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Prevalence and Pathological Characteristics of Tubercle Lesions from Slaughtered Camels in Maiduguri, Nigeria

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ABSTRACT

One-humped camels *(Camelus dromedaries*) are almost exclusively domesticated species that are commonly used as beasts of burden and food animals for meat and milk production in arid areas. This study appraised the prevalence and pathological characteristic of tubercle lesions isolated from camels slaughtered in Maiduguri Central abattoir. Two hundred and twenty-eight (228) camel carcasses comprising 124 (54.6%) males and 104 (45.6%) females were examined at slaughter to detect tuberculous lungs. Tubercle lesions were excised from the lungs, lymph nodes and liver after careful visual inspection and palpation of suspected nodules and granulomas. Tissue samples were processed using Zeihl Neelsen (ZN) microscopy and routine histopathological examination by means of Hematoxylin and Eosin (H&E) and Von Kossa staining techniques at the Veterinary Microbiology Laboratory, University of Maiduguri. Tuberculous lesions were detected in 15/228 (5.5%) of all the examined carcasses. Zeihl Neelsen staining further revealed 6.6% prevalence of tubercle lesion among the examined camels. Histopathology showed lymphocytic infiltration and calcium deposits in granulomatous lesions stained with H&E and Von Kossa, respectively. The results of this study which reveal typical gross and histopathological lesions of tuberculosis indicates prevalence of tuberculosis among camels slaughtered for human consumption in Maiduguri, Nigeria. Since the zoonotic potential of camel tuberculosis cannot be ruled out, it is necessary to step up prevention and control measures for camel tuberculosis to enhance public health safety.

Keywords: Camel; Granulomas; Histopathology; Maiduguri; Tuberculosis

INTRODUCTION

The dromedary camels have an estimated world population of 18 million across the arid and semi-arid environments of African and Asian countries (Baumann and Zessin, 1992). Africa has about 15 million dromedary, which accounts for 74% of the world's population and 60% of Africa's camels are found in East Africa, south of the Sahara (Kudi *et al*., 2012). There are approximately 87,000 camels in Nigeria with 30.9% located in Borno state because of their high adaptation to the semi-arid environments (Egbe-Nyiwi *et al*., 2016). Camels serve as an important source of milk and meat for many people, performs work and helps to conserve the environment in Northern Nigeria (Jaji *et al*., 2017). However, camels are faced with many undocumented health constraints in the region, including tuberculosis and other important zoonoses (Beyi *et al*., 2014).

Tuberculosis is a chronic bacterial disease of livestock, wildlife, pets, and man, which is characterized by the progressive development of granulomatous tubercle lesions in affected tissues. The disease affects all age groups and cause more deaths throughout the world than any other bacterial disease known today (Buss *et al*., 2016). Tuberculosis is still one of the most relevant and devastating diseases of man and animals caused by *Mycobacterium tuberculosis* complex. Various species of birds and wild animals participate in the epidemiological cycle of tuberculosis as vectors (Wernery and Kinne, 2012). Tuberculosis, caused by members of *Mycobacterium tuberculosis* complex (MTBC), is re-emerging as a significant health problem in humans and animals (Thoen *et al*., 2016). Tuberculosis is rare in camels managed under a free-range system, unless if kept in repeated contact with the source of infection. Tubercle bacilli such as *M. bovis*, *M. caprae*, *M. tuberculosis*, and atypical mycobacteria have been isolated from tuberculosis-like lesions in dromedary camels Pate *et al*., 2006; Mamo *et al*., 2011; Zerom *et al*., 2013).

The threat posed by increasing and prolonged periods of drought particularly in arid and semi-arid areas of Africa has forced pastoralists to undergo adaptive strategies such as herd

diversification, where emphasis on camel husbandry is becoming a priority. This coupled with camels' ability to be drought resistance more than most food animals as well as increased demand for protein has made camel meat a highly sought-after delicacy. Since it is kept in close proximity with other livestock that are known to carry TB, a contagious disease, it is necessary to investigate its susceptibility (Egbe-Nyiwi *et al*., 2016). This study was designed to appraise the prevalence and pathological characteristic of tubercle lesions isolated from camels slaughtered in Maiduguri Central abattoir.

MATERIALS AND METHODS

Study Area

The Maiduguri Central Abattoir is situated near the Maiduguri International Cattle Market (Kasuwan Shanu) in Maiduguri Metropolitan Council (MMC) Local Government Area. The Central Abattoir is the main slaughter facility where cattle, sheep, goats, and camels are slaughtered for human consumption in the city and environs. Maiduguri is situated between Latitude 11.50 North, and longitude 30.050 East at an Altitude of 34m above the sea level (Alaku and Moruppa, 1993).

Study Design

An Abattoir survey was conducted from January to March 2020 to appraise the prevalence and pathological characteristic of tubercle lesions by postmortem inspection of camel carcasses slaughtered for human consumption at the Maiduguri Central abattoir. Two hundred and twenty-eight (228) camel carcasses comprising of 124 male and 104 females were inspected. The camels were physically examined prior to dressing to access their body condition scores. Body condition score was done based on Faye *et al*., (2001) and 44 were found to be medium size while 184 were fat. Age-wise, the camels included in the study were 53 young and 175 adults. Aging was determined according to Oliphant, (1883) and Eze, Adamu, and Bukar, (2012). Young camels are those camels that have not attained full permanent teeth while adult camels are those had attained full permanent teeth; usually fromseven years and above. Post-mortem examination of camel carcasses was performed by physical inspection through palpation, virtual examination, and incision under bright light to detect tubercule lesions in parenchymatous organs (lungs, spleen, liver, heart, kidneys) and lymph nodes (bronchial, retropharyngeal, hepatic, mesenteric, mandibular, and mediastinal).

Sample Collection

Samples were collected during the daily routine postmortem meat inspection of camel carcasses at the Maiduguri Central abattoir. Carcasses were examined grossly, and suspected tubercule lesions were collected for further analysis. Briefly, samples of suspected tissue specimens from the lungs, lymph nodes, liver, kidney, and spleen were collected from the animals. The samples were obtained after careful visual palpation and incision procedure for nodules and granulomatous lesions (OIE, 2012). After collection, lesion bearing tissues from suspected carcasses were placed in a labeled sterile screw-capped, leak-proof specimen container on ice packing and transported to Microbiology laboratory, Faculty of Veterinary Medicine, University of Maiduguri for processing.

Laboratory Examination

Tissue Procession and Acid-Fast Staining

All tissue processing steps were performed in a biosafety cabinet. The tissues were first homogenized with a mortar and pestle followed by decontamination and digestion in a 15ml centrifuge tube containing equal amounts (2ml each) of 4% NaOH (OIE, 2012). The mixture was allowed to stand in a test tube until the specimen was liquefied before centrifugation at 3000 x g for 20min. The supernatant was carefully decanted, and two drops of the sediment was transferred onto a clean glass slide using sterile pasture pipette to make a thick film.

The smear was air dried and fixed by flinging in open flame. Fixed smears were then flooded with strong Carbol fuchsin for ten minutes with intermittent steaming. The stain was washed off and decolorized with 3% acid-alcohol for one minute before counter staining with malachite green for thirty seconds. After staining, the slides were washed under running tap water and air-dried before examination under oil immersion (x100) objective lens of a compound microscope for the presence of characteristic acid-fast bacilli (Payeur, 2014).

Hematoxylin Eosin Staining

Tissue sections for histopathology were first fixed in 10% formalin solution for 14 days before embedding in paraffin wax. Paraffin-embedded tissues were sliced into 4µm sections with a microtome and routinely stained with Harris Hematoxylin and Eosin (H&E). Dehydration was done in ascending grades of alcohol: 50%, 70%, 80%, 95% (twice), 100 % (twice), followed by clearing with xylene and mounting with DPX (Metchock *et al*., 2005).

Von Kossa Staining

Embedded slides were deparaffinized with xylene, dehydrated through alcohols and rinsed in running tap water. Afterwards the slides were rinsed through two changes of distilled water followed by 5% Silver Nitrate for 45 minutes and exposed to an ultraviolet light source. The slides were rinsed again in distilled water and placed into 5% Sodium Thiosulphate for 2 minutes. This was later washed in tap water and placed in Nuclear Fast Red stain for 5 minutes, rinsed in running water and dehydrated through three changes of fresh alcohol and also cleared through three changes of xylene and finally the stained slides were mounted with DPX (Sheehan and Hrapchak, 1980).

Data Analysis

Data such as sex, age group, body condition and site of lesions were entered in to excel spread sheets and exported into SPSS version 16 for analysis. The prevalence of TB lesions was calculated by dividing the number of camels that tested positive for tuberculosis (d) by the total number of camels examined (n) and multiply by 100 (p=d/n x 100). Chi square test was used to determine the associations between

prevalence of TB and the independent variables (sex, age group and body condition score). P values less than 0.05 were considered statistically significant.

RESULTS

Gross Lesions and Zeihl-Neelsen microscopy

The results of the demographic data of the study population were presented in Table 1.

Findings from the gross examination showed fifteen (5.5%) had signs of tubercle lesions, out of which 1 (6.6%) was positive for Zeihl-Neelsen stain as shown in Table 2

Gross lesions were more prevalent among male (8.1%) as compared to 4.8% among the female. Pathological lesions observed among young camel (7.5%) is higher than those found among the adult (6.3%). Carcasses of medium sized camel have more gross lesions (11.4%) as compared to those observed among the fat (5.4%) (Table 2). Tuberculosis lesions were observed predominately in the lung; while other organs were least affected.

Table 1: Demographic Variable of Study Camels

Variable	Frequency	Percentage
SEX		
Male	124	54.6
Female	104	45.6
BSC		
Thin	0	0
Medium	44	19.3
Fat	184	80.7
AGE		
Young	0	0
Young Adult	53	23.2
Adult	175	76.8
TOTAL	**228**	**100**

Table 2: Gross pathologic lesions and Acid-fast test of tubercle lesions of camels slaughtered in Maiduguri Central Abattoir

Variable	No. examined	Gross Lesions	χ^2 (p – value)	AFB Positive (%)	AFB Negative (%)
SEX					
Male	124	10 (8.1)	0.976 (0.424)	1 (10.0)	9 (90.0)
Female	104	5 4.8)		0 (0.0)	5 (100)
AGE					
Young	53	4 (7.6)	0.105 (0.754)	1 (25.0)	3 (75.0)
Adult	175	11 6.3)		0 (0.0)	11 (100)
BSC					
Medium	44	5 (11.4)	2.031 (0.175)	0 (0.0)	5 (100)
Fat	184	10 (5.4)		1 (10.0)	9 (90.0)
Total	228	**15 (6.6)**		**1 (6.7)**	14 (93.3)

Gross lesions encountered include a yellowish nodular lesion after incision in the lung (Figure 1).

Hematoxylin and Eosin and Von Kossa Staining

Samples from 15 suspected tubercle lesions were subjected to H & E staining and one showed granuloma characterized by fibroplasia and lymphocytic infiltration (Figure 2).

Staining with Von Kossa showed scanty presence of calcium deposits appearing as black spots around a granulomatous lesion

DISCUSSION

An abattoir survey was conducted to appraise the prevalence and pathological characteristics of tubercle lesions isolated from slaughtered camels in Maiduguri Central abattoir. It was hypothesized at the beginning of this study that camels are potential carries of TB in Maiduguri and environs. Detailed postmortem examination has revealed 6.6% apparent prevalence of tuberculosis lesions among slaughtered dromedary camels in Maiduguri Central abattoir. The result obtained in this study exceeds the 0.3% prevalence of TB reported among camels from the same abattoir in 2011 (Bala *et al*., 2011) and the 0.1% reported in Niger Republic (Boukary *et al*., 2012). However, the presently observed prevalence of TB among camels in Maiduguri is lower than previous report (17%) from Kano in Northwestern Nigeria (Kudi *et al*., 2012). Also, our result is lower than 12.3% and 19.5% previously reported prevalence of TB based on gross lesions observed in slaughtered dromedaries in Ethiopia (Zerom *et al*., 2013) and in India, respectively (Narnaware *et al*., 2015). The observed variations in the prevalence rates of TB reported by various authors may be due to differences in livestock production systems, diagnostic techniques, sample collection techniques, transmission pathways, endemic indices, TB control measures, and geographic conditions.

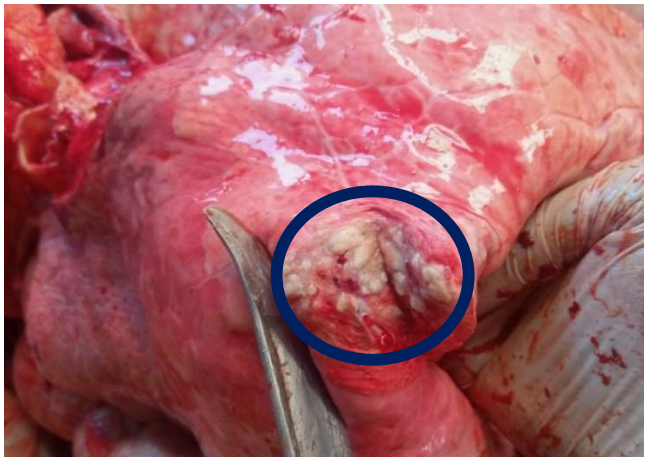

Figure1: Photograph of the lung showing an incised tubercle with caseous material.

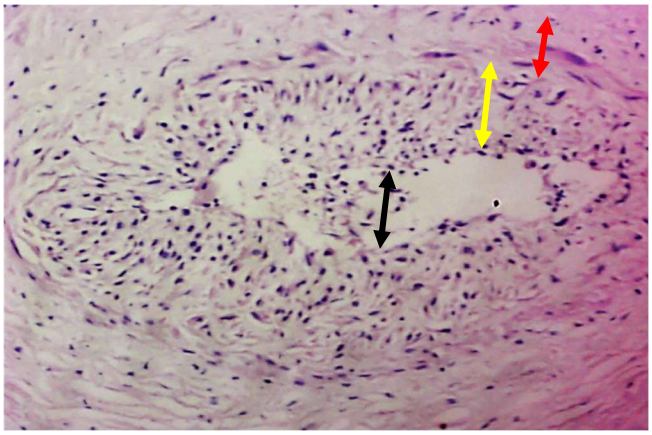

Figure 2: Photomicrograph of the lung showing an area of glanulomatous inflammation composed of a caseous center (black arrow), neutrophils and lymphocytes (yellow arrow), surrounded by fibrocytes (red arrow), H & E x 200.

The higher prevalence of TB lesions recorded among male camels in this study was lower than a previous study in Ethiopia (Beyi *et al*., 2014). Other studies reported significantly higher prevalence of TB lesions in female than male camels in Ethiopia (Mamo *et al*., 2011; Zerom *et al*., 2013). Moreover, less female camels were slaughtered at the abattoir in the study area because female animals are traditionally kept for reproduction and milk production purposes until their reproductive potential is exhausted (Ejeh *et al*., 2014b). The higher prevalence with age reflects the chronic nature of the tuberculosis (Ejeh *et al*., 2014a). The predominance and high severity of TB gross lesions were observed in the lungs. This significant finding support previous studies that had reported higher prevalence of tubercle lesions in the lungs than other organs in animals (Cadmus, 2019; Ejeh *et al*., 2014b; Macháčková *et al*., 2004; Pesciaroli *et al*., 2014). This is because tubercle bacilli have preference for macrophages in the lungs and associated lymph nodes (Ameni *et al*., 2013; Rodriguez-Campos *et al*., 2014). Also, the route of transmission of tubercle bacilli is primarily through inhalation of infectious droplets (Palmer *et al*., 201).

Granuloma formation is primarily a host-defense mechanism for containing the bacteria and limiting the infection, hence its presence as observed in the H and E as shown in Figure 2 is characteristics of tuberculosis. In the histopathological study of the sample, we observed tubercle granuloma with central necrosis and calcification. The central area is made of necrotic cellular debris, calcium deposits, and connective tissue capsule walled off the granuloma from the surrounding tissue as reported by (Jirata *et al*., 2019). The next layers from center to outer were made up of lymphocytes, macrophages, epithelioid macrophages distributed under connective tissue layers (Figure 2). Figure 6 shows calcium deposits appearing as black spots surrounding the granuloma.

The calcification observed in the histopathological lesion is characteristic of TB infection and other infectious agents like histoplasmosis, Nocardiosis and some fungi infection (Ejeh *et al*., 2014c). The most frequent causes of calcification are healed primary granulomatous lesions (Ghon focus) which consist of a densely calcified focus situated anywhere in the lung, commonly in the upper lung, (Chartier *et al.*, 1991). It has also been shown that Tuberculosis can develop hypercalcemia caused by excessive production of endogenous vitamin D, thus further predisposing to dystrophic pulmonary calcification (Rendon *et al*., 2017).

The poor calcium deposit observed could be due to host immune response due to the chronic nature of the infection; or it could also be because of poor fixing and staining technique used probably because Von Kossa staining technique is not commonly used routinely in our laboratories. It may also depend on the age of the lesion.

The detection of Mycobacteria tubercle from slaughtered camel in Maiduguri, Borno State confirms the presence of camel tuberculosis and hence the risk of transmission of zoonotic tuberculosis from camels to humans due to high rate of consumption of camel meat and meat products in Maiduguri metropolis.

Conclusion

The present study revealed the prevalence of camel tuberculosis lesions in Maiduguri. However, the current pathological findings are not conclusive. Therefore, further cultural isolation and molecular characterization of strains causing tuberculosis in camels is recommended.

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Conflict of Interest

The authors declare that they have no conflict of interest.

Authors Contribution

The study was conceived and designed by FEE and YJ. DLM, HIG, FAL and BTP carried out the study, analysed the data and wrote the manuscript. The study was overseen by FEE. The final manuscript was read and approved by all author.

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