Original Article

Molecular Identification of Mycobacterium species Based on Genotype MTBC in Goats Slaughtered at Kano Abattoir, Kano State, Nigeria

Abubakar UB[1], Tekdek LB[1], Saidu SNA[1], Usman AA[3], Surajo M[2], Lawson L[4], Abdulkadir IA[1]*

1. *Department of Veterinary Medicine, Faculty Veterinary Medicine Ahmadu Bello University, Zaria.*
2. *Department of Biology, Federal College of Education, Zaria*
3. *Department of Library and Information Sciences, Faculty of Education, Ahmadu Bello University, Zaria*
4. *Zankli Medical Centre, Abuja*

**Corresponding author: belbuba_tongo@yahoo.com*

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ABSTRACT

Background: Mycobacterium tuberculosis complex have been on increasing concern and a threat to public health especially in developing countries like Nigeria. This study aimed to identify Mycobacterium species based on Genotype MTBC in goats slaughtered at Kano abattoir in Kano State, Sahel part of Northern Nigeria.

Methods: Post-mortem meat inspection for tuberculosis -like lesions, culture, acid-fast staining, TB Ag MPT64 (SD- bioline) and genotype MTBC were carried out.

Results: A total of 500 slaughtered goats were examined, out of which 27 have gross TB lesions, which were subjected to culture, acid- fast staining, SD-bioline and genotype MTBC for detection of Mycobacterium spp. Three out of seven (42.9%) were identified as M. bovis, 1/7 (14.3%) was identified as M. tuberculosis and 3/7 (42.9%) were identified as M. bovis sbs caprae. However, it is worth noting that 2 M. bovis and 1 M. tuberculosis species were traced to male goats while the remaining 1 M. bovis and 3 M. bovis sbs caprae species were traced to female goats.

Conclusions: The study highlighted the importance of tuberculosis in Goats and its potential public health implications and calls for prompt action towards controlling the disease in Kano abattoir and Nigeria in general.

Key words: Mycobacterium species, prevalence, genotype, tuberculosis

INTRODUCTION

Tuberculosis (TB) is a chronic infectious and contagious disease of domestic animals , wild animals and humans [1]. It is characterized by the formation of granulomas in tissues especially in the lungs , lymph nodes , intestines , liver and kidneys [2]. It is caused by pathogenic members of the genus Mycobacterium which are commonly known as members of Mycobacterium tuberculosis complex (M. tuberculosis , M. bovis , M. africanum , M. bovis sbs caprae , M. microti and M. cannetti)[3].

Goat (Capra hircus) as known in different parts of the world is one of the smallest domesticated ruminants which have been with mankind for many generations.

The important role of goats in the production of milk, wool and manure has been well documented [4]. Goats are prolific and require low input for moderate level of production . They reach maturity at an early age and are nutritionally and financially profitable to keep for production[5].

Tuberculosis in goats has not been extensively investigated in Nigeria unlike TB in cattle. Tuberculosis in goat and sheep is caused by members of *Mycobacterium tuberculosis* complex predominantly M. bovis and M. caprae[6], and in some cases by *M. tuberculosis*[7].

In goats, the disease normally spreads 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 rendering the milk infective until it 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[8]. The predilection site for TB in goats is the lower respiratory tract and the associated lymph nodes[9]. Epidemiological studies have indicated that TB in goat and sheep has a wide global distribution ; it has been reported in several countries of the world including New Zealand , Sudan , Spain , Nigeria , the United Kingdom , Italy , Algeria and Ethiopia [10]. Caprine tuberculosis poses a risk to goat health and production in the developing world[7]. There has been recent increase in caprine tuberculosis in several countries; even among those practicing a long standing test and slaughter policy [11]. It is reported that the infection is widespread in Africa where goats co-graze with cattle that were not subjected to TB test and slaughter protocols[8]. Goats may also become infected with *M. bovis* when sharing pastures with infected cattle , at watering points , market places and shared night shelters [12]. A report on possible TB in goats in Nigeria was made on the basis of gross lesions without culture confirmation [13]. 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 [13]. This practice poses a threat to goats in Nigeria because of several reports on bovine TB in cattle in Nigeria[14-17].

Although there have been several reports of TB in cattle in Nigeria , Reports on diagnosis of TB in goats in Nigeria are scanty[7].

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

There is also failure or inadequate implementation of control policies for animal TB , 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[7]. In addition traditional practices exist in the Sahel part of northern Nigeria whereby goats are reared and kept in close proximity to their owners which could facilitate the transmission of TB between goats, cattle and humans during grazing and watering [17]. Furthermore , goats are usually slaughtered in abattoirs and slaughter houses where the butchers wear minimal protective clothing and process meat with their bare hands[18].

The absence of any epidemiological data on *Mycobacterium spp* in goat at Kano abattoir in Nigeria and the lack of any regulation with regard to processing and sale of goat meat and meat products other than the general meat inspection regulation has created an environment that can enhance TB transmission . This study aimed to identify Mycobacterium *spp* based on Genotype MTBC in goats slaughtered at Kano abattoir in Kano State, Sahel part of Northern Nigeria.

METHODS

Study Area

This 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 14o North and latitude 9 to 11o East in Kano state . Kano state shares boundaries with Jigawa state to the East , Kaduna /Bauchi states to the South and Katsina state to the Southwest [19]. 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 selecting 500 slaughtered goats for inclusion in this study . The goats were identified by serially numbering them with a permanent marker before they were slaughtered.

Meat Inspection/tissue sample collection

The organs of the slaughtered goats were examined grossly for TB lesions. Visceral organs and lymph nodes were inspected through careful visualisation, palpation and incision procedures for nodules and granulamatous lesions as previously described [20]. 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 [21, 20].

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 previously described [1,22], to detect AFB from granulomatous tissue samples collected during the post-mortem (PM) examination.

SD-BiolineTB Ag MPT64

This is a rapid immunochromatographic identification test for the M. tuberculosis complex that uses mouse monoclonal anti-MPT64. These test kits can be easily used for rapid identification of the M. tuberculosis complex in combination with culture system based on liquid or solid media without any technical complexity in clinical laboratories . The test was conducted as described by the manufacturers.

Genotype® MTBC for Molecular Identification of Mycobacterium tuberculosis Complex Species

The GenoType MTBC assay (HainLifescience GmbH , Nehren Germany) a new Deoxyribonucleic Acid (DNA) strip assay for the rapid molecular identification of members of the *M. tuberculosis* complex . The GenoType MTBC assay is based on MTBC specific 23 S ribosomal DNA fragment , gyrB NDA sequence polymorphisms and RD 1 deletion . The assay was performed according to the manufacturer's instructions and as previously described [23,24].

The four steps ; involved in this assay are DNA extraction by chemical method, amplification reaction , hybridization , and evaluation and interpretation of results.

In terms of evaluation and interpretation of result , strips were pasted and protected from light ; an evaluation sheet was used for the interpretation of result . Developed strips were pasted in the designated fields by aligning the bands CC and UC with the respective lines on the sheets. Each strip had a total of 13 reaction zones. Species were determined with the help of the interpretation chart (Figure 1) and name of the identified species.

1. The first band contained the conjugate control designed to indicate that the conjugate had been effectively united with the substrate , thereby facilitating correct visualization.

2. The second band includes a universal control designed to detect all known mycobacteria and members of the group of gram-positive bacteria with a high G+C content. This band was used for checking the presence of the amplified product after hybridization.

3. The third band contained a sequence that amplified a fragment of the 23 S rRNA region, which is common to all known members of the *M. tuberculosis* complex.

4. Amplification band 4 – 13 includes probes specific for each of the tuberculosis complex species.
5. The combination of these bands enabled identification of the different species within the complex.

RESULTS

A total of 500 slaughtered goats were examined, out of which 27 had gross TB lesions, which were sampled and subjected to culture, AFB staining, SD-bioline, genotype MTBC for isolation and identification. A total of Seven (7) mycobacterial pathogens were isolated from the samples processed in which 3 (42.9%) were identified as M. bovis, 1 (14.3%) was identified as M. tuberculosis and 3 (42.9%) were identified as M. bovis sbs caprae (Table 1). However, it is worth noting that 2 M. bovis and 1 M. tuberculosis isolates were traced to male goats while the remaining 1 M. bovis and 3 M. bovis sbs caprae isolates were traced to tissues of female goats.

Table 1: Identification of tubercle bacilli from goat tissue isolates using genotype MTBC

Sex	Isolates (%)	*M. bovis* (%)	*M. tb* (%)	*M. bovis sbs caprae* (%)
Total				
Male	3(42.86)	2(66.67)	1(100.0)	0(0.0)
Female	4(57.14)	1(33.33)	0(0.0)	3(100.0)
Total	**7**	**3(42.9)**	**1(14.3)**	**3(42.9)**

M. tb = Mycobacterium tuberculosis

DISCUSSIONS

The isolation and identification of *M. tuberculosis* from goat is intriguing; while human-to-cattle transmission of *M. tuberculosis* has been reported [25.20,26,27].

It is generally held that TB disease in goats due to *M. tuberculosis* is less severe than that caused by *M. bovis*[11]. 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 *M. bovis* from goat observed in this study is of serious public health importance. This finding suggests that there is an association between goat infection and the disease in cattle. It further demonstrates the importance of *M. bovis* in goat infection and shows a typical animal-to-animal transmission. A similar finding was made in other studies conducted in Nigeria [11]. This can also justify a recommendation that any preventive measure for TB in the cattle population in Nigeria should be associated with the same level of measures in the goat population.

Similarly, the isolation and identification of *M. bovis sbs caprae* species from goats is of interest. While it is virulent for goat, it has rarely been isolated from this host. However, Cadmus and colleagues reported a similar finding in a study in Ibadan, Nigeria[7].

This study has demonstrated the effectiveness of Genotype MTBC method used in the diagnosis of TB. Additionally, the Genotype MTBC might be more efficient, accurate and faster at least for field and epidemiological purposes as well as for conducting some PCR-based molecular methods like the spoligotyping and VNTR which do not need high quality DNA and also can be conducted directly from clinical samples.

This study should be interpreted in the context of its limitations. Since the sources of the animals were unknown, we could not determine whether the organisms were imported from a neighbouring country. In addition, we lacked information on the breed and condition of the animals. However, we have identified *M. tuberculosis, M. bovis and M. caprae sbs bovis* as causes of TB in goats in Kano abattoir, Nigeria.

DECLARATION

Competing interests: None

Funding: None

Authors contributions : AUB , TLB and SSNA designed the study. AUB, UAA and SM collected the samples , AUB , TLB , SSNA , LL and AIA analyzed the samples , AUB and conducted data analysis and drafted the initial manuscript . All authors reviewed the manuscript and approved the final version.

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Ethical approval : Approval was granted by the abattoir management before samples were obtained.

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