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Tuberculosis in Sheep and Goats: pathological characteristics based on abattoir Study in South Darfur State, Sudan

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ABSTRACT

The status of tuberculosis in Sudanese goats and sheep has not been studied yet; hence this study was designed to investigate the prevalence and pathological characteristics of tuberculosis in small ruminants in South Darfur State during the period October 2015 to February 2017. Slaughtered goats (3200) and sheep (3260) were examined for pathological tubercle lesions and histopathological changes. Grossly, tubercle like lesions which appearance as embedded in the parenchyma or bulging on surface of organs and tissues in variable sizes with white colour were observed in 119 (3.72%) goats and 93 (2.85%) sheep. Histopathological examination showed central caseous necrosis with or without calcified areas surrounded by epithelioid cells and langhan's giant cells with fibrous capsule infiltrated by lymphocytes and plasma cells with or without scattered acid fast bacilli in the granulomatous regions. Our findings indicate that presence of tuberculosis in the Sudanese goats and sheep, and *Ambboror* sheep and *Baggara* goats were the reservoirs of tuberculosis among the livestock in the region and and respiratory pathway was the most site of infection in goats and sheep. Therefore, proper implementation of meat inspection procedures at abattoir with public awareness are important to control tuberculosis in South Darfur State. In addition, large scale surveillance is needed in different parts of the Sudan to estimate the apparent and true prevalence of tuberculosis among animals and animal workers.

INTRODUCTION

In Sudan, goats were estimated at 42.5 million head forming about 18.2% goats in Africa and 5.3% of the world goat population (FAO, 1999; Yousif and Fadl El-moula, 2006). Moreover, goats and sheep play an important integral component in most traditional production system. Goats rearing is the main source of milk for children and both them are rearing for meat purposes, skin and cash income from sales (Ageeb, 1992).

Tuberculosis (TB) is an infectious, granulomatous, contagious and chronic disease in wide range of domestic and wild animals and also in humans (O'Reilly and Daborn, 1995). Its caused by the members of *Mycobacterium tuberculosis* complex (MTBC), which including *Mycobacterium tuberculosis*, *M. bovis, M. bovis BCG strain, M. caprae*, *M. africanum*, *M. microti* and *M. canetti* (Prodinger *et al*., 2002; Erler *et al*., 2004). However, *M. bovis* is the most universal pathogen among mycobacteria that affects many vertebrate animals of all age groups including humans, although cattle, goats and pigs are found to be most susceptible, while sheep and horses are showing a high natural resistance (Radostits *et al*., 2000; Thoen *et al*., 2006). Inhalation is the most common route of infection but ingestion of contaminated material can also cause infection (Neill *et al*., 2001; Biet *et al*., 2005).

TB in goats and sheep is caused predominantly by *M. bovis* and *M. caprae* (Cordes *et al*., 1981; Hiko and Agga, 2011) and few caused by *M. tuberculosis* (Cadmus *et al*., 2009; Tschopp *et al*., 2011). Although TB in small ruminants is considered an infrequent disease, it has been recognized for many years in some Mediterranean countries (Gutiérrez *et al*., 1995), particularly in goats. Recent reports of TB in both goats and sheep in several EU countries (Álvarez *et al*., 2008; Daniel *et al*., 2009; Quintas *et al*., 2010; Sharpe *et al*., 2010) have renewed attention to these domestic species as possible TB reservoirs for cattle (Napp *et al*., 2013).

Caprine and bovine TB are closely related in regard to the immune response and pathological characteristics (Marianelli *et al*., 2010). In natural infections, TB in goats and sheep, as in cattle, is primarily a chronic infection that causes exudative granulomatous caseous inflammatory lesions in the lungs and associated Lymph nodes. Occasionally, tuberculous lesions may also be found in the upper respiratory tract lymph nodes and other organs, such as the spleen, liver, or mesenteric lymph nodes (Dean *et al*., 2005; Daniel *et al*., 2009). Goats exhibit a strong tendency to develop liquefactive necrosis and caverns inside tuberculous granulomas that is remarkably similar to that observed in human TB. Histologically, the lesions are similar to those observed in cattle and humans. Typical tuberculous granulomatous necrotizing lesions are observed, characterized by central caseous necrosis, often with some mineralization, surrounded by macrophages, foamy macrophages, numerous giant cells, lymphocytes, and a fibrotic capsule. Acid-fast bacilli are usually present inside the caseous necrosis in very low numbers (Cvetnic *et al*., 2007; Marianelli *et al*., 2010).

In Sudan, previous studies in cattle of different regions indicated natural endemic of TB (Sulieman and Hamid, 2002; Manal *et al* 2005; El Tigani *et al*., 2013; Aljameel *et al*., 2014). The status of TB in goats and sheep has not been studied, although they have common watering and grazing points with cattle that might favor the transmission

of *Mycobacterium* ssp. among these domestic animals. Therefore, the present study was designed to estimate the prevalence of TB in goats and sheep and characteristic the pathological lesions based on abattoir study in South Darfur State, Sudan.

MATERIALS and METHODS

Study area

This study was conducted at Nyala abattoir, Nyala locality, South Darfur, Sudan during the period October 2015 - February 2017 for detecting the prevalence of tuberculosis in slaughtered small ruminants .

Pathological examination

During the study period, a total of (3200) goats and (3260) sheep of varying breed were presented for slaughtering at Nyala abattoirs. Postmortem inspection was performed in detail as previously described (Corner, 1994; Ameni *et al*., 2007; Biffa *et al*., 2010). Particular emphasis was given during examination to certain organs and lymph nodes that were carefully inspected for the presence of suspected TB lesions.

All lobes of the two lungs were inspected externally and palpated. Then, each lobe was sectioned into (2 cm) thick slices to facilitate the detection of lesions. Similarly, lymph nodes, namely, the parotid, mandibular, submaxillary, retropharyngeal, mediastinal, bronchial, mesenteric, prescapular, medial iliac, supramammary, portal and inguinal lymph nodes, were sliced into thin sections (about 2mm thick) and inspected for the presence of visible lesions. Moreover, organs such as liver, spleen, kidneys rumen and small intestine the cut surfaces were examined in detail during post-mortem under a bright-light source.

Whenever gross lesions suggestive of TB were detected in any of the tissue, the tissue was classified as having lesions. Furthermore, the distribution, size, color and component of the lesions beside the breed, age and sex were recorded. In addition, a part of suspected TB lesions were fixed in buffered neutral formalin, dehydrated in graded ethanol and embedded in paraffin wax. Sections (5 µm) were stained with haematoxylin and eosin (H&E), and Ziehl-Neelsen (ZN) stain techniques for histopatholgical examination (Hewitson and Darby, 2010).

RESULTS

Prevalence of tuberculosis

The overall prevalence of tuberculosis in goats and sheep slaughtered at Nyala abattoirs during the study period were 3.72% (119/3200) and 2.85% (93/3260) respectively. Out of the infected animals, in goats 89.08% (106/119) were localized tuberculosis and 10.92% (13/119) were generalized tuberculosis, while 92.47% (86/93) were localized tuberculosis and 7.53% (7/93) were generalized tuberculosis in sheep (Table.1). Moreover, by breeding in goats 94.12% (112/119) were (*Baggara*) goats, 2.52% (3/119) were saanaen goats and 3.36% (4/119) were shamian goats, while in sheep, 68.82% (64/93) were (*Ambboror*) sheep and 31.18% (29/93) were (*Baggara*) sheep.

Distribution of lesions

In goats, localized cases showed that 78.30% (83/106) were in thoracic cavity and 21.70% (23/106) were in abdominal cavity, while 90.70% (78/86) were in thoracic cavity and 9.30% (8/86) were abdominal cavity in sheep. Moreover, in both carcasses of goats and sheep the lungs was the predominant organ showed tubercles (17.11%, 19.41%) respectively, followed by lung's associated lymph nodes (bronchial and mediastinal), retropharyngeal lymph nodes, liver, mesenteric and Portal lymph nodes (Table. 2). In addition, in most infected goats and sheep in thoracic cavity,

the majority of lesions were observed in right lobes.

Grossly in both small ruminants, the tubercles appearance as embedded in the parenchyma and sometimes bulging on the surface of the infected organs with thick fibrous capsule in white or pale yellowish colour. Furthermore, they were variable in sizes ranged between 0.1mm to 2 cm and containing caseous, caseo-calcified, calcified or sticky or gritty material (Fig. 1 and 4). Histopathologically in both small ruminants, most the sections showed central caseous necrosis with or without calcification surrounded by epithelioid cells and langhan's type giant cells with a fibrous capsule infiltrated by lymphocytes and plasma cells (Fig. 2). in addition, acid fast bacilli were seen in some sections in both small ruminants but in goat was more than sheep (Fig. 3).

DISCUSSION

In Sudan, especially in South Darfur, control of the disease through the test-and-slaughter policy has not been adopted yet due to the lack of knowledge on the actual prevalence of the disease, the absence of cattle identification and control of animal movements, and prevailing technical and financial limitations. The control of bovine tuberculosis is only based on the detection of gross lesions in abattoirs and subsequently partial or total condemnation of carcasses. Moreover, an abattoirs based study on bovine tuberculosis was conducted in cattle in different regions of Sudan. The status of TB in goats and sheep has not been studied although they have common watering and grazing points with cattle that might favor the transmission and spread of the disease among these small ruminants.

In the present study, the overall prevalence of tuberculosis were 3.72% and 2.85% among goats and sheep respectively at Nyala abattoir based on grossly detected of tubercle-like lesions. This finding was similar to the prevalence reported in previous studies by Benti *et al*, (2013) who conducted that, the prevalence of goats TB like lesions was 3.5%. Furthermore, in sheep was observed 1.4% prevalence of TB at 2 mm cut-off point (Cordes *et al*., 1981). Our prevalence in sheep was higher than the previous study, this might be due to the high spread of TB among different animal species in one region through pasture and watering points.

In this study, the distribution and development of tubercle-like lesions were almost exclusively observed in the thoracic cavity followed by abdominal cavity of the infected goats and sheep. This result was in agreement with the previous study showed that TB in goats and sheep as the same as in cattle, the caseous inflammatory lesions were found in the lung and associated lymph nodes and the lesions may also be found in the upper respiratory tract lymph nodes and other organs such as liver, spleen or mesenteric lymph nodes (Dean *et al*., 2005; Daniel *et al*., 2009; Naima *et al*., 2011). In addition, the lungs was the predominant organ (17.11%, 19.41%) followed by bronchial and mediastinal lymph nodes (16.93%, 19.41%) showed tubercles in goats and sheep respectively. This findings was consistent with the previous reports showed that 97% of the gross TB like lesions were observed in the lungs and associated lymph nodes (Benti *et al*., 2013). This which may indicate that, the small ruminants acquire the infection mainly via the respiratory route.

In the present study, the macroscopic lesions in goats and sheep appearance as embedded in the parenchyma and sometimes bulging on the surface of the infected organs with thick fibrous capsule in white or pale

yellowish colour and variable in sizes ranged between 0.1mm to 5 cm containing caseous, caseo-calcified, calcified or sticky or gritty material. This findings was in agreement with previous reports conducted that the tubercle lesions embedded in the parenchyma or bulging from the surface in nodules shape and white to yellowish-grey colour and consistency caseous or caseous gritty and sometimes calcified material (Cordes *et al*., 1981; Davidson *et al*., 1981; Radostits *et al*., 2007). Histologically, the most granulomatous lesions containing central caseous necrosis with or without calcification surrounded by epithelioid cells and langhan's type giant cells with fibrous capsule and infiltrated by lymphocytes and plasma cells. This results was similar to Davidson *et al*, (1918) how conducted that the granulomatous lesions showed central caseous area with calcification surrounded by epitheliod cells and langhan's giant cells with a fibrous capsule and infiltrated by lymphocytes and plasma cells. in addition, acid fast bacilli were seen in some sections but in goat was more than sheep in this study. This findings was disagree with Malone *et al*., (2003) who reported that the acid fast bacilli were very few in the sections and sometimes were found within the langhan's giant cells.

In conclusion, detection of TB among the slaughtered goats and sheep indicate the presence of TB in the Sudanese animals, while *Ambboror* sheep and *Baggara* goats were the reservoirs of TB among the livestock in the region and respiratory pathway was the most site of infection in goats and sheep. Therefore, proper implementations of meat inspection procedures at abattoir with public awareness are important to control TB in South Darfur State. In addition, large scale surveillance is needed in different parts of the Sudan to estimate the apparent and true prevalence of TB among animals and animal workers.

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Table.1: Prevalence of tuberculosis in slaughtered goats and sheep in Nyala locality, South Darfur State, Sudan during the period 2015 - 2017.

Type of animal	Slaughtered	Infected	Localized	Generalized	Percentage
Goats	3200	119	106 (89.08%)	13 (10.92%)	**3.72%**
Sheep	3260	93	86 (92.47%)	7 (7.53%)	**2.85%**

Table.2: Distribution of lesions in different organs of infected goats and sheep in Nyala locality, South Darfur State, Sudan during the period 2015 - 2017.

Organs	Goats	Sheep
Lungs	96 (17.11%)	**85 (19.41%)**
Liver	36 (6.41%)	**15 (3.42%)**
Spleen	30 (5.35%)	**12 (2.74%)**
Kidneys	3 (0.53%)	**3 (0.68%)**
Intestine	28 (4.99%)	**8 (1.83%)**
Rumen	13 (2.32%)	**8 (1.83%)**
Abomasums	13 (2.32%)	**9 (2.05%)**
Mesentery	9 (1.60%)	**11 (2.51%)**
Ribs	4 (0.71%)	**2 (0.46%)**
Pleura	8 (1.43%)	**7 (1.60%)**
Peritoneum	4 (0.71%)	**7 (1.60%)**
Retropharyngeal lymph node	83 (14.80%)	**77 (17.58%)**
Mediastinal lymph nodes	83 (14.80%)	**85 (19.41%)**
Bronchial lymph node	95 (16.93%)	**80 (18.26%)**
Mesenteric lymph node	36 (6.41%)	**14 (3.20%)**
Portal lymph node	20 (3.57%)	**15 (3.42%)**
Total	561(100%)	**438 (100%)**

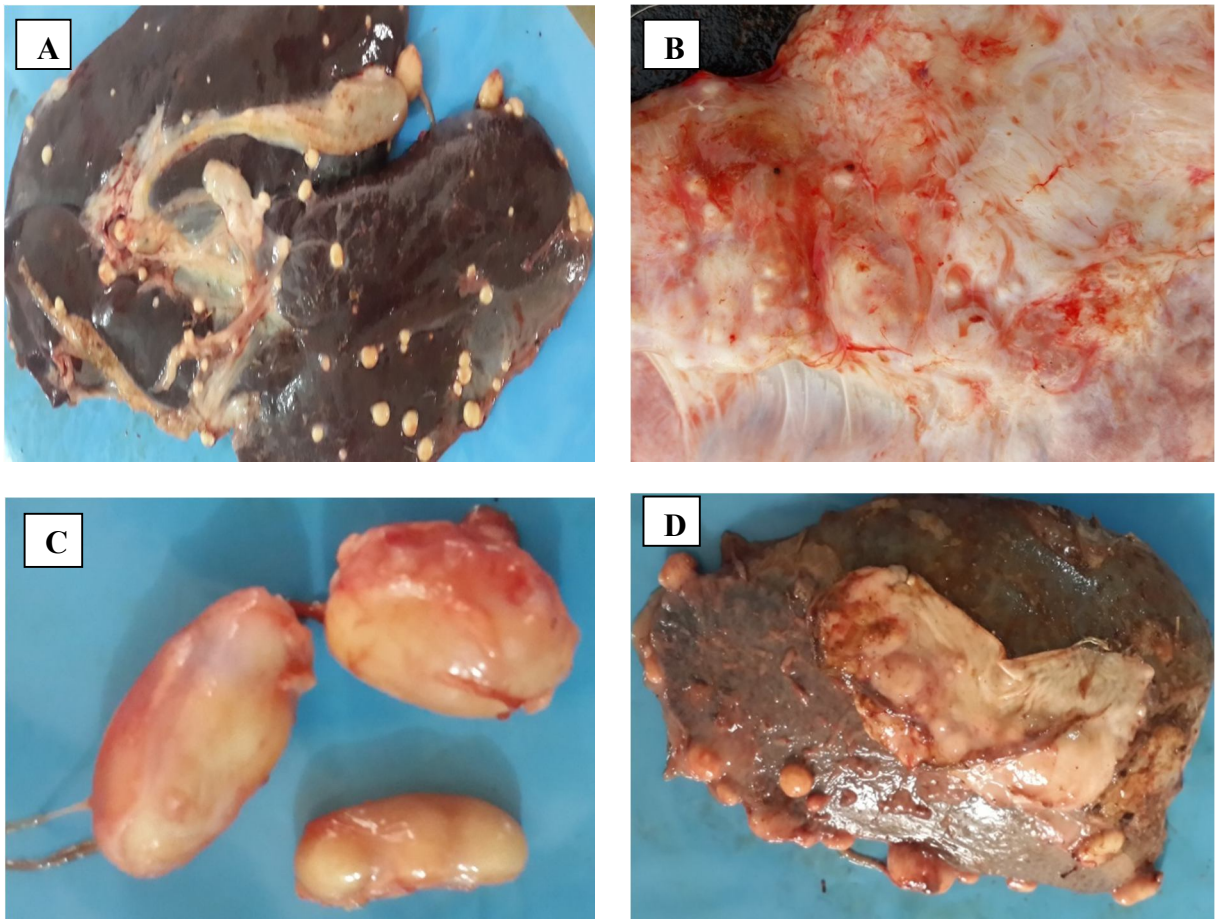

Figure.1: Liver (A), lung (B), lung's associated lymph nodes (C), spleen (D) of small ruminants showing white or pale yellowish nodules with thick fibrous capsule bulging on the surface of the organ and variable in sizes.

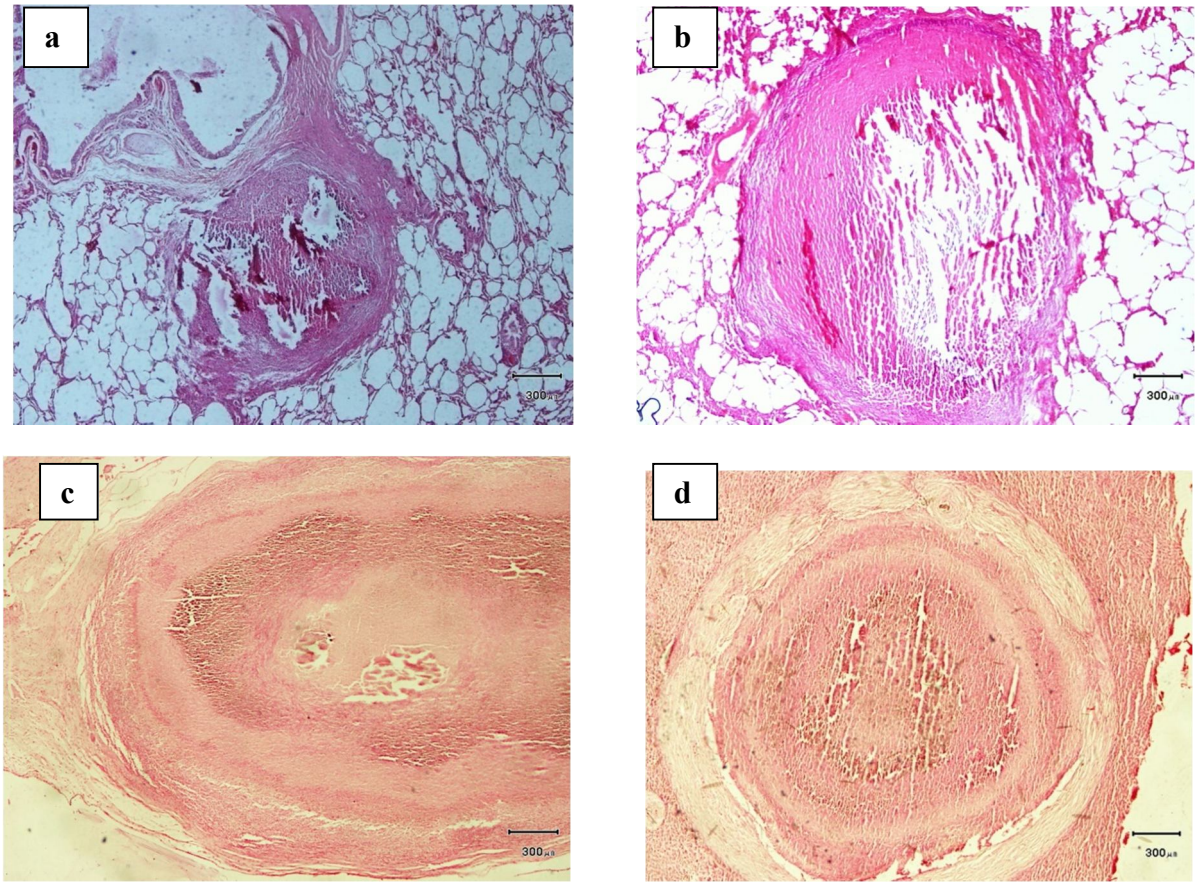

Figure.2: Histopathological sections of small ruminants, lungs (a) and (b), livers (c) and (d) showing central caseous necrosis with calcification surrounded by epithelioid cells and langhan's type giant cells with a fibrous capsule infiltrated by lymphocytes and plasma cells. H&Ex40.

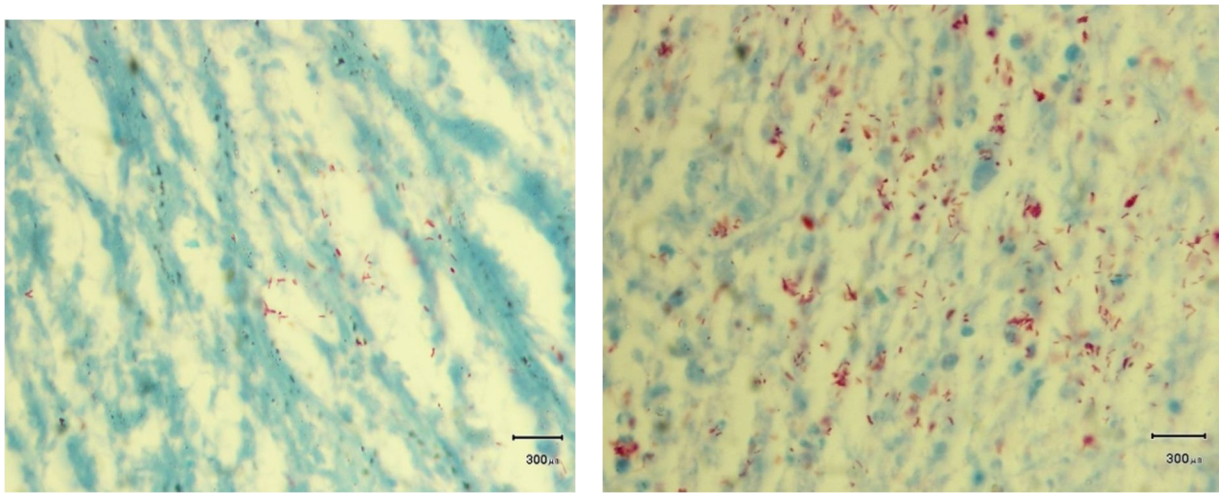

Figure.3: Histpathological section of small ruminant liver showing scattered acid fast bacilli in the granulomatous region. Ziehl-Neelsen's stain (100 x oil immersion objective).

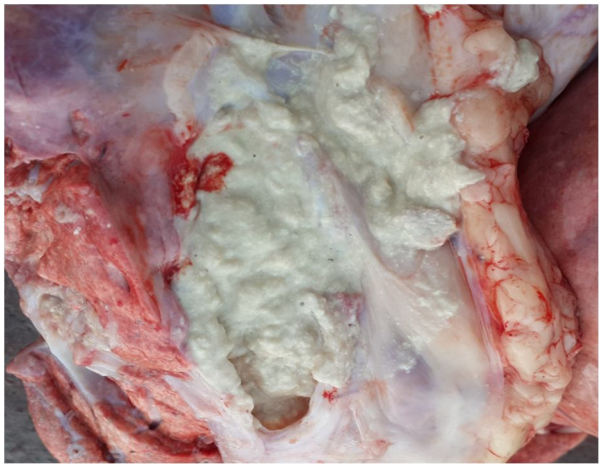

Fig. 4: lung of small ruminant showing granulomatous lesion containing caseous gritty material.