Prevalence of *Mycobacterium tuberculosis* complex in Goats Slaughtered at Kano Abattoir, Kano State, Nigeria

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Abstract: Pathogenic *Mycobacterium* infection has been on increasing concern and a threat to public health especially in developing countries like Nigeria. A cross sectional study was carried out at Kano abattoir in Kano State in the Sahel part of Northern Nigeria. The aim of the study was to determine the Prevalence and detect*Mycobacterium tuberculosis* complex in Goats Slaughtered at Kano Abattoir, based on Post-mortem meat inspection for TB-like lesions, culture, acid-fast staining, and TB Ag MPT64 SD-bioline. A total of 500 slaughtered goats were examined during post-mortem meat inspection, out of which 27 have gross TB lesions with an overall prevalence of 5.4%. The males were 11 with a prevalence of 5.8%, while females were 16 with a prevalence of 5.1%. The chi-squire ($\times^2$) test of significance based on sex shows the difference was not statistically significant (P>0.05). The 27 gross TB lesions obtained/sampled, were subjected to culture, acid- fast staining and SD-bioline in which 5(38.5%), 5(41.7%) and 3((42.9%) males were culture, acid-fast stain and SD-bioline positive respectively while on the other hand 8(61.5%), 7(58.3%) and 4(57.1%) females were culture, acid-fast stain and SD-bioline respectively. The study highlighted the importance of tuberculosis in Goats and its public health implications and calls for prompt action towards controlling the disease in goats in Kano abattoir and Nigeria in general.

Keywords: Prevalence, Isolation *Mycobacterium* species, Goats

INTRODUCTION

Tuberculosis (TB) is a chronic infectious and contagious disease of domestic animals, wild animals and humans (Radostits *et al.,* 2010). It is characterized by the formation of granulomas in tissues especially in the lungs, lymph nodes, intestines, liver and kidneys (Shitaye *et al.,* 2007). It is caused by pathogenic members of the genus *Mycobacterium* which are commonly known as members of *Mycobacterium tuberculosis* complex (*Mycobacterium tuberculosis, Mycobacterium bovis, Mycobacterium africanum, Mycobacterium bovis sub caprae, Mycobacterium microti Mycobacterium cannetti and Mycobacterium ulcerance)* (Collins and Grange, 1983; Pefeiffer, 2003).

Goat (*Capra hircus*) as known in different parts of the world is one of the smallest domesticated ruminant which have been with mankind for many generations. The importance role of goats is in production of milk, wool and manure is well documented (Devendra, 1999). Goats are prolific and require low input for moderate level of production. They reach maturity at early age and are nutritionally and financially profitable to keep for production (Devendra & Burns, 1970). Tuberculosis (TB) in goats has not been extensively investigated in comparison with cattle in Nigeria. Tuberculosis in goat and sheep is caused by members of *Mycobacterium tuberculosis* complex predominantly *Mycobacterium bovis and Mycobacterium caprae* (Crawshaw *et al.,* 2008) and in some cases by *Mycobacterium tuberculosis* (Cadmus *et al*., 2009).

In goats, the disease normally spread through head to head contact, which will include sharing of contaminated haystacks and water bowls as well as infected aerosols spread from breath. Tuberculosis can affect the udder in which case the milk is infective until or unless is pasteurized. Infected sputum coughed up can be swallowed and thus infect the gastrointestinal tract. Most commonly in goats, the cough is usually seen as a chronic cough which is unresponsive to treatment and may be accompanied by gradual loss of weight and sometimes diarrhoea (Aranaz *et al.,* 2003). The Predilection site for tuberculosis in goats is the lower respiratory tract and the associated lymph nodes (Daniel *et al.,* 2009). Epidemiological studies have indicated that tuberculosis in goat and sheep has a wide global distribution, being reported in several countries of the world including New Zealand, Sudan, Spain, Nigeria, the United Kingdom, Italy, Algeria and Ethiopia (Aranaz *et al.,* 1999). Caprine tuberculosis poses a risk to goat health and production in developing world (Cadmus *et al.*, 2009). There has been recent increase in caprine tuberculosis in several countries; even among those practicing a long standing test and slaughter policy (Cadmus *et al.,* 2009). It is reported that the infection is widespread in Africa where goats co- graze with cattle that were not subjected to tuberculosis testing and slaughter protocols (Aranaz *et al.,* 2003). Goats may also become infected with Mycobacterium bovis when sharing pastures with infected cattle, at watering points, market places and shared night shelters (Naima *et al.,* 2011). Report on possible tuberculosis in goats in Nigeria was made by Ojo (1994) on the basis of gross lesions without culture confirmation. Livestock owners in Nigeria normally graze cattle and goats together, and this practice poses a high risk for transmission of bovine TB among these animals (Ojo, 1994). This practice is especially a threat to goats in Nigeria because of several reports on bovine TB in cattle in Nigeria (Alhaji, 1976; Cadmus *et al.,* 2006; Abubakar, 2007; Danbirni *et al.,* 2010). However, reports on diagnosis of TB in goats in Nigeria are scanty (Cadmus *et al.*, 2009).

In Nigeria, information on the epidemiology and public health significance of goat tuberculosis is very scanty. The only available information is mostly on limited surveys carried out on individual basis and scanty records from abattoirs. These disjointed studies, make understanding of the magnitude of the problem difficult. The epidemiology and public health significance of goat tuberculosis in Nigeria remain largely unknown. What is known about goat tuberculosis mostly comes from the granulomatous lesions found at slaughter houses/abattoirs.

There is also failure or inadequate implementation of control policies for animals tuberculosis, such as rigorous meat inspection to control and monitor epidemiology of the disease. This is largely due to politico-economic reasons, such as the high cost of sustainable compensation program, inadequate trained veterinary personnel and the political instability in the country.

In addition traditional practices exist in the Sahel part of northern Nigeria whereby goats are reared and used in close proximity to their owners which could facilitate the transmission of tuberculosis between goats, cattle and humans during grazing and watering. Furthermore, goats are usually slaughtered in abattoirs and slaughter houses where the butchers wear minimal protective clothing and process meat with their bare hands (Cadmus *et al.*, 2007).

The absence of any epidemiological data on *Mycobacterium* species in goat at Kano abattoir in Nigeria and the lack of any regulation with regard to processing and sale of goat's meat and meat products other than the general meat inspection regulation has left that transmission route of direct contact wide open.

MATERIALS AND METHODS

Study Area

The fieldwork study was carried out at Kano main abattoir located in the Sahel part of Northern Nigeria (where goats are slaughtered daily). Kano City is located between longitude 12 to 14^{o} North and latitude 9 to 11^{o} East in Kano State which shares boundaries with Jigawa State to the East, Kaduna/Bauchi States to the South and Katsina State to the Southwest to the north (Kano State Diary, 2007). The initial laboratory work (staining, culture and isolation) were carried out at the Tuberculosis and HIV laboratory of Zankli Medical Center, Abuja, Nigeria; while molecular analysis were conducted at the Tuberculosis Laboratory of ECWA Bingham University, Nasarawa State, Nigeria.

Goat sampling

Non-probability sampling technique was used (Judgemental/purposive) in which 500 slaughtered goats were sampled. The goats were identified by serially numbering them before they were slaughtered with a permanent marker which was applied on the side of the thoracic cavity.

Meat Inspection/tissue sample collection

The organs of the slaughtered goats were examined grossly, at meat inspection for TB lesions. Visceral organs and lymph nodes were inspected through careful visualisation, palpation and incision procedures for nodules and granulamatous lesions as described by (Abubakar,2016). Tissue samples were collected from goats with suspected TB lesions in sterile screw-capped containers (with normal saline solution to keep them moist) and transported on ice to the laboratory where they were processed.

Laboratory Processing of Goat Tissue Samples (Culture)

Tissue samples were decontaminated prior to culture as described in the Veterinary Laboratory Manual (Anonymous 1970; Corner 1988, Abubakar, 2016). Tissues were processed as follows:

The sample was removed from the freezer and submerged in diluted locally produced household bleach (Jik, 3.5% NaOCl) (Reckitt Benkiser, Nigeria Ltd). It was left at room temperature before rinsing with freshly diluted bleach for culture.

Specimens were thawed at room temperature and then trimmed of excess tissue and fat.

Tissue samples were homogenized (individually) in a sterile blender for 2mins in 50ml nutrient broth containing phenol red. Five milliliters of the aliquot homogenate were transferred into 50 ml screw capped centrifuge tube and 20ml of 4% NaOH was added and shaken for few seconds.

The sample was allowed to stand for 40 mins at room temperature before adding 6NHCL drop-wise to neutralize until first colour change (purple to pink or muddy-pink) and then centrifuged for 30 mins.

The supernatant was discarded leaving about 2 mls, which was mixed with the pellets and inoculated on two Lowenstein-Jensen slants, glycerol and pyruvate enriched, using sterile pipette applicator.

The tubes were then incubated at 37°C for a minimum of 8 weeks (Plate 2).

Primary Isolation

Cultures were examined weekly for colonies with a hand lens; the growth time and colonial characteristic were noted. A representative colony was smeared and stained by the Zeil Nelsen stain technique for presence of acid-fast bacilli (AFB) and cellular morphology was noted.

Acid-Fast /Zeihl-Neelsen (ZN) Stain

Zeihl-Neelsen staining was carried out using standard protocol as described by ((Anonymous 1970; Corner 1988, Abubakar, 2016) to detect acid-fast bacilli from granulomatous tissue samples collected during the Post-Mortem meat inspection.

An impression smear was made using new, clean and labelled grease-free slide and the slide was air-dried and heat fixed by passing it through a flame (Over a bursen-bunner) with the specimen side up. This is to fix the specimen to the slide and preserve the bacterial morphology.

The slide was then flooded with carbol fuschin and then steamed gently with the flame from underside. It was then rinsed off with water and decolorized with 5% acid alcohol until the red colour is gone.
The slide was rinsed again with water and counter-stained with methylene blue.
Additional rinsing with water was applied to remove excess colour and air dried. It was then examined under a microscope with oil emersion lens at x 100 to look for acid fast bacilli (AFB). The bacilli appear red, straight or slightly curved rods occurring either singly or in groups while non-acid-fast microorganisms stained blue (Plate 3).

SD-BiolineTB Ag MPT64

This is rapid immunochromatographic identification test for the *Mycobacterium tuberculosis* complex that uses mouse monoclonal anti-MPT64. This test kits can be easily used for rapid identification of the *Mycobacterium tuberculosis* complex in combination with culture system based on liquid or solid media without any technical complexity in clinical laboratories.

Test procedure

3 – 4 colonies were suspended in 200 ul of extraction of buffer prior to test
Remove the test device from the foil pouch and place it on a flat, dry surface
Add 100ul of suspended solid cultures in buffer in to the sample well
As the test begins to work a purple colour moved across the result window in the centre of the test device
Interpret the result in 15 minute after sample application

Interpretation of the test result

A colour band appears in the left section of the result window indicates that the test was working properly. This band was the control line (C)
The right section of the result window indicates the test result (T)
The presence of only one purple band within the result window indicates a negative result (Plate 5).
The presence of two purple bands ('T' band and 'C' band) within the result window indicates a positive result (Plate 4).
If the purple band colour was not visible within 15 minutes after performing the test, the result was considered invalid. It was recommended that the specimen be re-tested.
*Marketed as (SD-TB Bioline Ag MPT64)

Data analysis

The chi-square (x^2) was used to calculate appropriate degrees of freedom (df) and to specify the level of significance or association between variables.
Prevalence was calculated using the formula:

$$\text{Prevalence} = \frac{\text{Number of animals positive}}{\text{Total animals tested}} \times 100$$

RESULTS

The result of post-mortem meat inspection conducted at Kano central abattoir is presented in (Table 1). A total of 500 slaughtered goats were examined, out of which 27 had suspected gross TB lesions with an overall prevalence of 5.4%. The males were 11 with a prevalence of 5.8%, while females were 16 with a prevalence of 5.1%. The chi-squire ($\times^2$) test of significance based on sex shows the difference was not statistically significant ($P>0.05$). Results based on Culture, Acid-fast Stain and SD-Bioline were presented in (Table 2). The 27 gross TB lesions obtained were subjected to culture, acid- fast staining and SD-bioline, (consisting of 11 males and 16 females), 5(38.46) males were culture positive, 5(41.66) were acid-fast stain positive and 3(42.85) were SD-bioline positive. On the other hand 8(61.53%) females were culture positive, 7(58.33%) were acid-fast stain positive while 4(57.14%) were positive for SD-bioline.

Table 1: Prevalence of Caprine TB in Goats based on gross lesions at Kano Central Abattoir.

Sex	Number Examined	Number Positive	Prevalence(%)	P-Value
Male	188	11	5.85	
0.143				
Females	312	16	5.12	
Total	**500**	**27**	**5.4**	

Table 2: Isolation of *Mycobacterium* Species from Gross Tissues based on Culture, Acid-fast-Stain and SD-Bioline at Kano Central Abattoir

Sex	Tissue Samples	Culture Positive	Acid-fast stain	SD-Bioline
No(%)	**No(%)**	**No(%)**		
Male	115(38.46)	5(41.66)	3(42.85)	
Female	168(61.53)	7(58.33)	4(57.14)	
Total	**27**	**13(48.15)**	**12(44.44)**	**7(25.93)**

KEY: Values in parenthesis are percentages

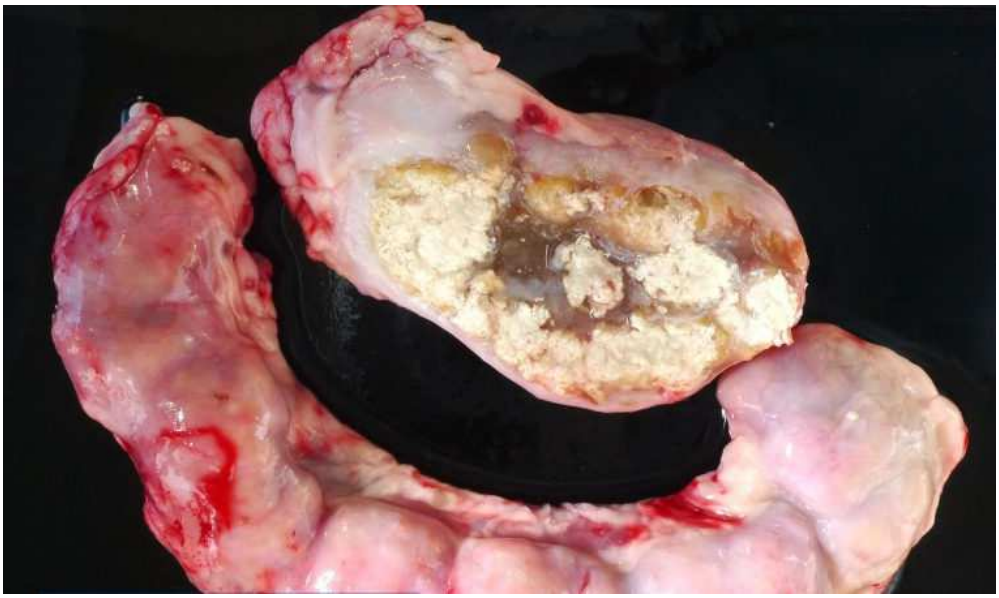

Plate: 1. Gross TB lesion in intestines from slaughtered Goat at Kano central abattoir

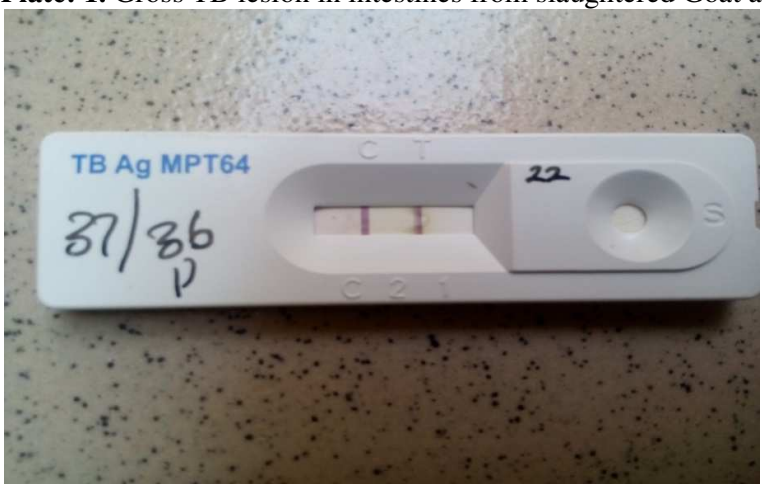

Plate 2; SD TB Bioline Ag MPT64, indicating positive result for *Mycobacterium tuberculosis* complex obtained from goat at Kano central abattoir.

DISCUSSION

The result obtained in this study showing 5.46% for post-mortem meat inspections in slaughtered goats at Kano Central Abottoir, Kano State is of great epidemiological and public health significance. This is important because traditional practice exist in the Sahel part of Northern Nigeria that could facilitate the transmission of Tuberculosisbetween goats, sheep, camels, cattle and humans, during watering and grazing. This practice involves rearing and using goats in close proximity and mixing with their owners thereby giving increased avenues for zoonotic transmission. The habit of people eating undercooked meat and improperly roasted meat in the form of "suya" and "kilishi" is gaining ground in Nigeria (Bale, 1991) leading to potential zoonotic transmission of the disease, although, no study was undertaken to isolate the organism in goat "suya" and "kilishi" in the country.

The prevalence rate reported in this study is slightly high compared with the previous report of 4.5% for TB in goats in Ibadan by Cadmus *et al.,* (2009). This, however, is not surprising since there is no record of any proper control programme in other livestock in the country, which could serve as source of infection to goats. There is high prevalence of TB infection reported (14.%) in other domestic species especially cattle (Abubakar, 2007, Ibrahim, 2016, Danbirni, 2016 and Abubakar, 2016). Other possible contributing factors to this prevalence could be the nomadic nature of the "Fulani" who own most of the Nigerian cattle disseminating the organisms to other animals including goats. Also the porosity of Nigerian borders allowing the influx of livestock from neighbouring countries as earlier observed (Ocholi, 1990) could be partly responsible for the rate of the disease observed in this study. The detection of gross TB lesions in slaughtered goats at the abattoir poses great danger of contacting the disease by the public, especially abattoir workers and butchers (Plate 1). This is because goats are usually slaughtered in abattoirs where the butchers wear minimal protective clothing and process meat with their bare hands. It further, confirms that control measures are not in place or inadequately applied; because in countries where control of TB infection is in place, detection of gross lesions at the abattoir during meat inspection is usually very minimal. This finding also agrees with an earlier suggestion that abattoir monitoring could be an essential element in the national tuberculosis control campaign and the most effective means of detecting residual infection especially in countries that have achieved control of the disease (Corner *et al*., 1990). The association between male and female goats examined for gross TB lesions was not statistically significant ($P<0.05$). This is in agreement with previous studies (Omer *et al.,* 2001; Asseged *et al.,* 2004; Teklu *et al.,* 2004; Cleaveland *et al.,* 2007; Abubakar *et al.,* 2011).The detection of pathogenic *Mycobacterium species* from goat is intriguing; while human-to-animals transmission of Pathogenic *Mycobacterium tuberculosis complex* has been reported (Ayele *et al.* 2004, Abubvakr, 2016, Danbirni, 2016 and Ibrahim, 2016), Cadmus *et al.,* 2009). However, the possibility of cross-contamination especially from infected animal handlers or abattoir workers to the goat cannot be ruled out.

This is also of public health importance as consumers of infected meat stand the risk of getting infected. Most importantly, the isolation and identification of *Mycobacterium* species from goat observedin this study is of serious public health importance. This finding reveals that there is a definite association between goat infection and the disease in cattle and in other animals. It further demonstrates the importance of *Mycobacterium* species in goat infection and shows a typical animal-to-animal transmission. A similar finding was made in other studies conducted in Nigeria(Cadmus *et al.* 2009). This can also justify a recommendation that any preventive measure for TB in cattle population in Nigeria should lean heavily and associated with the same level of measures in goat population.

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