratic Republic of the Congo, India, Indonesia, Nigeria, Pakistan, and the Philippines) were found to be responsible for two third of the global burden of Tuberculosis (WHO, 2023). Africa has been disproportionately affected by the growing burden of Tuberculosis. In 2016 a quarter of the 10.4 million tuberculosis cases occurred in Africa. Similarly, a quarter of the 1.7 million deaths reported globally within the same period occurred in Africa (Africa CDC, 2023; World Health Organization, 2019).

Stakeholders in the one health subsector posited that the shortfall in the global budget on essential TB services from US$6 billion in 2019 to US$5.4 billion in 2021 which was less than half of the global annual target of US$13 billion may have contributed to the increase in the incidence of global Tuberculosis (Africa CDC, 2023). It's worthy of note that between 2000 and 2014, 10 million lives were saved in Africa through TB diagnostic screening and treatment (Africa CDC, 2023). These findings underscore the importance of the Abuja declaration of 2013 where stakeholders pledged and adopted policies targeted at eliminating tuberculosis in 2030. However, this global drive may remain a mirage if concerted efforts by the tripartite one health subsector are not target-driven at eliminating tuberculosis via sustained screening for TB in animals, and Human, surveillance, diagnosis, and treatment of affected individuals. Donkey meat is a staple in Ebonyi State Nigeria, Its low fat, cholesterol, rich iron, protein, and cost-benefit may have endeared its meat to an increasingly health-conscious population as an alternative to beef. The epidemiological importance of this was that between the 1st of January and to 31st of December 2023, 57 suspected cases of tuberculosis were identified during routine post-mortem meat inspection of carcasses at the abattoir which were not laboratory-confirmed. The purpose of this study was to determine the burden of confirmed cases of tuberculosis in the post-slaughter matrix of Donkeys at the slaughter slab in Ebonyi State Nigeria to provide information for public health action.

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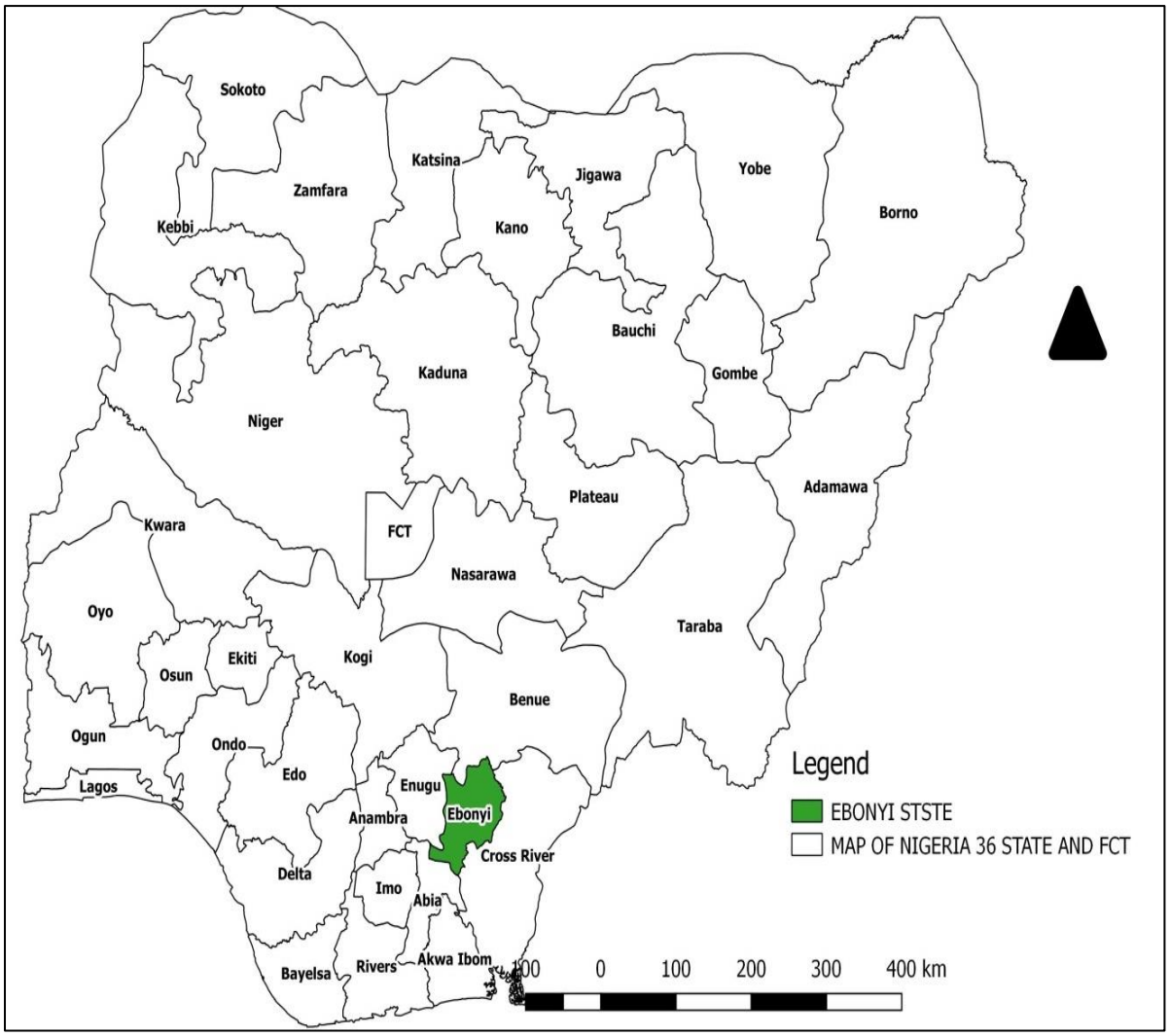
METHOD

Study Area

Ebonyi State is located in the southeast of Nigeria on Latitude. 6°15′N, Longitude. 8°05′E, bordered by Enugu State to the west, Cross River State to the east, Benue and Abia State to the North and South(Ebonyi online, 2021) (*Figure 1*). Farming is the primary occupation of the people of Ebonyi State. The Nkwo-Izhia donkey market is located along the Enugu Abakaliki expressway in the 'Ezzamgbo Ohaukwu' Local Government area (LGA) of Ebonyi State in an area of about 900 square meters. It has three sections, the Donkey paddock, the slaughter slab, and the meat market. Donkeys slaughtered at the abattoir were sourced from Maiduguri, Sokoto, Katsina, Kano, Kebbi, Adamawa, Northern Cameroun, and Niger Republic (FDVS and PC, 2022).

Figure 1: Map of Nigeria showing the Study Area



Case Definition of Suspected Case of Tuberculosis

The presence of granulomatous lesions localized or disseminated on the organ (*Plate 1*).

Differential Diagnosis

Corynebacterium pyogenase, Liver fluke infestation

Study Design

A survey was conducted at the abattoir between the first of January 2024 and 31st of March 2024,

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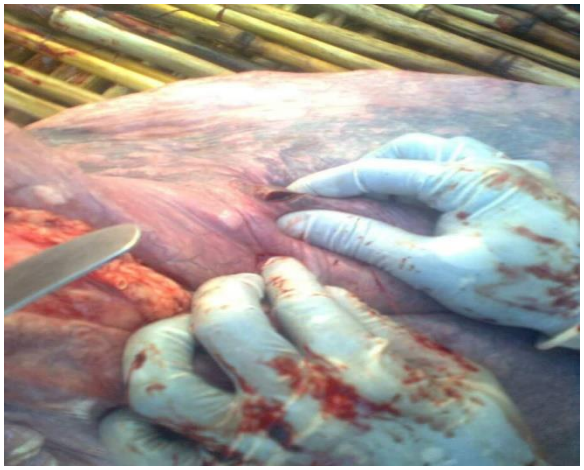
Tissue samples (Lung, spleen, and liver) which met the case definition for a suspected case of Tuberculosis were purposively collected for laboratory tests.

Plate 1: Suspected Tuberculosis infected lungs



Source: Original image from suspected TB lungs of Donkey

Study Population

A total of 4200 animals were slaughtered and their organ was inspected during the study period.

Sample Collection

Tissue samples were collected from suspected carcasses in a sterile universal sample bottle and transported to the laboratory for the Tuberculosis diagnostic at the University Teaching Hospital in a Giostyle maintained at 4-5 degrees centigrade.

Laboratory Test

Tissue was stored at -20 ^{O}C until processing. Acid-fast bacilli were identified using the Ziehl-Neelsen stain and techniques described earlier by Ahmad et al. (2019). The laboratory testing was carried out with utmost precision and accuracy, ensuring that the results were reliable

RESULT

A total of 4200 carcasses were inspected within the period of study, 26 organs met the case definition for suspected TB cases (*Table 1*). On Laboratory analysis, 8 carcasses tested positive for Acid-fast bacilli of these spleen accounts for 2(25%). Liver 2(25%), and Lungs 4(50%). The prevalence of tuberculosis in the carcass of slaughtered donkeys during the study period was 0.2%.

Table 1: Distribution of suspected TB samples obtained during postmortem inspection

Organ	Number of Suspected Samples	Number of Laboratory confirmed Sample
Spleen	6	2
Liver	8	2
Lung	12	4
Total	26	8

DISCUSSION

The prevalence of Acid-fast bacilli in the post-slaughter matrix of donkeys was low 0.2% compared to 30% reported by Chukwu et al. (2013) in Cattle and Goats in Jos plateau State, 16.4% by Ahmad et al. (2019) in cattle in Zamfara State Nigeria. However, Ahmad et al. (2019); Ameen et al. (2008); Cadmus et al. (2009); Damina et al. (2011); Gong et al. (2021) have reported a consistent increase in TB prevalence (0.54% – 6.1%) at various abattoirs in Nigeria. The adjusted concentration of acid-alcohol solution (5% instead of the regular 3%) and extended decolonization time of 10 min employed during the staining procedure in the present study increased the specificity of the test reagents to rule out weak acid-fast non-tuberculous mycobacteria (Ahmad et al., 2019). It is pertinent to note that laboratory screenings for Tuberculosis were rarely conducted at the abattoir in Nigeria except for research purposes consequently, screening of carcasses for suspected TB cases by clinicians, and subsequent judgment were based solely on the gross morphological lesions seen on the carcass of the slaughtered animal. The epidemiological importance of this finding is that the carcass of an animal with latent Tuberculosis may be passed as fit for consumption with the attendant risk of TB

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zoonosis. Although Krajewska et al. (2017) reported a high prevalence of tuberculosis in areas where animals and Humans live in close proximity. This study shows that infection may occur irrespective of the niche and demography of the individual or animal involved due to poor laboratory screening practices for tuberculosis in meat destined for human consumption at various slaughter points/abattoirs within the country. However, further study may be required to characterize the burden of the *Mycobacterium spp*. Involved according to species.

CONCLUSION AND RECOMMENDATION

The risk of TB zoonoses remains high due to the absence of laboratory screening equipment and facility to screen for suspected TB in carcasses consequently, animals with latent infection may be passed as fit for human consumption at the abattoir. Similarly, non-TB cases may be rejected based on inconclusive gross morphological lesions. We recommend the immediate establishment of dedicated tuberculosis screening facilities in all abattoirs within the state to enhance the detection and confirmation of suspected cases in meat meant for human consumption.

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