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Research

Molecular characterization of *Mycobacterium bovis* isolated from camels slaughtered for human consumption in Northeastern Nigeria and the public health implication

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Abstract

Introduction: although, camels serve as sources of food, transportation, work and income in Northern Nigeria, they are also a potential source of zoonotic diseases such as tuberculosis (TB). Camel TB is an important emerging public health problem in sub-Saharan Africa. However, there exist limited epidemiological data on the disease in Nigeria. **Methods:** we conducted a cross-sectional study among camels in Maiduguri, Northeast Nigeria. Lesions suggestive of TB were collected from camels slaughtered at Maiduguri Abattoir following post-mortem examination. The lesions were subjected to acid fast microscopy (AFB) and culture. Furthermore, isolates were tested for *Mycobacterium tuberculosis* complex (MTBC) using SD Bioline and confirmed by spoligotyping. **Results:** twenty (16.26%) out of 123 camels inspected had gross TB lesions; 50% of which were positive for AFB and 60% were identified by culture. Animals with poor body conditions had higher TB lesions ($P < 0.05$). There was no significant difference in the occurrence of TB lesions between male and female camels. SD Bioline identified four of the 12 isolates as MTBC which were further confirmed by spoligotyping to be *M. bovis*, belonging to the clonal complex SB0944 and lack spacer 30, that is dominant in West Africa. **Conclusion:** the isolation and confirmation of the unique dominant *M. bovis* strain from camels underscore the importance of molecular epidemiology of tuberculosis in camels and the possibility of cattle to camel transmission of tuberculosis. We strongly recommend implementation of One Health policies towards addressing threat posed by tuberculosis in camels in Nigeria.

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Introduction

Camel population in Africa is concentrated in East and North African countries [1]. In Nigeria, there has been a tremendous increase in camel population in the last 20 years Bourn *et al*. [2] reported a population of 90000 in 1994 while The National Bureau of Statistics/ Federal Ministry of Agriculture and Rural Development Collaborative survey on National Agriculture Sample Survey, [3] reported an estimated camel population of 277727 heads in 2012. Camels are mainly found in Northeast and Northwestern Nigeria 4 where it serves as source of food, income and transportation for people in the region. Clashes between cattle herdsmen and farmers constitute a serious security treat as a result of dramatic climate change in the region, human population explosion, poverty and decrease in available land for grazing has led to displacement of people and their animals from their place of origin. Thereby, introducing diseases that were previously unknown in the region as well as acquiring new diseases as they move about and contacting people and animals from other places [3-5] . Therefore, in view of the increasing important of camel as source of food, transportation and work there is urgent need to monitor zoonotic diseases that may be transmitted from camels to human. Particularly, tuberculosis in camels in Nigeria.

Borno state accounts for a significant production of camels in the Northeast Nigeria [6], due to its geographical location which provides suitable ecological conditions that favours camels and other livestock production [5,7]. Camel production serves as a major source of food proteins, income for farmers and other stakeholders in the livestock industry [6,8]. However, camels rearing in the Sahel region of Nigeria is constrained by various factors, notable among which is Boko haram insurgency that have rendered over 2.4 million people in the Northeastern Nigeria internally displaced and 4.5 million people facing acute food insecurity. This situation has rendered the people vulnerable to re-emerging infectious diseases such as zoonotic tuberculosis [5,9]. Livestock including camels are equally exposed to tuberculosis [5]. Tuberculosis is a debilitating disease that affects organs in the thoracic and abdominal cavity. It is characterized by formation of granulomatous lesions [10]. Camels were considered highly resistance to tuberculosis [11]. However, in recent time there have been increased cases of tuberculosis in camels in many countries [12]. Tubercle lesions in camels are predominantly distributed in the mesenteric lymph nodes than in the thoracic lymph node and that the right cardiac lobe of the lung is more affected than the other parts [13].

In Africa, the first case of tuberculosis was reported in Egypt [14]. In 2006, Pate *et al*. [15] reported an outbreak of tuberculosis caused by *M. caprae* in a zoological garden. Kinne *et al*. [16] also reported a case of tuberculosis in adult dromedary bull and the causative agent was identified as member of the antelope clade of MTBC. Also, a case of tuberculosis in *camelus dromedarius camelus* caused by *M. bovis* was reported in Mauritania [17]. In Ethiopia, a prevalence of 4.52% of tuberculosis in camels was reported in a study conducted on apparently healthy camels slaughtered for human consumption at Abaki abattoir [18]. And *M. bovis*was isolated from camels in Ethiopia 30. In Nigeria, there is no information on the causative agent of camel tuberculosis. Although, available data indicates that tuberculosis is present in camels in Nigeria [19,20]. However, these studies were based on serological test and acid-fast microscopy. Hence, the aim of this study was to provide information on the molecular characterization of *M. bovis*isolated from camels slaughtered for human consumption and the public health significance of camel tuberculosis in Borno Northeast, Nigeria.

Methods

Study Setting and Animal: this study was carried out to analyze for the first time, *Mycobacterium bovis* isolated from camels in Nigeria with the aid of bacteriology and molecular methods. The study animal comprises of 55 and 68 apparently healthy male and female camels slaughtered for human consumption at the Maiduguri Central abattoir from April 2017 to June 2017. Camel slaughtered in the abattoir originate from Chad, Niger, Sudan and Yobe state and from within Borno state, Nigeria. Approximately, 30-40 camels are slaughtered at the Maiduguri Central abattoir on daily basis.

Abattoir meat inspection: post-mortem examination of camel carcasses was performed as previously described [13,21]. Briefly, physical inspection was done by palpation, virtual examination and incision procedure was carried out under bright light. Body condition scores were assigned based on physical examination. Animal with fat body condition were assigned 3, medium as 2 and thin or poor body condition as 1 [22]. Organs inspected include lymph nodes (bronchial, retropharyngeal, hepatic, mesenteric, mandibular, and mediastina), lungs, liver, kidneys, heart, spleen, and intestines. Camel organs with lesions suggestive of tuberculosis were collected in to polytene bags and labeled appropriately. Samples were then transported in ice parked to the Department of Veterinary Microbiology Laboratory, University of Maiduguri and stored at -20^{o}C. Samples were later shipped to Zankli TB Research Laboratory at the Bimghan University, Nasarawa state for mycobacteriological culture and SD Bioline assay.

Tissue processing and bacteriological culture: tissue processing and culture was performed in a biosafety cabinet according to previously described methods [13,22,23]. Samples collected were sectioned with a sterile blade and ground in to paste using a ceramic pestle and mortar. 2ml of the homogenate was transferred in to a 15ml centrifuge tube. It was followed by decontamination and digestion by adding equal volume of NALC-NaOH (N-acetyl, L-cysteine-NaOH) and vortexed. The mixture was allowed to stand at room temperature for 15 minutes. Then it was neutralized by adding prepared phosphate buffer to the 15ml mark on the centrifuge tube and mixed gently. Thereafter, the mixture was centrifuged at 3000 x g for 20 minutes. The supernatant was decanted, and the pellet was resuspended in 2ml phosphate buffer with a pH 6.8. The suspension was vortexed gently and inoculated on to Lowesteine-Jenseen media agar slant containing pyruvate and or glycerol and incubated at 37^{o}C for 8-12 weeks. Colonies suspected of mycobacterium were tested for acid-fast bacilli by Ziehl-Neelsen microscopy. Isolates were harvested in duplicate in to 2 ml 7H9 Middlebrook broth and stored at -20^{o}C for further identification.

Crude DNA extraction: crude DNA extraction was carried out by scraping 2 loopful of the colonies into 200µl of molecular graded water and heating at 90^{o}C by immersing the tuber completely in a water bath for 30 minutes. Thereafter, the heated sample was centrifuged at 15000xg for 2 minutes. The supernatant containing the DNA was harvested in to a 2 ml tube by pipetting and stored at -20^{o}C until tested.

SD BIOLINE TB Ag MPT64 Rapid test: the SD BIOLINE TB Ag MPT64 rapid test (Cat. # 08FK50-04-1; Standard Diagnostic, Republic of Korea) is an immunochromatographic identification test for M. tuberculosis complex. It was used to identify all the isolate as members of the MTBC as described by the manufacturer. Briefly, the test device was removed from the foil pouch and placed on a flat, dry surface. 100µl of suspended solid culture in buffer was added to the specimen well. The result was interpreted after 15 minutes. The presence of band in the control and test

window was interpreted as positive while the absence of band in the test window and presence of band in the control window was interpreted as negative [20,24].

Spoligotyping: spoligotyping was done on genomic DNA as described previously Kamerbeek 37, 38 H37Rv was used as positive control while DNA free water was used as negative control. This procedure was carried out at Genoscreen (Lille, France). The results were recorded as "n´ for spacers present and "o" for spacers deleted. It was later transformed in to number where; n = 1 and o = 0. Spoligotype names were obtained from the global database by comparing the patterns to that in the database.

Data analysis: data such as sex, age group, body condition and site of lesions were entered in to excel spread sheets and exported in to SPSS version 16 for analysis. Prevalence was calculated by dividing the number of camels that are positive for tuberculosis by the total number of camels examined and multiply by 100. Chi square test was used to determine association between prevalence and parameter like sex, age group and body condition. P- Value less than 0.05 was considered as significant.

Ethical Approval: the permission to conduct this study was obtained from the Ahmadu Bello University Research and Ethic Committee.

Results

Gross lesions and Ziehl-Neelsen microscopy: a total of 123 camels slaughtered for human consumption were examined for tuberculosis, 20 (16.26%) had gross tubercle lesions. 10 (8.13%) of the total camel carcasses examined were positive for tubercle bacilli by Ziehl-Neelsen microscopy. Occurrence of tuberculosis and body condition were significantly different ($P < 0.05$) Camels with poor body condition were more affected by tuberculosis than camels with medium and fat body condition (Table 1). Macroscopically, tubercle lesions were more common in the lungs and lymph nodes than other organs Figure 1.

Mycobacterial culture and SD Bioline assay: from a total of 23 suspected TB lesions collected from 20 camels, 12 (60.00%) showed growth on both pyruvate and glycerol enriched Lowenstein-Jensen medium agar slants (Table 2). Culture positive was highest (69.23%) among camels with poor body condition. Also, adult camels had high (71.43%) positive culture than young adult (33.33%). Out of the 12 isolates, 4 (33.33%) were identified as *Mycobacterium tuberculosis* complex (MTBC) by using SD Bioline assay (Table 2).

Spoligotyping: The four isolates that were identified as MTBC by SD Bioline were further characterized using spoligotyping. Spoligotyping results were compared with global *M. bovis* database. All 4 (100%) isolates were confirmed to be SB0944 and lack the unique spacer 30 which is specific for African 1 clonal complex. This spoligotype pattern had been previously reported in Cameroon, Chad and Nigeria (Table 3).

Discussion

In general, there was a total absence of information on the molecular characterization of *M. bovis* isolated from camels in Nigeria. The prevalence of tuberculosis in camels in the present study was in agreement with previous report in Nigeria were Kudi *et al*. [19] reported a prevalence of 17% based on detection of antibodies in sera collected from camels slaughtered at an abattoir in northern Nigeria. Similar

results was reported in camel slaughtered in abattoirs in Ethiopia [13]. However, Abubakar *et al*. [20] reported a higher prevalence (22.6%) of camel tuberculosis in Kano Northwest, Nigeria by detecting antibodies in sera using lateral-flow technology. Camels with poor boy condition had higher prevalence of tuberculosis than camels with medium or good body condition. This observation was in agreement with Ejeh *et al*. [22] who reported higher prevalence of bovine tuberculosis in cattle with poor body condition slaughtered for human consumption in Makurdi and Otukpo, Northcentral Nigeria. It has also been observed that animals with poor body condition have poor immunological response than animals with medium or good body condition39. Results obtained in this study was in contrast with previous studies on the prevalence of camel tuberculosis in Ethiopia in which there was no significant difference between body condition and occurrence of tuberculosis [13,18,25].

Distribution of tuberculosis lesion in this study were more commonly seen in lymph nodes and lungs than other organs in the body. This finding was consistent with other researchers [13,18]. The relatively high tubercule lesions in the lungs and lymph nodes further corroborated inhalation as the primary route of transmission of tuberculosis in camels [26]. The results of bacteriological culture in this study was higher than previous result of culture of tuberculous lesions from camels [18]. However, this results was lower than bacteriological culture of TB suspected lesion in cattle slaughtered for human consumption in North Central Nigeria [21]. The low rate of bacteriological isolation of tubercule bacilli from camel may be due to excessive calcification observed in camel tubercule lesions. SD Bioline assay reults in this study revealed that 4 out of 12 isolates were identified as Mycobacterium tuberulosis complex (MTBC). Hence, the remaining isolates were assumed to be non-tuberculous mycobacteria (NTM) since NTM had been isolated from other animals in Nigeria [21] and other parts of the World [27,28]. In this study we report for the first time, the isolation of the spoligotype SB0944 from camels in Nigeria. All isolates lack spacer 30 which is specific for a clonal complex referred to as African 1. SB0944 strains had been isolated from cattle [23,29] and pigs [30]. The strain had also been isolated from humans infected with zoonotic tuberculosis in Nigeria, Burkina Faso and Ghana [28,30-33]. It is the most dominant spoligotype in West Africn countries such as Nigeria, Chad, Cameroon and Mali [34]. The isolation of the spoligotype SB0944 from camels in Northeastern Nigeria emphasis the strain dominant of *M. bovis* strains in West Africa and possible cattle to camel transmission or vice visa and a potential for zoonotic transmission of the strain to humans are at risk of zoonotic tuberculosis.

Conclusion

In conclusion, the isolation and conformation of *M. bovis* from camels slaughtered for human consumption underscore the importance of comprehensive abattoir inspection of camel carcasses. Since camels rearing and consumption in the northeast and northwest are on the increase due to increase population and high demand for camel meat and as an alternative to beef. Again, camel's ability to utilize the scanty fodder resources of the arid zones of Nigeria for body maintenance, growth and milk production makes this animal a potentially important source of animal proteins. Also, following the displacement of over 4.5 million people, increased animal raiding, massive environmental population, poverty, malnutrition and increased HIV/AIDS epidemics as a result of insurgency in the northeastern parts of Nigeria, there is urgent need for one health approach in the control and prevention of tuberculosis in camels, cattle and human population.

What is known about this topic

- Tuberculosis is endemic in cattle slaughtered at abattoir in Maiduguri;
- Zoonotic tuberculosis had been reported in Southwest and Benue state in Nigeria;
- The causative agent of tuberculosis in camel in Nigeria is unknown.

What this study adds

- *Mycobacterium bovis* is the cause of tuberculosis in camels slaughtered at Maiduguri Central Abattoir;
- *M. bovis* isolated from camels in Maiduguri Central Abattoir belong to African 1 clonal complex;
- This study report the first isolation and molecular characterization of M. bovis from camels in Nigeria.

Competing interests

The authors declare no competing interest.

Authors' contributions

All the authors have read and agreed to the final manuscript.

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Figure 1: distribution of tubercle lesions in camels slaughtered at Maiduguri Abattoir ZN = Ziehl-Neelsen

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Table 1: gross lesions and Ziehl-Neelsen Microscopic Examination of Tissue Samples from Camels Slaughtered in Maiguguri Abattoir

Parameters	**No of samples**	**Gross Lesions (%)**	**P- Value**	**ZN – Microscopy positive (%)**	**P- Value**
Sex					
Male	55	6 (10.91)	0.306	2 (3.64)	0.152
Female	68	14 (20.59)		8 (11.76)	
BCS					
Lean	45	13 (28.89)	0.008	6 (13.33)	0.129
Medium	46	5 (10.87)		3 (6.52)	
Fat	32	2 (6.25)		1 (3.13)	
Age					
Adults	82	14 (17.07)	0.294	7 (8.54)	0.415
Young adult	41	6 (14.63)		3 (7.32)	
Total	123	20 (16.26)		10 (8.13)	
P- Value > 0.05 (fisher exact test)					

Table 2: mycobacterial culture and SD Bioline Assay of Suspected Tubercle Lesions

Parameter	**No Gross lesions**	**Culture positive (%)**	**SD Bioline positive (%)**
Sex			
Male	6	4 (66.67)	1 (25.00)
Female	14	8 (57.14)	3 (37.50)
BCS			
Poor	13	9 (69.23)	3 (33.33)
Medium	5	2 (40.00)	0 (0.00)
Good	2	1 (50.00)	1 (100.00)
Age			
Adults	14	10 (71.43)	4 (40.00)
Young adult	6	2 (33.33)	0 (0.00)
Total	20	12 (60.00)	4 (33.33)

Table 3: spoligotype profile of mycobacterium isolated from camels Slaughtered in Maiduguri Abattoir					
SB Number	**Binary**	**Clonal complex**	**Sex**	**BCS**	**Age**
SB0944	11011111011111101111111111111101111111100000	M. bovis, African 1	F	P	AD
SB0944	11011111011111101111111111111101111111100000	M. bovis, African 1	F	P	AD
SB0944	11011111011111101111111111111101111111100000	M. bovis, African 1	M	P	AD
SB0944	11011111011111101111111111111101111111100000	M. bovis, African 1	F	G	AD
H37RV	11111111111011111110011111111111100001111111	Euro-America			
Negative ctl	00000000000000000000000000000000000000000000				
BCS = body condition score, F = Female, M = Male, P = poor body condition, G = good body condition and AD = Adults					

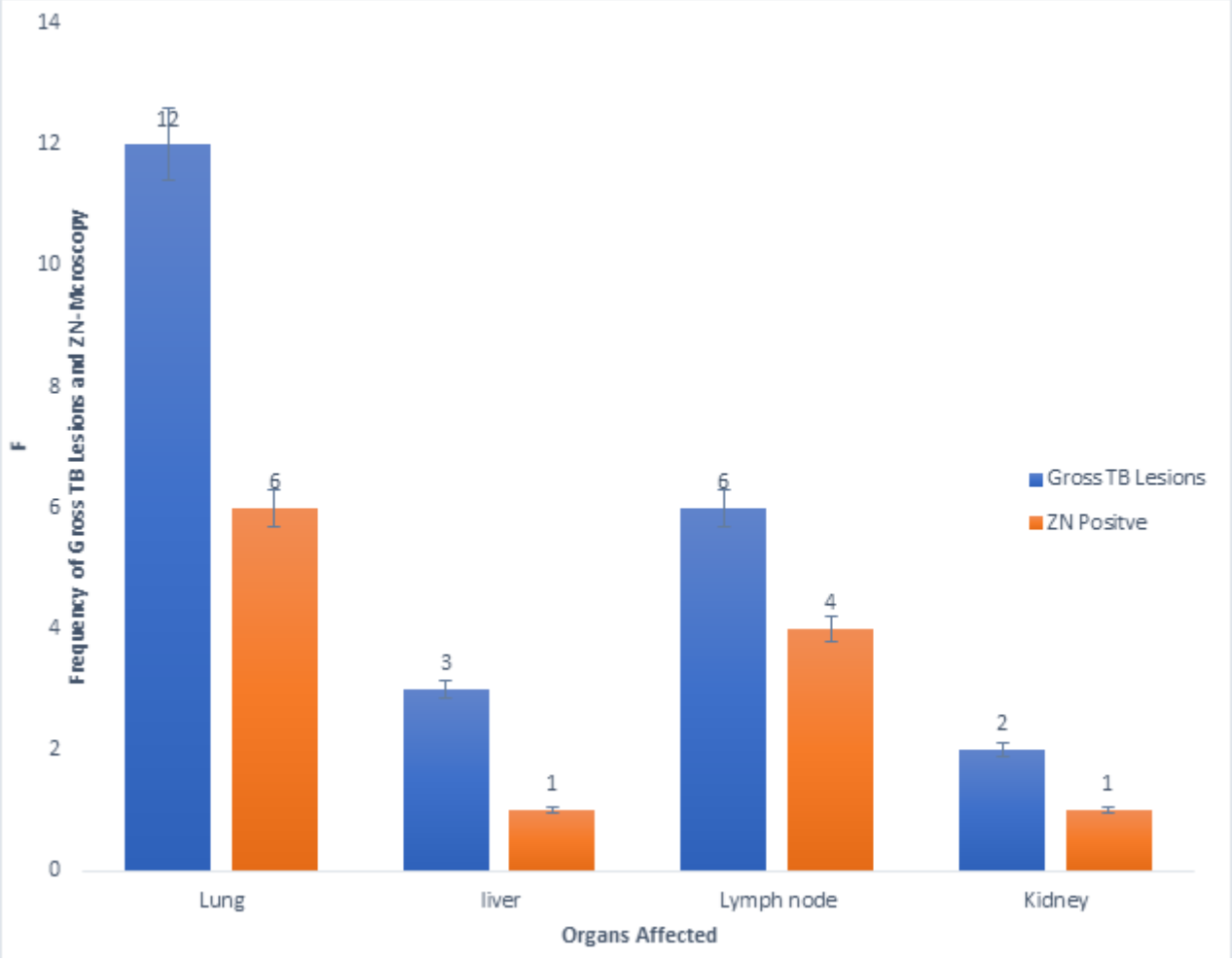

Figure 1: distribution of tubercle lesions in camels slaughtered at Maiduguri Abattoir ZN = Ziehl-Neelsen