Nig. J. Anim. Prod. 2017, 44(4): 71 - 80
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Nigerian Journal of Animal Production

Prevalence and Pathology of Bovine Tuberculosis among Cattle Population Slaughtered in Abeokuta Abattoir

Mshelbwala, F. M[1]*., Oladotun, O. O[1]., Kadiri, A. K. F[2]., Abakpa, S. A. V[3]., Nyillah, E. L[4].
[1]*Department of Veterinary Pathology, College of Veterinary Medicine, Federal University of Agriculture, Abeokuta*
[2]*Department of Veterinary Medicine, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria*
[3]*Department of Veterinary Medicine, College of Veterinary Medicine, Federal University of Agriculture, Abeokuta*
[4]*Department of Veterinary Pathology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria*
*Corresponding author: Tel. 08109064065;
mshelbwalafakilahyel@yahoo.com

Abstract

The prevalence of bovine tuberculosis among cattle population slaughtered in Lafenwa, Abeokuta, abattoir was determined and the pathology associated with the disease was described in the present study. The ages, sexes and total number of cattle slaughtered and examined was recorded over a period of six months (October, 2015 to March, 2016), out of which suspected cases of tuberculosis were sorted out. The prevalence of bovine tuberculosis was calculated as percentage of occurrence as overall, age and sex-specific; and gross lesions were described. Tissue samples of the lungs, liver, Intestine, lymph nodes were collected from the affected animals in two parts; one for Ziehl Nelson staining and the other was fixed in 10% buffered formalin for histopathology. Among the 25,875 cattle examined, an overall prevalence of 8.72% was recorded. Monthly prevalence varied from 5.28% in October to 11.33% in December, 2015. Sex-specific prevalence was higher in females (89.94%) than in males (1.0.06%). Gross lesions observed were severe and diffused necrosis in the lung parenchyma with marked creamy caseous yellowish nodules of variables sizes in 100% of the cases. In some cases, similar lesions were observed in the liver, intestine, heart, kidneys and lymph node. Histopathological lesions in the lung and extra-pulmonary organs were severe necrosis with calcified centers; and there were granulomatous inflammation. Ziehl Neelsen stain demonstrated reddish bacilli suggestive of Mycobacterium organisms. There was an increase in the cases of tuberculosis among cattle population slaughtered in Lafenwa abattoir when compared with those reported in previous studies.

Keywords: Cattle, Abattoir, histopathology, bovine tuberculosis

Introduction

Sustainable agricultural development is one of the main prerequisites for obtaining growth and stability of the economy of an agrarian nation like Nigeria, in which livestock production play an important role (Oluwafemi *et al*., 2001; Amanfu, 2006). They also provide great inputs to crop production as most farms in the developing world are too small to own or use a tractor, and the alternatives are animal power or human labour (Amanfu, 2006). Cattle are one of the major livestock produced in Nigeria, because of their high adaptation to different environmental conditions (Cadmus *et al*., 2004; Amanfu, 2006). However, cattle production in Nigeria is faced with many diseases challenges, among which Bovine tuberculosis takes a high place. Bovine tuberculosis is a chronic bacterial disease of

animals and humans mostly caused by *Mycobacterium bovis* (Smith *et al*., 2006) but can also be caused by other members of the Mycobacterium tuberculosis complex (MTbC) (Brosch *et al*., 2002). It has been reported that *Mycobacterium tuberculosis* as well as other members of the MTbC are causes of tuberculosis in cattle in Nigeria (Cadmus *et al*., 2006; Cadmus *et al*., 2010; Jenkins *et al*., 2011). *Mycobacterium bovis* is an infectious agent that can also cause tuberculosis in other domesticated animals and certain free or captive wildlife species (OIE, 2009). It is usually characterized by formation of granulomas known as tubercles (Shitaye *et al*., 2007). Although commonly defined as a chronic debilitating disease, bovine tuberculosis can occasionally assume a more progressive course. It can affect any tissue of the body, but lesions are most frequently observed in the lymph nodes (particularly of head and thorax), lungs, intestines, liver, spleen, pleura and peritoneum (OIE, 2009). Other members of the *M. tuberculosis* complex, that were previously considered to be *M. bovis*, have been accepted as new species despite identical 16s RNA sequences and over 99.9% identity of their genome sequences. These include *M. caprae* (Aranaz *et al*., 2003) (in some countries considered to be a primary pathogen of goats) and *M. pinnipedii* (Cousins, 2001), a pathogen of fur seals and sea lions. These two new species are known to be zoonotic. In central Europe, *M. caprae* has been identified as a common cause of bovine tuberculosis (Prodinger *et al*., 2005). Disease caused by *M. caprae* is not considered to be substantially different from that caused by *M. bovis* and the same tests can be used for its diagnosis (OIE, 2009). Consumption of unpasteurized milk is the most important route of contracting the disease in developing countries (Ayanwale, 1984; Shehu, 1988; Abubakar, 2007; Wilkins*et al*., 2008). The human cases of tuberculosis associated with *Mycobacterium bovis* infection, both pulmonary and extra pulmonary has been on the increase in Nigeria (Idigbe *et al*., 1986; Cadmus *et al*., 2004; Abubakar, 2007). Clinical signs vary when they are present; lung involvement may be manifested by dyspnea, pneumonia, cough, which can be induced by temperature changes or applying manual pressure on the trachea. Lymph nodes (of the head and neck) are often affected and may rupture and drain (OIE, 2009). Tuberculosis can be difficult to diagnose based on the clinical signs. In developed countries, few infections become symptomatic; most are diagnosed by routine testing including serology, or found at the slaughterhouse (CFSPH, 2007). Gross lesions of tubercles (De Lisle *et al*., 2001) and histopathological lesions of granulomatous reaction are highly diagnostic for tuberculosis (Shitaye *et al*., 2007; Marais *et al*., 2008). Ziehl Neelsen stain has also been used for the diagnosis of bovine tuberculosis, with highly suggestive result (Marais *et al*., 2008), but confirmation can be done by isolation and identification of the Mycobacterium organism (Sam *et al*., 2011). Most of the cattle presented for slaughter in Abeokuta abattoirs are brought from different places such as the far northern Nigeria, Niger Republic, Chad, Benin Republic and other African countries without any clinical history (Cadmus *et al*., 2004). While Awosile *et al*. (2012) reported a prevalence of 1.78% in Lafenwa abattoir, Abeokuta, the gross and histopathological lesions were not described; and there is no recent work to ascertain the situation of tuberculosis among cattle slaughtered in Abeokuta, Ogun State, Nigeria.

Materials and methods

Study area

The study was conducted in Abeokuta, Ogun State. The state is bounded by Lagos, Osun and Oyo states. The vegetation of south west Nigeria is made up of tropical rain forest (Ogunsesan *et al*., 2003). Nigeria has a humid climate with average annual temperature and rainfall of 29-39°C and 2000mm/hg respectively with the relative humidity of 41-67% (Omogbai, 2010; Olorunfemi and Fasimirin, 2011). The administrative capital of Ogun State is Abeokuta where samples were collected from cattle presented for slaughter at the abattoirs and sent to the Department of Veterinary Pathology, Federal University of Agriculture, Abeokuta for analysis.

Population characteristics of cattle slaughtered in Lafenwa, Abeukuta abattoir

Cattle presented in Abeokuta Abattoir for slaughter were examined to determine their ages and sexes for a period of six months (October to March, 2016). The total number of cattle slaughtered in every month of examination was retrieved from the record book of the abattoir.

Prevalence of tuberculosis

Suspected cases of tuberculosis were sorted out from the total number of cattle slaughtered according to the month of examination. The prevalence of bovine tuberculosis was determined by calculating the percentage of occurrence of tuberculosis among the cattle population slaughtered in the Lafenwa, Abeokuta abattoir; as overall, age-specific and sex-specific.

Gross Pathology

The carcasses of the cattle were examined for pathological lesions. Gross lesions of suspected cases of bovine tuberculosis encountered were described. Tissue samples of the lungs (suspected pulmonary tuberculosis) and liver, Intestine, lymph nodes (suspected miliary tuberculosis) making a total of 36, were collected from the affected animals in two parts; one for Ziehl Nelson staining and the other was fixed in 10% buffered formalin for histopathology.

Histopathology

The tissue samples of lung, lymph node, liver and intestine that were fixed in 10% buffered formalin were processed and cut into 6µm thick with rotary microtone machine and stained with Haematoxylin and Eosin stains as described by Awvioro (2002). The stained tissues were examined with light microscope for histopathological lesions.

Acid fast stains (Ziehl Neelsen)

Procedure for acid fast staining

Ziehl Neelsen staining was carried out to determine the acid fast organisms, according to the method described by various workers (Morello *et al*., 2006; Newton *et al*., 2008; Robert, 2009). Smears from suspected tissue samples were made on clean glass slides and air dried for 10 minutes. The smears were floaded with carbol fuchsin (primary stain) and a flame from spirit lamp was held beneath the slides containing the smear until steam appeared over it. The slides were allowed to cool for 5 minutes and the smear were rinsed with tap water. The smears were flooded with 20% sulphuric acid and allowed to stay for 1 minute in order to decolourize the carbol fuchsin stain, then counter stained with methylene blue and allowed to stay for 1 minute. The smears were then rinsed with tap water; air dried, and viewed with light microscope under x100 oil immersion lens.

Results

The total number of cattle slaughtered in Lafenwa abattoir, Abeokuta during the six months study was 25,875. The population of cattle slaughtered in Lafenwa abattoir, Abeokuta was mainly female (89.94%),

(Table 1). The overall prevalence of tuberculosis in cattle slaughtered in Lafenwa abattoir, Abeokuta was 8.72%. Monthly prevalence varied from 5.28% in October to 11.33% in December (Table 1). Age-specific prevalence was higher among adult cattle (7.51%) while lower prevalence was recorded among young cattle (1.21%) (Table 2).

Table 1: Overall prevalence of tuberculosis among cattle population slaughtered in Lafenwa abattoir Abeokuta; between October, 2015 and March, 2016

Month	Number of animals slaughtered	Number of animals with lesions	Prevalence (%)
October, 2015	4449	235	5.28
November, 2015	4484	438	7.09
December, 2015	4582	519	11.33
January, 2016	3933	378	9.61
February, 2016	4175	353	8.46
March, 2016	4252	334	7.86
Overall	25875	2257	8.72

Table 2: Age-specific prevalence of tuberculosis among cattle population slaughtered in Lafenwa abattoir

Age	Number of cattle slaughtered	Number of cattle with tuberculosis	Prevalence (%)
Adult	23271	1943	7.51
Young	2604	314	1.21
Overall	25875	2257	8.72

Sex-specific prevalence of tuberculosis among cattle population slaughtered in Lafenwa abattoir, Abeokuta from October, 2015 to March, 2016 was higher in females (89.94%) than in males (1.0.06%)(Table 3).

Table 3: Sex specific population characteristic of cattle slaughtered between October 2015 and March 2016 in Lafenwa abattoir, Abeokuta,between October, 2015 to March, 2016.

Sex	No. Of cattle slaughtered	Number of cattle with tuberculosis	Percentage (%)
Male	8604		10.06
Female	17271		89.94
Overall	25875		100

Gross Pathology

Gross lesions observed were severe and diffused necrosis of the lung parenchyma with marked creamy caseous yellowish nodules of variables sizes in 100% of the cases. The nodules were observed on the pleural surfaces and deep in the lung parenchyma.

In some cases, extrapulmonary lesions were observed in the liver, intestine, heart, and the kidney (miliary tuberculosis) (Figures 1 and 2).

Histopathology

Microscopic lesions were severe, diffused and extensive necrosis of the lung parenchyma, surrounded by inflammatory cells, mainly macrophages and giant cells. The lesions were surrounded by fibrous connective tissue, with infiltration of lymphocytes and plasma cells in 100% of the cases examined. The central areas of necrosis were calcified in 70% of the cases, giving it the characteristic granuloma lesion (Figures 3 and 4).

Ziehl Neelsen stain

All the 36 tissue samples of lung, lymph node liver and intestine stained with Ziehl Neelsen stain demonstrated reddish bacilli suggestive of Mycobacterium species (Figure 5).

Figure 1: Photograph of the lung of a cow slaughtered in Lafenwa abattoir showing creamy yellowish nodules in the pleural surfaces and in the cut surface of the parenchyma (arrow).

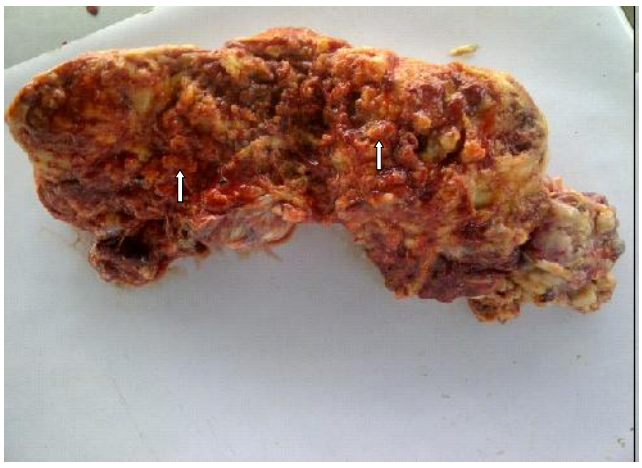

Figure 2: Photograph of the lung of a cow slaughtered in Lafenwa abattoir showing creamy yellowish nodules in the pleural surfaces and in the cut surface of the parenchyma (arrows).

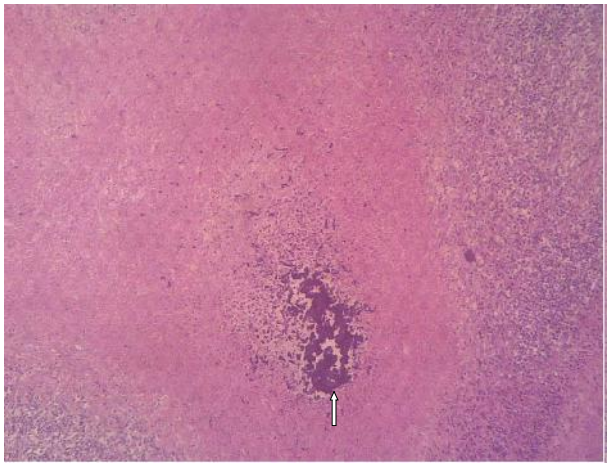

Figure 3: Section of the lung of a cow slaughtered in Lafenwa abattoir, Abeokuta showing severe necrosis of lung parenchyma with calcified center (arrow) (x100; H&E)

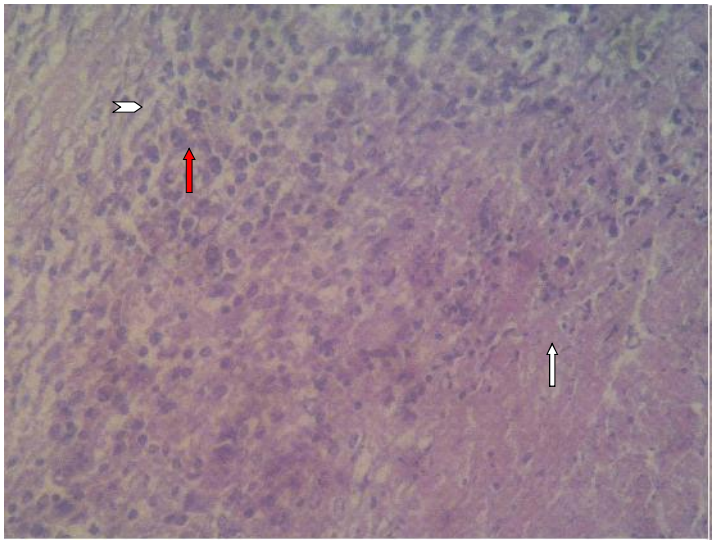

Figure 4: Section of the lung of a cow slaughtered in Lafenwa abattoir, Abeokuta, showing severe necrosis of lung parenchyma (white arrow), infiltration by macrophages, giant cells and lymphocytes (red arrow) with fibrous connective tissues (arrow head) (x400; H&E)

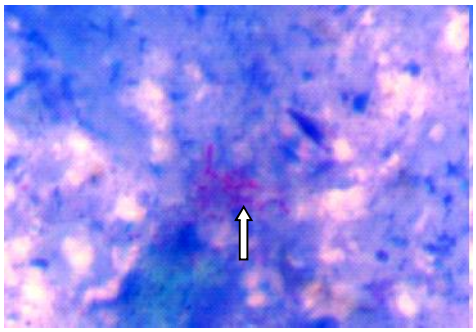

Figure 5: Photomicrograph of a stained tissue smear made from the lung of a cow slaughtered at Lafenwa abattoir, Abeokuta, showing acid fast bacilli (arrow) (x1000; Ziehl Neelsen stain)

Discussion

The cattle population slaughtered at Lafenwa abattoir, Abeokuta between October and March, 2016 were mostly adult cows (89.94%). These could be cows that are thought to have spent their productive live span. However, massive culling of adult cows could have deleterious implications on the economy of Nigeria at large, because of the milk loss, calves lost (in pregnant animals), hides and skin loss and other prospective losses, which could have been of economic benefit had the animals been kept alive and in the production system. The action of culling adult cows could also account for the protein malnutrition in some African countries and a possible constraint to future livestock populations in the continent (Nwakpu and Osakwe 2007; Ademola 2010; Cadmus and Adesokan, 2010). The act of maternal slaughter (in most cases) also tends to frustrate the efforts of breeders and geneticists as it poses the risk of widening the gap of animal protein requirements by meat consumers (Khan and Khan 1989). Poor financial condition of the farmers and ignorance of the pregnancy state of the

animals have been reported to be the reasons for culling and slaughtering pregnant livestock (Sanusi *et al*., 2006; Muhammad *et al*., 2009).

The overall prevalence of tuberculosis in Lafenwa abattoir, Abeokuta increased from the 1.78% reported by Awosile *et al*. (2012) to 8.72% (between October, 2015 and March, 2016). This implies that there is continuous exposure of apparently healthy animal flocks and even humans because of the zoonotic importance of the disease. The increase in the prevalence of tuberculosis agreed with the report of Amanfu (2006) that the situation is worsened and the control programme in farm animals has not been effective and this poses a serious health hazard to the human population. Nwanta *et al*. (2011) reported the occurrence of tuberculosis in cattle and human population in Enugu, Ngeria. The occurrence of the disease increased during the dry season (Nov-Feb) with notable increase in December, 2015 which agreed with the report of Auda (2011) who attributed the increase in December to the extreme dryness of the weather which aids aerosolization of the organisms and easy transmission through dust particles.

Ejeh *et al*. (2014) estimated the economic loss to bovine tuberculosis in Makurdi with prevalence of 0.9% in 2008 and 4.04% in 2012 at ₦$2.91x10^6$and ₦$3.56x10^5$. Kwaghe *et al*. (2015) also estimated the economic loss to bovine tuberculosis in Maiduguri with a prevalence of 8.08% in females and 1.02% in males respectively to be ₦4,841,879.2, ₦349,580,199.89 and a total of ₦349,580,199.89 which indicated that bovine tuberculosis in Nigeria is not just a threat to the economy of the livestock industry but also to food security, food safety and the health of the public.

The gross lesions of severe and diffuse areas of necrosis with yellowish creamy and caseous nodules which were seen in the lung, lymp node, liver, kidney, heart and intestine are consistent findings in bovine tuberculosis (De Lisle *et al*., 2001). *Mycobacterium bovis* is a facultative intracellular pathogen known to cause granulomatous inflammation in the lung, liver and other organs (Kubica *et al*., 2006). The histopathological lesions of necrotic and calcified center surrounded by numerous macrophages, fibrous connective tissues and giant cells are also consistent with bovine tuberculosis (Nair *et al*., 2006), and correlated well with the gross lesion.

Ziehl Neelsen stain has been used in the diagnosis of bovine tuberculosis (Newton *et al*., 2008). The stain was found to be highly sensitive in detecting *Mycobacterium bovis* in tissues and should be applied where isolation and identification is not possible.

Conclusion

In conclusion, most of the cattle slaughtered in Lafenwa abattoir, Abeokuta abattoir were female (89.94%) and this pose serious economic loss. The overall prevalence of 8.72% of bovine tuberculosis in Lafenwa abattoir, Abeokuta recorded in this study was higher than the ones reported earlier. Gross lesions of bovine tuberculosis correlated well with the microscopic lesions which were diagnostic for bovine tuberculosis; and Ziehl Neelsen stain was highly sensitive in the diagnosis of bovine tuberculosis.

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Received: 10th August, 2017
Accepted: 1st December, 2017