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Occurrence of tuberculosis in cattle slaughtered at Ikpa abattoir, Nsukka, Enugu State, Nigeria

Akwoba J. Ogugua [1], Chisom O. Nwobi [1], Lynda O. Majesty-Alukagbarie [1], Nzubechukwu E. Ezeakor [1], Charity A. Agada [2] and Chinwe U. Chukwudi [3]

[1] Department of Veterinary Public Health and Preventive Medicine, Faculty of Veterinary Medicine, University of Nigeria, Nsukka, Nigeria.

[2] Department of Veterinary Public Health and Preventive Medicine, Faculty of Veterinary Medicine, Joseph Sarwuan Tarka University, Makurdi, Nigeria.

[3] Department of Veterinary Pathology, Faculty of Veterinary Medicine, University of Nigeria Nsukka, Nigeria.

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Abstract

Tuberculosis (TB) is a chronic zoonotic disease affecting humans and animals. Scientific information on the occurrence of the disease in Nsukka, Enugu State, Nigeria is scarce in available literature. The present study is a cross sectional survey of the occurrence of tubercles in organs and acid fast bacilli in tubercles observed in organs of slaughtered cattle at Ikpa abattoir, Nsukka, Enugu State Nigeria. Post mortem meat inspection was conducted on 538 slaughtered cattle to detect tubercles. Smears made from the tubercles were subjected to Ziehl Neelsen (ZN) staining. Results showed that tubercles were found in 2.79% (15/538) of the cattle carcasses. Highest occurrence was recorded in cattle of 4½ years (5.15%), among the females (4.94%) and the White Fulani breed (3.10%). The occurrence of tubercles in the body organs of the slaughtered cattle was found to be significantly associated with sex – significantly more females had tubercles than males. The ZN staining of tubercle smears showed the occurrence of acid fast bacilli in the tubercles of 2.23% (12/538) of the cattle. The occurrence of acid fast bacilli in tubercles was highest among cattle of 4½ years old (5.15%), the females (4.32%) and the White Fulani breed (2.66%). Statistically significant association was recorded between presence of acid fast bacilli in tubercle smears and age as well as sex. There is need for further studies on the definitive organisms associated with tubercles in cattle slaughtered at Ikpa abattoir and also further molecular characterization of the acid fast bacilli detected in tubercles from cattle in the study area.

Keywords: Tuberculosis; Tubercles; Acid fast bacilli; Slaughtered cattle; Ikpa abattoir; Nsukka Nigeria.

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***Correspondence:** Akwoba J. Ogugua; E-mail: ogugua.akwoba@unn.edu.ng; Phone: +2348033844475

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Introduction

In 2022, tuberculosis (TB) was the world's second leading cause of death due to a single infectious agent in humans (behind the corona virus disease (COVID-19), causing almost twice as many deaths as the HIV/AIDS (WHO, 2023). Remarkably, it has overtaken HIV/AIDS to become the most prevalent infectious disease causing death in humans, and represents a substantial contributor to fatalities associated with antimicrobial resistance (Liebenberg *et al*., 2022). The WHO (2023) report showed that, globally, Nigeria was the 6th country with leading high burden TB cases and was the top in Africa. The country is among the eight leading TB burden nations and amongst those having two thirds of global TB cases. It is also in the list of the world top five countries with low treatment coverage and, with gap between notification and estimated incidence cases. This report negates the end-TB-2030 joint programs of the WHO/FAO/OIE and the existing Roadmap for Zoonotic Tuberculosis (zTB) that target to drastically reduce TB incidence in the world (WHO/FAO/OIE/IUATLD, 2017).

Human zoonotic TB is defined as tuberculosis in humans caused by *M. bovis* which is traditionally the cause of bovine TB (WHO, 2017). It is estimated that there are 140,000 annual zoonotic TB cases that result in approximated 11,400 deaths (WHO, 2020). This is important given that *M. bovis* is traditionally resistant to pyrazinamide, one of the drugs used for the treatment of TB (Marianelli *et al*., 2015). Moreover, drug resistant strains have been reported to be circulating in livestock (Marianelli *et al*., 2015; de Val *et al*., 2021). With the reports of zTB circulating in the Nigerian populace and human TB circulating in livestock in Nigeria (Adesokan *et al*., 2019), it has become necessary that epidemiological TB studies in the country should involve the one health approach.

Tuberculosis due to *M bovis* or *M tuberculosis* are indistinguishable clinically, at post mortem, radiologically or microscopically (Torres-Gonzalez *et al*., 2016; Singh *et al*., 2022). Therefore, confirmatory tests and characterization of tuberculosis agents are quite important in the diagnosis of the disease. However, due to numerous constrains ranging from availability of equipment, reagents and manpower, this all important aspect of TB surveillance is rarely done in Nigeria. Therefore microscopy remains an important means of tuberculosis diagnosis in the country.

Although abattoir-based surveillance studies are not truly representative of the prevalence of a disease in a community (Ogugua *et al*., 2015), surveys on cattle herds which are mostly domiciled in rural areas are difficult or nearly impossible in Nigeria currently. Therefore many TB studies in livestock in Nigeria, are conducted at post mortem at the abattoirs. In affected animals, the pathognomonic post mortem lesions of tuberculosis include the presence of multiple foci of caseous necrosis (tubercles) in the lungs (Ramos *et al.,* 2015). In cases of generalized tuberculosis, tubercles may also be observed on various organs and the gastrointestinal tract, indicating the spread of the disease beyond the lungs (Cantres-Fonseca *et al.,* 2018).

In places where tuberculosis is prevalent in the livestock population, individuals at risk are those with regular contact with animals, such as livestock and abattoir workers, as well as individuals that habitually consume unpasteurized dairy products (Agada *et al*., 2019). Individuals and communities with a higher risk of contracting zoonotic tuberculosis (ZTB) are those who consume unpasteurized dairy products or consume undercooked meat products containing *Mycobacterium bovis* (Hambolu *et al*., 2013), as well as those in close contact with infected cattle (Adesokan *et al.,* 2012; Olea-Popelka *et al.,* 2017). Factors

such as poverty, malnutrition, and co-morbidities such as HIV/acquired immune deficiency syndrome (AIDS) are also significant considerations in the epidemiology of tuberculosis in humans (Cleaveland *et al.,* 2007).

Tubercles, which are nodular granulomas lacking blood vessels, may develop in cattle upon infection with the *Mycobacterium* organism (OIE, 2018). Tubercles are usually grossly observed during post mortem inspection at the abattoir. The diagnosis of bovine TB can be supported by the examination of tissue sections under the microscope. Further confirmation is through cultivation and primary isolation on Löwenstein–Jensen (LJ) medium (Elbir *et al.,* 2008). *Mycobacterium bovis* can also be visualized using microscopic techniques on clinical samples and prepared tissue materials. The Ziehl-Neelsen (ZN) stain and its variations have historically played a crucial role in the identification of Mycobacteria (Kradin *et al.,* 2017). The classical ZN method relies on the ability of Mycobacteria to resist decolourization of a basic dye when treated with mineral acid or an acid-alcohol solution (Prakoeswa *et al*., 2022). The ZN stain specifically aims to detect acid-fast bacilli. Consequently, it can be employed to screen for various Mycobacteria and may be necessary for the detection of certain atypical mycobacteria like *Mycobacterium leprae* (Kradin *et al.,* 2017). In the 19th century, ZN stain was the gold standard for diagnosis of tuberculosis and leprosy (Prakoeswa *et al*., 2022).

Bovine tuberculosis was first documented in Nigeria in a study conducted by Manley in 1929, with the tuberculin, post-mortem and laboratory procedures (Cadmus *et al*, 2004). Although, the emergence of molecular techniques have relegated the ZN diagnostic technique to the background, it retains its relevance in resource poor countries where molecular diagnosis is unaffordable. Surveillance with ZN remains a useful tool towards the attainment of the goals of the end-TB-2030 program (Swai *et al*., 2011).

In a study in North East Nigeria, a prevalence of 1.34% was recorded in cattle by gross pathological observation and these were confirmed as *M. bovis* by molecular techniques (Damina *et al*., 2023). In Maiduguri Nigeria, 32.5% of 160 lesions found in slaughter cattle were positive for tuberculosis, and 3.7% sputum samples from abattoir workers were also positive for TB (Kwaghe et al., 2023). Out of the isolates from cattle, 50% belonged to the *M. tuberculosis* complex and 65.4% were *M. bovis* (Kwaghe *et al*., 2023). In a retrospective study conducted in slaughter cattle at Plateau State, Nigeria, an average yearly prevalence rate of 9.1% was reported, fluctuating from a peak of 16.3% in 2007 to a low of 3.1% in 2012 (Okeke *et al*., 2016). Nwanta *et al.* (2011) and Ogugua *et al*. (2021) reported prevalence of 1.4 and 0.7% respectively in slaughter cattle in Enugu State, Nigeria. Despite the occurrence and transmission of the disease between animal and human populations in Nigeria, there is a notable dearth of scientific reports on animal populations in Nsukka, Enugu State, Nigeria. The present study evaluated the occurrence of tubercles and acid fast bacilli in the tubercles found in cattle slaughtered at Ikpa abattoir Nsukka, Enugu State, Nigeria.

Materials and Methods

Study Area: The study was conducted at Ikpa Abattoir, Nsukka, Enugu State, Nigeria. Nsukka is located at Latitude 06°52' N, Longitude 07°24 E and altitude 447.26 m. The study area has a typical tropical climate characterized by two distinct seasons: a rainy/wet and a dry season. The relative humidity is low at 14% and yearly average rainfall ranges from 168 mm to 170 mm and prevalent vegetation type is guinea savanna (Ogugua *et al.,* 2024). A 2006 estimate put the human population in

Nsukka at 309,448 (National Population Commission, 2006). The primary occupation of the local residents is crop farming, although some engage in subsistence food animal production, such as poultry and piggery. The Muturu breed of cattle is traditionally the reared cattle in the area, but most cattle slaughtered at the abattoir are from northern Nigeria and neighboring countries.

Study Design: The study adopted the cross-sectional study design.

Study Population: The study population was cattle slaughtered at Ikpa abattoir, Nsukka, Enugu State, Nigeria. Sample size was calculated using the appropriate/standard formula.

Ethical approval: Ethical approval for the study was obtained from the Anambra State Ethics Committee (MH/AWK/M/321/344).

Sampling and Sample collection from the Cattle: The Ikpa abattoir is the major slaughterhouse in Nsukka and all cattle slaughtered in each day of visit was sampled. The Ikpa abattoir was visited three times weekly for a period of three months (July to September, 2023). Gross tuberculosis diagnosis was based on identifying typical granulomatous lesions in the organs of slaughtered cattle through a visual observation, palpation and incision. The carcasses were systematically inspected, specifically, the lymph nodes, lungs, spleen, liver, heart and intestines for any indications of tuberculosis lesions. The organs were assessed based on their visual appearance and texture; observing for organ enlargement, lumps, pus formation, caseation and tubercles. Specimens were carefully collected from suspected organs, and the breed, age (determined by dentition method according to Verma *et al*., 2019), sex and organ of the affected animal noted. The samples were then transported to the Veterinary Public Health and Preventive Medicine Laboratory, Faculty of Veterinary Medicine, University of Nigeria, Nsukka where they were stored in the refrigerator for further analysis.

Microscopy (Ziehl-Neelsen staining method): In a biosafety Level II cabinet (Airstream Class II Biosafety Cabinet, Model AC2-4E1), a loopful thin smear of the sample was made on clean, grease-free properly labeled slide. It was left to air dry naturally overnight, fixed over a Bunsen flame and the slide was stained using Ziehl-Neelsen technique.

The slide with the fixed smear was placed on a staining rack over a sink. Freshly prepared carbol fuchsin was poured over the slide to completely cover the smear. The slide was then gently heated from the below using a Bunsen burner flame until steam rose, and this was allowed to continue for about five minutes. The stained slide was washed with distilled water under a running tap, and excess water on the slide was drained by tilting it. The slide was then placed back on the rack, and a decolorizer (acid-alcohol) was poured over it to cover the smear. This was allowed to act for five minutes, and then washed with running tap water. The counter stain (methylene blue) was poured onto the slide, left for about one to two minutes and washed with tap water. It was drained, the back cleaned with a cotton wool and arranged in a vertical position on a slide rack to air dry. The stained slide was examined using the oil-immersion objectives of a binocular microscope. Acid-fast bacilli appeared brick red against a blue background (Prakoeswa *et al*., 2022).

Data Analysis: Data generated were subjected to descriptive statistics. Chi-square was used to determine the association between demographic variables and presence of tubercles in cattle and acid fast bacilli in the tubercle smears, using STATA version 12 software. Probability values less than 0.05 were regarded as significant.

Results

Demographics of cattle examined at Ikpa abattoir, Nsukka, Nigeria: Most of the cattle slaughtered at Ikpa abattoir during the study period were of about 3½ years of age (44.80%), males (69.89%) and were of the White Fulani breed (83.83%) (Figure 1).

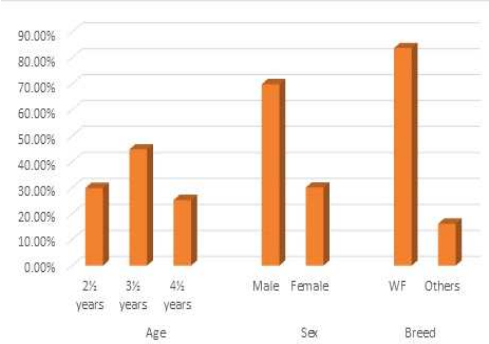

Figure 1. Demographic characteristics of cattle slaughtered at Ikpa abattoir Nsukka, Enugu State, Nigeria, during the study period (July to September 2023). [WF = White Fulani; Others = Mixed, Muturu and Red Bororo]

Occurrence of grossly observable tubercles in organs of cattle slaughtered at Ikpa abattoir, Nsukka, Nigeria: Out of the 538 cattle examined post-mortem after slaughter, 15 (2.79%) had tubercles (Figure 2). Tubercles were found more in animals of 4½ years of age and above (5.15%) as compared to cattle of about 2½ and 3½ years of age, with tubercle occurrence of 0.62% and 2.90%, respectively (Table 1). There was no significant association (p = 0.061) between presence of tubercles in the organs of the slaughtered cattle and their age (Table 1). The occurrence of tubercles was found to be significantly associated with sex (p = 0.047); the occurrence being significantly higher in the females (4.94%) than males (1.86%) (Table 1). The occurrence of tubercles in the slaughtered cattle was also higher in the White Fulani breed of cattle than other breeds, though the occurrence of tubercles was not significantly associated (p = 0.311) with breed (Table 1).

Occurrence of acid fast bacilli in tubercles found in organs collected from cattle slaughtered at Ikpa abattoir Nsukka, as detected with Ziel Neelsen stain: Out of the 15 cattle in which tubercles were found, acid fast bacilli was observed in the Ziel Neelsen stained smears of the tubercles of only 12 of them (2.23% of 538). Cattle of 4½ years of age and above showed higher occurrence of acid fast bacilli in smears (5.15%), with the females and White Fulani breed recording occurrence of 4.32 and 2.66%, respectively (Table 2). The occurrence of acid fast bacilli in the tubercles found in the organs of the cattle was significantly associated with age (p = 0.011) and sex (p = 0.031) (Table 2).

Figure 2. Tubercles observed on organs of cattle slaughtered at Ikpa abattoir Nsukka, Nigeria.

Table 1. Distribution of occurrence of tubercles in cattle slaughtered at Ikpa abattoir Nsukka Nigeria, based on age, sex and breed. [WF= White Fulani; Others = Mixed, Muturu and Red Bororo]

Characteristics	Variables (years)	No. with tubercles (%)	No. without tubercles (%)	Chi-square	p-value
Age	2½	1 (0.62%)	160 (99.38%)	5.59	0.061
	3½	7 (2.90%)	234 (97.10%)		
	4½	7 (5.15%)	129 (94.85%)		
Sex	Male	7 (1.86%)	369 (98.14%)	3.95	0.047
	Female	8 (4.94%)	154 (95.06%)		
Breed	WF	14 (3.10%)	437 (96.90%)	1.03	0.311
	Others	1 (1.15%)	86 (98.85%)		

Table 2. Distribution of occurrence of acid fast bacilli on stained smears of tubercles found in cattle slaughtered at Ikpa abattoir Nsukka Nigeria, based on age, sex and breed. [WF= White Fulani; Others = Mixed, Muturu and Red Bororo]

Characteristics	Variables (years)	No. with acid fast bacilli (%)	No. without acid fast bacilli (%)	Chi-square	p-value
Age	2½	0 (0.00%)	161 (100.00%)	9.00	0.011
	3½	5 (2.07%)	236 (97.93%)		
	4½	7 (5.15%)	129 (94.85%)		
Sex	Male	5 (1.33%)	371 (98.67%)	4.64	0.031
	Female	7 (4.32%)	155 (95.68%)		
Breed	WF	12 (2.66%)	439 (97.34%)	2.37	0.124
	Others	0 (0.00%)	87 (100.0%)		

Discussion

The meat inspection system at abattoirs encompasses the evaluation of bisected carcasses, organs and their respective lymph nodes. The primary objective of abattoir meat inspection is to guarantee the suitability of slaughtered animals for human consumption in terms of safety and wholesomeness. Furthermore, abattoir meat inspection has contributed valuable epidemiological insights concerning animal and zoonotic diseases, notably bovine tuberculosis, globally (Jenkins *et al.*, 2011; EFSA, 2016; Adesokan *et al.*, 2019).

In this study, the 2.79% occurrence of tubercle lesions in cattle carcasses meant for human consumption concurs with the earlier reports of 2.8% by Igbokwe *et al.* (2001) and Agbalaya *et al.* (2020). However, the occurrence recorded in the present study is lower than the 9.3% reported by Lawan *et al.* (2020) at the Maiduguri Central Abattoir and 29.16% by Saidu *et al.* (2015) in slaughtered cattle in Bauchi State, Nigeria. In contrast, the 2.79%

recorded in the present study is higher than the 0.71% prevalence noted by Ogugua *et al.* (2021) in the same Nsukka study area in 2021, the 1.4% prevalence reported by Nwanta *et al.* (2011) in Enugu State in 2011, and the 1.9% reported by Ejeh *et al.* (2014) in Makurdi, Benue State, Nigeria. The recorded occurrence of tubercles in organs of slaughtered cattle in this study may be attributed to the lack of adequate control measures for bovine tuberculosis in Nigeria (Adesokan *et al.*, 2019). The variations in prevalence recorded in different parts of the country may be due to differences in meat inspection capability and capacity to detect bovine TB lesions which differ between individual inspectors (Okeke *et al.*, 2016). However, differences in sample size may also have contributed to the variations, given that small sample size potentially overestimates prevalence (Ramos *et al.*, 2020). In addition, diversity of sources of the slaughter animals may have contributed to the observed differences in prevalence.

The higher occurrence of tubercles in older animals relative to the younger ones in the present study concurs with earlier reports by Awah-Ndukum *et al.* (2012), Okoro *et al.* (2014), and Ahmad *et al.* (2017), that reported a greater prevalence of bovine TB in older cattle when compared to the young. This outcome can be attributed to the insidious and chronic nature of bovine tuberculosis and the remarkable resilience of *Mycobacteria*, which may incubate for extended periods and only become apparent in advanced stages and in older animals (Garba, 2002).

The significant association recorded between occurrence of tubercles in cattle and sex in the present study is consistent with earlier reports of similar investigations conducted in Enugu (Nwanta *et al.*, 2011), Imo (Opara *et al.*, 2012), and Sokoto States of Nigeria (Garba, 2002). Conversely, a higher prevalence among males was documented in Maiduguri, Borno State (Abubakar *et al.*, 2011). However, Ameen *et al.* (2008) reported an absence of association between sex and the presence of bovine TB lesions in Oyo State. The higher prevalence among females in this study may be attributed to the usual extended keeping of females within herds, as more females than males are retained for breeding in herds. Longer period of existence increases chances of exposure to the infection as well as possible manifestation of the disease both ante- and post- mortem. This higher prevalence in cows implies a more extended duration of shedding of infectious agents. This situation increases the risk of disease transmission to other animals as well as to handlers and the general public via the consumption of unpasteurized milk, proximity and regular contact with infected animals (Agbalaya *et al.*, 2020). This calls for sustained surveillance for bovine TB in herds and at the abattoir to protect consumers and in-contact persons who are mostly at the risk of infection.

The current study found the highest prevalence of tuberculosis within the White Fulani breed, which aligns with reports from previous work by Garba (2002) in Sokoto State, Okoro *et al.* (2014) in Enugu State, and Ahmad *et al.* (2017) in Zamfara State. However, it is in contrast with the observation of Ofukwu (2006), who reported the highest prevalence in Sokoto Gudali breed. The higher prevalence observed among the White Fulani breed may be attributed to preponderance of the breed among cattle slaughtered in the Ikpa abattoir (Ogugua *et al*., 2021).

The present study recorded that the occurrence of tubercles as determined through gross lesions (2.79%) was higher than the proportion of tubercles with acid fast bacilli (2.23%) as observed with the Ziel-Neelsen microscopy. This may be attributed to the fact that some tubercle lesions observed during routine meat inspection may be as a result of infections by other pathogens, such as Norcadia, Corynebacterium, and Streptococcus species (Lawan *et al.,* 2020). This difference in occurrence necessitates the

need for further studies on the subject in the study area, using better definitive diagnostic techniques such as isolation and molecular characterization.

Conclusion and Recommendations: The study recorded the presence of tubercles in 2.79% of cattle slaughtered at Ikpa abattoir, with significantly higher number of females having tubercles. With ZN staining, acid fast bacilli were detected in the tubercles of 2.23% of the cattle, and the occurrence of acid fast bacilli was significantly associated with age and sex of the animals. Screening of abattoir workers for TB is recommended. There should be massive public health awareness campaigns for workers at Ikpa abattoir and the general public in Nsukka on the risks of zoonotic TB. Individuals like butchers, veterinarians, meat sellers, in the abattoir should be informed to imbibe the habit of using personal protective equipment while on duty.

Limitations of the study: The study did not isolate the organisms nor did it use molecular methods to characterize the acid fast bacilli. This would have confirmed the organisms detected by microscopy. However, ZN stain is traditionally used to diagnose TB in most parts of Nigeria.

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