بسم الله الرحمن الرحيم

Sudan University of science and Technology

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Pathological Study of Bovine Tuberculosis in Sheep, Goats and Cattle Slaughtered at Gadarif Abattoir-Sudan

دراسة مرضية لمرض السل البقري في الاغنام والماعز والأبقار المذبوحة بمسلخ القضارف ـ السودان

A thesis submitted to the Sudan University of science and Technology in Fulfillment of the requirement for the Master degree of Veterinary Medicine in Pathology

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DEDICATION

This work is dedicated to my parents,

My brothers, sister and my friends

With great love

Aladeen

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First, I am grateful to God for giving me assistance, health, and patience to complete this work.

My gratitude to my supervisor Professor : Amel Omer Bakhiet College of Veterinary Medicine for her guidance, valuable advice, and unfailing help

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ABSTRACT

This study was carried out at Eastern Sudan Gadarif state to detect the pathological characteristics of Tuberculosis (TB) lesions in carcasses of small ruminants (sheep and goats) and Cattles. A total of 4560 small ruminant and 4010 Cattle were examined in ante-mortem (afternoon) and postmortem (morning) between June 2017 and December 2018. The animals were brought from different localities of Gadarif State. The general body condition score (BCS) of the animals were observed and looked apparently active and healthy. Postmortem inspection was performed based on the principle of meat inspection, visualization, palpation, and incision. 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 and the lymph nodes were inspected externally and palpated for the presence of visible lesions. Moreover, organs such as liver, spleen, kidneys, rumen, and small intestine were examined. Any suspected tuberculous lesions with yellowish appearance, caseous, purulent, caseo-calcareous or calcified in consistency were collected. The results revealed that overall prevalence of granulomatous lesions in small ruminants was 0.22% while 0% in the Cattles. The detected granulomatous lesions were typical caseous necrosis, with a whitish or yellowish color. They were of various sizes and enclosed in light grey fibrous tissue. The inspection in sheep and goats showed that 80% of the lesions were localized mainly in the abdominal cavity, thoracic cavity 20%, however, the predominant organs have tubercles in liver and lungs (55.5%, 16.7%) respectively, followed by mesenteric lymph node, mediastinal lymph node, intestine, kidney.

ملخص الدراسة

أجريت هذه الدراسة بولاية القضارف بشرق السودان للكشف عن الخصائص المرضية لآفات السل في المجترات الصغيرة (الأغنام والماعز) والابقار. تم فحص حوالي 4560 رأس الأغنام والماعزو 4010 من الابقار بأعمار مختلفة قبل الذبح (المساء) وبعد الذبح (الصباح) في الفترة بين يونيو 2017 وديسمبر للحيوانات والتي تبدو نشطة وصحية ثم تم إجراء الصفة 2018. لوحظت درجة حالة الجسم العامة التشريحية للحيوانات التي تم ذبحها في المسلخ حيث تم التركيز بشكل خاص أثناء فحص بعض الأعضاء والغدد الليمفاوية التي تم فحصها بعناية لوجود آفات السل المشتبه بها. تم فحص جميع فصوص الرئتين والعقد الليمفاوية خارجيًا وتحسسها بحثًا عن وجود آفات مرئية. كما تم فحص أعضاء مثل الكبد والطحال والكلى والكرش والأمعاء الدقيقة. تم جمع أي آفات سلية مشتبه بها ذات مظهر مصفر، جبني، قيحي، حالة الجيرية أو متكلسة. أظهرت النتائج أن معدل انتشار الآفات الحبيبية في جميع الذبائح التي تم فحصها كان من الابقار. كانت الآفات الحبيبية المكتشفة عبارة عن نخر في المجترات الصغيرة بينما 0% 0.22٪ تجبني، مع لون أبيض أو أصفر. كانت بأحجام مختلفة ومحاطة بنسيج ليفي رمادي فاتح. أظهر الفحص في الأغنام والماعز أن 80٪ من الآفات كانت موضعية بشكل رئيسي في التجويف البطني، التجويف الصدري 20٪ ، أما الأعضاءالتي توجد فيها درنات السل هي الكبد والرئتين (55.5٪ ، 16.7٪) على التوالي ، تليها العقد اللمفاوية المساريقية ، العقدة الليمفاوية المنصفية ، الأمعاء ، الكلى.

INTRODUCTION

Ant-mortem and post-mortem examination are significance method to prevent transmit of the illness and provide safe foods for humans. The responsibility for achieving this objective lies primarily on the relevant public health authorities who are represented by veterinarians at the abattoir stage. The results of the inspection of slaughtered animals and meat at slaughterhouse are indicator of health of food animals on farm level and at slaughterhouses (Adam, 2010).

As meat is the main source of protein to humans, it should be clean and free from diseases communicable to man such as tuberculosis. Meat is also convicted at slaughter to break the chain of some zoonoses which are not transmitted to man directly. Yet, meat is also condemned because of aesthetic values caused by disease during slaughtering procedures (Adam, 2010).

Tuberculosis is a bacterial chronic contagious disease that affects many species of animals (domestic animals and wildlife animals). This disease is a significant zoonotic disease that spreads among humans' population particularly in developing countries in the world (O'Reilly and Daborn, 1995; Aljameel *et al.,* 2017). The causative agent of tuberculosis is Mycobacteroum bovis Gram positive, acid-fast, often thin rods of varying length, non-motile, non-spore forming, aerobic and oxidative (Carter and Chengappa, 1991). It is usually characterized by the formation of nodular granuloma known as tubercles (Tageldin, *et al.,* 2007; Marianelli *et al.,* 2010). Any part from the body can be affected, but lesions are often observed in the lymph nodes (particularly of the head and thorax), lungs, intestines, liver, spleen, and peritoneum (OIE, 2009).

The global prevalence of tuberculosis in the human caused by M. bovis estimated to be 3.1% of all tuberculosis cases, show for 2.1% in the lungs and 9.4% the other organs in the body (Cosivi *et al.*, 1998). In Africa, the BTB is widespread and is affecting the animal industries and human health, posing serious public health threats (Cosivi *et al.,* 1998; Ayele *et al.,* 2004; Thoen *et al.,* 2010).

In Sudan, studies about tuberculosis are little. Aljameel *et al.,* (2014) reported that in South Darfur State necropsy was performed for 163 cattle. Forty (1.4%) of the animals were found infected, among which seven had generalized tuberculosis and 33 localized tuberculosis, mainly in the lungs, thoracic lymph nodes, liver, spleen, kidneys, and mesenteric lymph nodes.

Studies on bovine tuberculosis disease of slaughtered sheep, goats and cattle in the Gadarif State have not been studied comprehensively before, therefore, the aim of this study was to assess the prevalence of tuberculosis disease in cattle and small ruminants slaughtered in Gadarif city abattoir.

The objectives of the present study were to:

- Study prevalence of Tuberculosis in Gadarif State.
- Study the gross examination of pathological lesions in organs of cattle and small ruminants slaughtered at Gadarif abattoir.
- Study the distribution of tuberculosis lesions in the thorax and abdominal cavity.

CHAPTER ONE

LITERATURE REVIEW

1.1 Historical background

Tuberculosis is considered one of scientific, medical, social, and political failures since TB remains one of the leading causes of mortality and morbidity (Chakrabarti *et al*, 2007).

Barberis *et al.,* (2017) stated that Tuberculosis (TB) is a contagious, infectious disease, due to Mycobacterium tuberculosis (MT) that has always been a permanent challenge over the course of human history, because of its severe social implications. It has been hypothesized that the genus Mycobacterium originated more than 150 million years ago".

Tuberculosis disease infected animals and humans since 2000 DC year in the world which confirmed recently by the discovering in the Egyptian mummy where Hippocrates believed that phthisis as fatal hereditary widespread disease (Morse *et al*., 1964). In the 18-19 centaury incidence of high prevalence of Tuberculosis was in the Europe and America (Morse *et al.,* 1964; Salo *et al.,* 1994; Arriaza *et al.,* 1995). Carter and Chengappa, 1991; Davies, 2006) stated that in 1800's TB has been described in cattle in slaughterhouses. Davies, (2006) reported that in 1865 Villemin showed that infected tuberculous material could be injected from one species to another to cause disease. Koch, pointed out that in 1882; there was a danger that TB could be transmitted from animals to humans. Davies, (2006) recorded that in 1902 Ravenel demonstrated Mycobacterium bovis in a child with tuberculous meningitis. Yet it was not until 1929 that the danger of animal to human transmission of TB received Government debate Davies, (2006). One can hypothesize that the genus Mycobacterium originated more than 150 million years ago (Barberis *et al,*2017). Mycobacterium ulcerans has specific habitat requirements and a current geographic distribution that separates its endemic regions widely. Those

regions were last in contiguity as part of the Gondwanaland continental land mass during the Jurassic period.

Gutierrez *et al.,* (2005) reported that modern techniques of molecular genetics and the sequencing of the genome of several strains of M. tuberculosis allow a more rigorous estimation of the time of origin of mycobacteria. This estimation is facilitated by the low mutation rate of M. tuberculosis. Gutierrez and her colleagues concluded that an early progenitor of M. tuberculosis was present in East Africa as early as 3 million years ago, and they suggest that it may have infected early hominids at that time (Gutierrez *et al.,* 2005). It is likely, however, that all modern members of the M. tuberculosis complex, including not only M. tuberculosis but its African variants Mycobacterium africanum and Mycobacterium canettii as well as Mycobacterium bovis, had a common African ancestor about 35,000–15,000 years ago (Brosch *et al.,* 2002; Gutierrez *et al.,* 2005). Modern strains of M. tuberculosis appear to have originated from a common ancestor about 20,000–15,000 years ago (Sreevatsan *et al.,* 1997). Currently circulating strains fall into six major lineages, or clades, all of which are present in East Africa; their global distribution varies, however (Gagneux *et, al.,* 2006). Analysis based on the known mutation rate of M. tuberculosis indicates that much of the present diversity among these strains originated between 250 and 1000 years ago (Hirsh *et al.,* 2004).

1.2 Tuberculosis

Tuberculosis is an infectious and chronic (slowly progressing) disease distributed in the domestic animals and wild animals (O'Reilly and Daborn, 1995). Historically the link between animal and human tuberculosis (TB) has always been strong (Davies, 2006). The principal microorganism associated with human tuberculosis is M. tuberculosis. M. bovis is the causative agent of Tuberculosis in animals used for production of food and accounts for a relatively small proportion of human cases (Hadush, 2015). Infection with these microorganisms is chronic and the infected human host may remain entirely asymptomatic or may have mild to moderate illness that does not come to medical attention for long periods. In a proportion of human or animal hosts

infected with these microorganisms, the infection may ultimately progress to severe systemic illness. Pulmonary disease is the classical feature and ultimately the disease may progress to death of the host if untreated. The classical pathological feature of the disease in humans is the caseating granuloma. This is an organized aggregation of macrophages surrounding an area of caseous necrosis (Food Safety Authority of Ireland (FSAI, 2008).

1.3 Causative agent of tuberculosis

Mycobacterium are thin rods of varying length (0.2-0.6 by 1.0-10.0μm) and sometimes branching filamentous, non-motile, non-spore forming, aerobic and oxidative (Tauro *et al.,* 1996; Quinn *et al.,* 2004). M. tuberculosis is straight or slightly curved rod, where as M. bovis is usually straighter, stouter, and shorter (Gupte, 2006). All Mycobacteria are acid fast and share a characteristics cell wall, thicker than many other bacteria, which is hydrophobic, waxy, and rich in mycolic acid/mycolates (Palomino *et al.*, 2007).

1.4 Properties of Mycobacterium bovis

Mycobacterium bovis grow slowly in the media (16- to 20-hour generation time) and also highly resistant environmental conditions capable of surviving in cattle faeces for at least 5 months in winter, 4 months in autumn, 2 months in summer and in soil for up to 2 years (Maddock, 1936).

1.5 Pathogenesis and Immunology

Tubercle bacilli invade the animal body through respiratory, alimentary, genital, cutaneous and genital routes. The first two are being the most observed routes of infection resulting in pulmonary and extra-pulmonary form of the disease, respectively. After infection the bacteria may localize in tissue related to the route of infection and associated lymph nodes (Menzies and Neill, 2000). Mycobacterial infection triggers a Th1-induced cell mediated immune response (CMI) which leads to release of cytokines of such as tumor necrosis factor-α, Interleukin-12 (IL-12) and interferon gamma (IFN-γ). This pathway is essential

to activate macrophages (Orme and cooper, 1999). Depending on the balance of cytokines involved, three outcomes are possible: 1) macrophages kill and eliminate the bacteria, 2) the bacteria lie dormant (latency), 3) the bacteria cannot be contained by the immune system and the disease develops to active TB (Welsh *et al.,* 2005).

Containment of the bacteria results in the formation of nonvascular nodular granulomas known as "tubercles". Lesions show typically a centre of caseous with some degree of calcification surrounded by a cell wall of epitheloid cells, lymphocytes, and neutrophils (Doherty *et al.,* 1996). Unlike in man, these primary lesions are rarely contained by the immune system in cattle and bacilli spread by lymphatic and hematogenous routes, resulting in tubercles in other organs (Neill *et al.,* 1994).

The initial CMI response is followed later in time by a humoral antibody response, which is caused by a shift of Th1 to Th2 cell activation (Dlugovitzky *et al.,* 2000). A state of energy may occur in advanced stage of the disease and a CMI response is no more detected. Initial pathological changes are associated with the onset of CMI response (Cosivi *et al.,* 1998). CMI response can be affected by the animal's nutritional state (e.g., deficiency in energy, protein and 12 micro nutrients), by stress or concurrent diseases, which lead to a reduction of the host resistance (Pollock and Neill, 2002).

1.6 Incubation period

The symptoms of bovine Tuberculosis take many months to develop in cattle. Infections can also remain recumbent and reactivate during periods of stress or in old age experimentally infected by the parenteral route, the incubation period is approximately three weeks; it is probably longer under natural conditions(corner,2001).

1.7 Descriptive epidemiology

1.7.1 Distribution of bovine tuberculosis in the world

In the world there are several studies survey about Tuberculosis in the cattles slaughtered at abattoirs (Tschopp *et al.,* 2009) reported that many areas in Ethiopia have bovine tuberculosis ranking between (1.2%) to (7.9%). (Enríquez-Cruz *et al.,* 2010) state that in the State of Tamaulipas, Mexico, bordering the United States of America. From January 1995 to March 1998, 599 carried out study to analyzed for the presence of Mycobacterium bovis which observe 15.36%, which was considerably higher in 1995 41.38% in comparison with 8.09%, 14.42%, and 10.14% for the years 1996, 1997, and 1998, respectively. in Kombolcha abattoir in northeastern abattoir At least one TB lesion was observed in 57 (5 per cent) of the cattle, of which 27 (47 per cent) yielded mycobacteria isolates (Ameni *et al.*,2010). (Youssef and Ahmed, 2014) mentioned that in Ismailia detected bovine tuberculosis lesions during meat inspection from cattle (0.7%) and buffalo (0.5%) for ten year (1996–2005). Of 55 African countries, 25 reported sporadic/low occurrence of bovine TB. Six reported enzootic disease, 2, Malawi and Mali, were described as having a high occurrence, 4 did not report the disease; and the remaining 18 countries did not have data. Ghana was among the countries that reported tuberculosis as enzootic disease. Of all nations in Africa, only 7 apply disease control measures as part of a test-and-slaughter policy and consider bovine TB a notifiable disease. The remaining 48 countries control the disease inadequately or not at all. Ghana does not control the disease at all. Only 15% of the cattle population is found in countries where bovine TB is notifiable, and a test-and-slaughter policy is used. Thus, approximately 85% of the cattle and 82% of the human population of Africa are in areas where bovine TB is either partly controlled or not controlled at all (Cosivi *et al*.,1998). In the Asia 16 countries reported have low occurrence of bovine TB, and one (Bahrain) described the disease as enzootic; ten did not report bovine TB; and the remaining nine did nothave data. Within the Asian region, seven countries apply disease control measures as part of a test-and-slaughter policy and consider bovine TB notifiable. In the remaining 29 countries, bovine TB is partly controlled or not controlled at all (Cosivi *et al*., 1998). In Latin American

and Caribbean Countries of 34 Latin American and Caribbean countries, 12 reported bovine TB as sporadic/low occurrence, seven reported it as enzootic, and one (Dominican Republic) described occurrence as (Cosivi *et al.*,1998). In Nigeria a total of 52, 262 cattle were slaughtered from 2007-2012, out of which 4, 658 (11.2%) had evidence of tuberculosis lesion at postmortem. The average yearly prevalence was 9.1% but varied from a high of 16.3% in 2007 to a low of 3.1% in 2012. Trend analysis showed that bovine tuberculosis had a seasonal variation and peaked mostly in July and August. The number of suggestive Tb lesion cases was highest in the month of August and lowest in the month of January, 2007-2012 (Okeke *et al.*, 2016). Pritchard et al., (1975) reported that in 281 cattle slaughtered in the Uganda 27% has lesions tuberculosis. In Cameroon were assessed. Slaughter inspection records from major cities revealed that BTB detection rates in cattle from 0.18% to 4.25% and BTB lesions were most common. Analyses of tissues and sera confirmed BTB in 31% (Ziehl-Neelsen), 51% (culture), and 60% (antibody detection) of test cattle. Among cattle handlers, 81.9% were aware of BTB, 67.9% knew that BTB is zoonotic, and 53.8% knew one mode of transmission but over 27% consumed raw meat and/or drank unpasteurized milk (Awah *et al.*, 2010).

1.8 Zoonotic importance of bovine tuberculosis

Approximately 85 per cent of cattle and 82 per cent of human populations in Africa live in areas where BTB is either partly controlled or not controlled at all (Cosivis *et al.,* 1998).The current increasing incidence of tuberculosis in humans, particularly in immune compromised humans, has given a renewed interest in the zoonotic importance of M. bovis, especially in developing countries and the ease and frequency of the spread of tuberculosis from animals to humans in an unhealthy environment makes this important zoonosis (OIE, 2009).

M. bovis can be responsible for 10 to 15% of human tuberculosis with higher rates in children in some areas. Infection in humans occurs largely through consumption of infected raw milk and raw milk products by children but spread can also occur by inhalation. Transmission to humans can be significantly

reduced by pasteurization of milk but only complete eradication of the disease can protect the farmer and his family Transmission from cattle to humans in developed countries is an unlikely event now a days but still occurs and resurgence of the disease in association with wildlife reservoirs has resulted in a spillover into human populations. The widespread occurrence of tuberculosis in exotic animals maintained in captivity adds to the public health importance of these infections (Radostits *et al.,* 2006).

1.8.1 Distribution of bovine tuberculosis in the Sudan

In Sudan, studies conducted in the 1960's and 1970's indicated that the incidence was high in the humid southern part of the country where the animals are in close contact, and low in the dry zones where the nomadic cattle of Western provinces, the seminomadic cattle of Eastern. (Sulieman and Hamid, 2002) found that 64 (53.3%) of 120 caseous lesions from cattle in Eastern and Central Sudan were due to acid-fast bacilli (AFB), whereas 56 (46.7%) were due to other causes. Growth on Lowenstein-Jensen slants was obtained in 52 of the 120 samples and identified as follows: 25 (48.1%) Mycobacterium bovis, 21 (40.4%) M. farcinogenes, 4 (7.7%) M. tuberculosis, 1 (1. 9%) M. avium, and 1 (1.9%) Nocardia sp.(Aljameel *et al.,* 2014: aljamel et al., 2017) reported that in South Darfur State perform necropsy for cattle's goats and sheeps were found 40 (1.4%), 119 (3.72%) and 93 (2.85%) respectively animals have tuberculosis lesion and also in the Kassala State which tested 569 animals (140 local breeds and 429 cross breed) by using comparative cervical tuberculin test (CCTT), Rapid test, Indirect ELISA and PCR. The results showed A prevalence of tuberculosis in Kassala 60% (Ayman *et al.,* 2014). In the North Upper Nile State confirmed the presence of M. bovis in 26 (65%) of milk samples and 12 (40%) of lymph node samples (Ishaga *et al.,* 2015). Shuaib *et al.,* (2018) stated that only 0.1% (n = 2) of the carcasses had suspicious TB lesions. These lesions were solely found in carcasses of sheep, in the liver, lungs, and peritoneal cavity.

1.9. Transmission

Disease spreads through contact with infected animals across animals' products such as milks and meats. The infected animals release the organisms through sputum at coughing and contact animals get infection through droplet inhalation. It is also transmitted through contaminated instruments, utensils, and beddings (OIE, 2009).

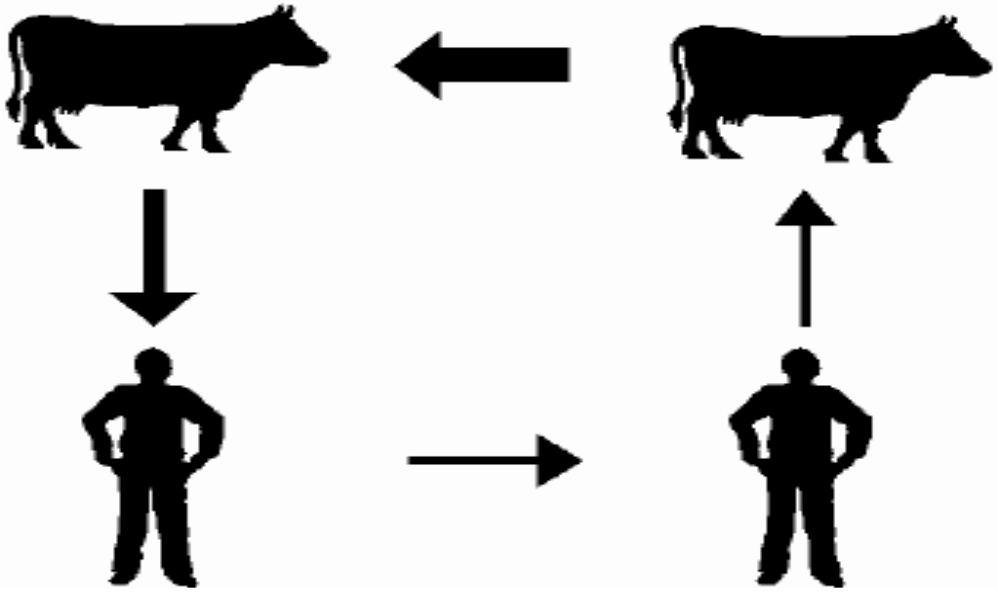

Fig.1. Cycle of M. bovis transmission between cattle and humans the thickness of arrows suggests level of probability. Source: Anaelom *et al.* (2010).

Jackson, (1995) mentioned that transmission of tuberculosis between possums occurs through two major and one minor pathway.

The first major pathway is pseudo-vertical transmission from mother to joey during the rearing process. The second major transmission mechanism is direct horizontal transmission among adult possums with available evidence suggesting that this takes place around the locality where a possum dens, probably during competition and threat/agonistic behaviour and during courting and mating activity. The third and probably least important pathway is indirect transmission among mature possums.

1.10 Diagnosis

1.10.1 Symptoms

Tuberculosis is usually a chronic debilitating disease in cattle, but it can occasionally be acute and rapidly progressive. common symptoms include progressive emaciation, a low–grade fluctuating fever, weakness and inappetence. Animals with pulmonary involvement usually have a moist cough that is worse in the morning, during cold weather or exercise, and may have dyspnea or tachypnea (Ramos and Dellagostin, 2015).

In the terminal stages, animals may become extremely emaciated and develop acute respiratory distress. In some animals, the retropharyngeal or other lymph nodes enlarge and may rupture and drain. Greatly enlarged lymph nodes can also obstruct blood vessels, airways, or the digestive tract. If the digestive tract is involved, intermittent diarrhea and constipation may be seen. In cervids, bovine tuberculosis may be a subacute or chronic disease, and the rate of progression is variable from animal to another. In some animals, the only symptom may be abscesses of unknown origin in isolated lymph nodes, and symptoms may not develop for several years (Ramos and Dellagostin, 2015).

1.10.2 Examination of gross Lesions

Bovine tuberculosis is characterized by the formation of granulomas (tubercles) where (MB) is found. Usually, the color of lesions tuberculosis become yellowish and either caseous and calcified often surround by encapsulated also tubercles found in many organs of the body such as lung, spleen, lymph nodes, liver, and the surfaces of body cavities (de Kantor and Ritacco, 2006). Wangoo *et al.,* (2005) reported that granulamatous lesion classified into many forms such as Type I granulomas consist of clusters of epithelioid macrophages with multinucleated Langerhans-type cells and a thin rim of lymphocytes, and no necrosis. In type II granulomas, epithelioid macrophages, multinucleated Langerhans-type cells, and lymphocytes are more numerous, and caseous necrosis starts to develop in the centers of the tubercles. In type III granulomas, caseous necrosis is well developed, but mineralization is minimal. Type IV granulomas are matured and have caseous necrosis with mineralization and development of fibrous encapsulations. Determining the type of granuloma helps in identification of the stage of disease and differentiating normal granulomas from those induced by vaccination and immunosuppressant (Wangoo *et al.,* 2005).

The suspected bovine TB lesions from the non-tuberculous granulomas caused by actinomycosis (lumpy jaw), actinobacillosis (‘wooden tongue’), paratuberculosis (Johne’s disease), neoplasms and other lesions based on visual inspection of a carcass (Frankena *et al.,* 2007).

1.10.3 Examination of histopathological lesions

These types of diagnosis made by light microscopic demonstration of acid-fast bacilli, as a complementary form of postmortem lesions diagnostic presumptive BTB. It is simple direct methods for diagnosis bacterium from sputum samples in humans and from tuberculosis organ lesions (generally in animals). The presence of mycobacteria in a given sample can be assessed by Ziehl-Neelsen staining followed by light microscopy (Marais *et al.,* 2008). These techniques are based on the properties common in mycobacteria and microorganisms of the

genus Nocardia, Rhodococcus and Corynebacterium, known as acid resistant bacilli. That is named because they can retain the fuchsine-heated material after treatment for alcohol-acid. In this type of coloring, alcohol acid resistant microorganisms can be observed under the microscope (Marais *et al.*, 2008).

1.10.4 Tuberculin testing of animals

Generally, in the field is most test used in diagnosis of tuberculosis in live animals and humans. In this test, tuberculin is injected intradermally; a positive test is indicated by a delayed hypersensitivity reaction (swelling). The tuberculin test can be performed using bovine tuberculin alone, or as a comparative test that distinguishes reactions to M. bovis from reactions to environmental mycobacterium (De la Rua-Domenech *et al*,2006).

1.10.5 Selective culture media

Culture of mycobacterium in the special media more commonly known as LJ medium Herrold's medium, Middlebrook 7H10 and 7H11 or Coletsos, with 1% sodium pyruvate added is used for the diagnosis of Mycobacterial infections. When grown on LJ medium, M. tuberculosis appears as brown, granular colonies (sometimes called "buff, rough and tough"). The medium must be incubated for a significant length of time, usually four weeks, due to the slow doubling time of M. tuberculosis (15–20 hours) compared with other bacteria. The identity of the organism can be confirmed with biochemical tests and culture characteristics (Cole, 2002; Kubica *et al.*, 2006).

1.11 Treatment, Control and Prevention

Several countries in the world have programme for remove of bovine tuberculosis usually component from two main line 'test and slaughter' test of live animals using the tuberculin skin test and carcass inspection in abattoirs for detection of bovine TB lesions Furthermore, the suspected lesions are submitted for laboratory confirmatory diagnosis of TB by microscopy, histology, and culture (Amanfu, 2006) The specific legistilation in each country are largely

dependent on the prevalence of the disease, the socioeconomic capacities of the country and the epidemiological scenario such as in the USA bovine TB eradication programs started in 1917 and are a joint venture of U.S. Department of Agriculture (USDA) Animal and Plant Inspection Services (APHIS), Food Safety and Inspection Services (FSIS), state health agencies and livestock producers (Kaneene *et al.*, 2006). Currently, slaughter surveillance is one of the important components to control bovine TB in USA. The program was first applied in the 1960s, when the prevalence of the bovine TB was low. The current bovine TB control program focuses on slaughter surveillance along with trace back investigations of infected herds (Humphrey *et al.,* 2014). In developing countries like Cameroon, abattoirs are controlled by government. Usually, they detect a more advanced stage of bovine TB infection. Previous prevalence studies identified that bovine TB cases were as low as 1% in Cameroon. The same studies, however, also suggest as high as 51% of the inspected lesions had the mycobacterial infections with detection of acid-fast bacilli (Egbe *et al.*, 2016). Similarly, in Ethiopia, a study conducted by Asseged *et al.*, (2004) revealed that 1.5% of cattle in Addis Abba had TB lesions.

1.12 Meat inspection:

The purpose of meat inspection to prevent spreading of zoonosis disease from the animals to human during nutritional chain and produce high quality food for consumers (Mitchel, 1980; Gracey, 1986).

1.12. 1 Procedure:

The routine meat inspection procedure divides the slaughtered animals into four parts i.e., the carcass, the pluck, the viscera, and the head. In addition to visual examination, post-mortem inspection involves palpation of tissues and organs laboratory tests (Adam, 2010).

1.13 Diagnosis during Postmortem examination

1.13.1 Detection of tuberculous lesions

post-mortem examination and culture are effective procedures for the diagnosis of bovine tuberculosis (Corner, 1994). However, postmortem meat inspection surveillance for detection of tuberculous lesions depends on the work load, time and diligence of the meat inspector (Comer *el al.,* 1990). The sensitivity of postmortem procedures to detect bovine tuberculosis is also affected by the presence of non-tuberculous parasitic granulomas, bacterial or mycotic pyogranulomas and bacterial abscesses which may be indistinguishable macroscopically from tuberculous granulomas (Liébana *el al.,* 2008; OIE, 2009). However, careful examination of as few as 6 pairs of lymph nodes, the lungs and the mesenteric lymph nodes can result in 95% of cattle with macroscopic lesions being identified (Corner, 1994). Lesions suspected to be tuberculous at necropsy should be submitted to the laboratory for bacteriological and histological examination (Corner, 1994).

CHAPTER TWO

MATERALS AND METHODS

2.1. Gadarif city abattoir

This abattoir located in the Gadarif State East Sudan established 30 years ago, except Wednesday each day, on average 340 head of cattle plus considerable numbers of goats, sheep are slaughtered. The abattoir has adequate electric light and water supplies as well as facilities to dispose condemned carcasses.

2.2. Study area

The study was conducted from June 2017 to Dec 2018 at the Gadarif State. This State was selected as it is one of the animal production sites in Sudan and exporting the animals located 410 km away from Khartoum located in the east direction, 12° 17' N and 34° 36' E A total population for this State of 363,945of men and women, livestock are main components as main factors for the livelihood of the community to undertake agricultural activities and as source of income. The cattle sheep and goat livestock population in the Gadarif State 5000000 head.

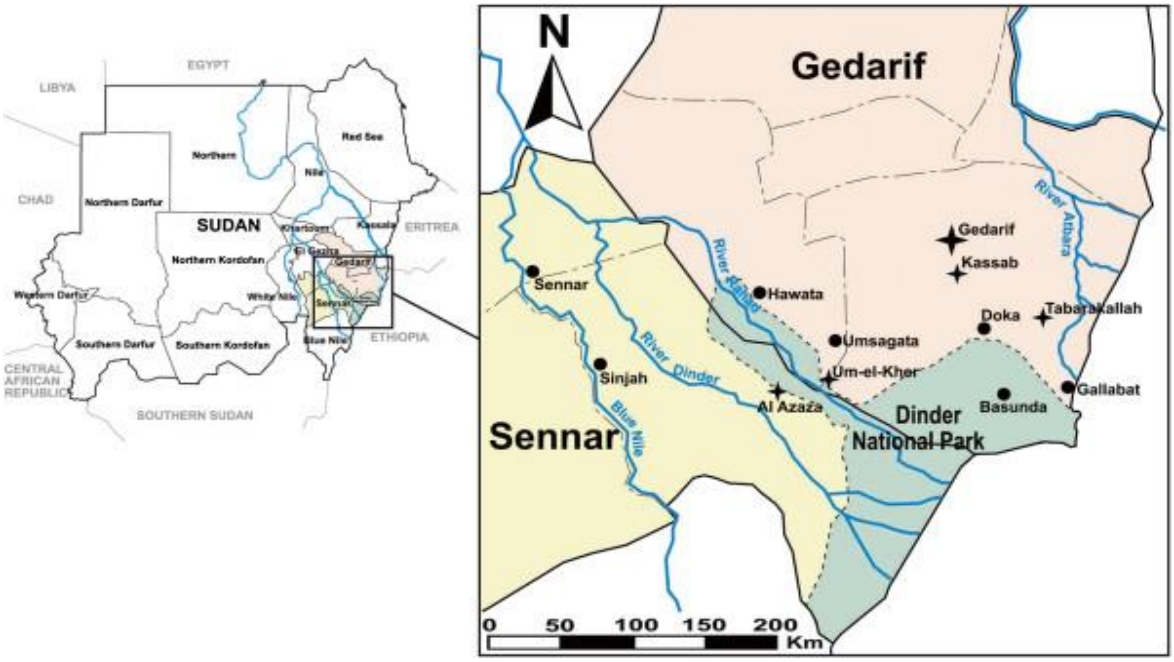

Fig.2. Map of the Sudan showing the selected District (Study site). Source: https://www.researchgate.net/.../Map-of-Gedarif-and-Sennar-States-Sudan

2.3 Animals under study

2.3.1. Ante mortem examination

In the afternoon, the animals were examined by eye in the abattoir of Gadarif State before 12 hours from sacrifice. Around (4010) cattle and (4560) sheep were tested between June 2017 and December 2018.The animals were brought from Sennar State and from the Gadarif State. The general body condition score (BCS) of the animals were observed and looked apparently active and healthy.

2.3.2 Post-mortem examination

Postmortem inspection was examined as described by Corner *et al.* (1990) and Corner (1994). Also was performed based on the principle of meat inspection – Visualization, Palpation, and Incision (Ameni *et al.,* 2007; Aljameel *et al.,* 2017). 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 (Aljameel *et al.*, 2017).

In goats the seven lobes of the two lungs, (left apical, left cardiac, left diaphragmatic, right apical, right cardiac, right diaphragmatic, and right accessory lobes) were inspected, palpated and incised. The carcass including internal organs such as liver, kidneys, mammary gland, intestines, and lymph nodes were inspected and palpated. Lymph nodes were not incised for fear of contaminating the carcasses and the environment. The cut surfaces of the organs were thoroughly examined for the presence of abscesses, cheesy masses and

tubercles as described by Corner (1994). Where gross lesions suggestive of tuberculosis were found in any of the tissues, the animal was classified as having tuberculosis-like lesions. Whenever gross lesions suggestive of TB were detected in any of the tissue, the tissue was classified as having lesions.

Any suspected tuberculous lesions with yellowish appearance, caseous, purulent, caseo-calcareous or calcified in consistency were collected. Type of organ or tissue in which the lesion was located was recorded as well as the nature of the gross pathological lesion. Observation of localized tuberculous lesion in the various parts of the carcass led to partial condemnation of affected parts while generalized infection led to total condemnation.

2.4. Sample collection, transport, and storage

Samples of affected lymph nodes and other tissues were collected individually into sterile plastic bags, labelled, secured to prevent any leakage, and placed into a cooler-box containing ice packs for transportation to the laboratory. In the laboratory fat and connective tissue were trimmed off using sterile surgical blade for each sample. All specimens were split into two parts depending on the size and consistency. One portion was placed in 100ml plastic containers and fixed in 10% buffered formalin for use in histological examination. The other portion was placed in sterile bijoux bottles for use in microbiological analysis. Specimens for microbiological analysis were preserved at -20°C.

2.5. Acid fast staining

This test was carried out in the laboratory of the College of Veterinary Science, University of Gadarif by using (TB-STAIN HOT KIT) contains TB Carbol Fuchsin reagent, TB Decolorier, Methylene Blue Loeffler reagent.

2.6. Prepare the sample for staining.

Impression smears in the clean glass slide from the suspect lesions were collected then two drops from saline solution were added and fixed by heat.

2.7. Sample staining procedure

1.	Cover the samples completely with the carbol fuchsin reagent and heated carefully until evaporation	5 min
2.	Rinse with tap water until the dye destains	
3.	Cover the samples using TB Decolorizer	30 second
4.	Rinse with tap water	
5.	Stain the sample using Methylene Blue Loeffler reagent.	30 second
6.	Rinse with tap water thoroughly	
7.	Dry the section	

2.8. Diagnosis

All the collected samples were examined under microscope (100 x oil immersion objective).

CHAPTER FOUR

RESULTS

4.1 Cattle

Four thousand and ten (4010) cattle were examined and tested for bovine tuberculosis using the anti-mortem and post-mortem examination.The animals were mostly of indigenous breeds, e.g. Amprro Gaash cattle; crossbreed cattle. All cattle were apparently health, and no clinical signs or symptoms were detected during ante mortem. At postmortem examination all cattle had no lesions consistent with bovine tuberculosis in Gadarif abattoir.

4.2 Small ruminants (sheep and goats)

In total 4560 small ruminants were slaughtered in Gadarif abattoir during the study period. The animals were mostly of indigenous breeds, e.g., Dubassy and Gaash sheep and Baladi, Garrage and Nubi goats. The overall prevalence of granulomatous lesions in all inspected carcasses was 0.22% (Table 2). The detected granulomatous lesions were typical caseous necrosis, with a whitish or yellowish color. They were of various sizes and enclosed in light grey fibrous tissue.

Distribution of lesions

The inspection in sheep and goats showed that 80% of the lesions were localized mainly in the abdominal cavity, thoracic cavity 20%, however, the predominant organs have tubercle is liver and lymph node (55.5%, 16.7%) respectively, followed by Mesenteric lymph node, mediastinal lymph node, intestine, kidney (Table 1).

Gross appearance showed lesion various sizes ranged between 0.3 mm to 4 cm capsulated by fibrous tissue and contain white, pale, yellowish cheesy calcified material in liver. Thick fibrous encapsulated yellowish central sticky mucoid pus

was seen in the liver (Fig 4). Also lesions of tuberculosis on the surface of liver of a sheep were observed (Fig. 5). Fig. (6) Show granulomatous lesions in the lungs (white color) in the sheep Fig. (7) Shows mesenteric lymphnode with yellowish caseous material in the sheep and Fig. (8) shows enlargement mesenteric lymph node with yellowish caseous material in a sheep.

Table (1) Distribution of TB lesions in organs of sheep and goats slaughtered in abattoir during the period 2017 – 2018.

Organ/tissue	No. TB –suspected lesions (%)
Lung	1 (6.25)
Mesenteric lymph node	2 (12.5)
Mediastinal lymph node	1 (6.25)
Liver	10 (62.5)
Intestine	1 (6.25)
Kidney	1 (6.25)
Total	16

Table (2) Prevalence of tuberculosis in slaughtered sheep and goats in Gadarif abattoir during the period 2017 – 2018

Animals	Slaughtered	Infected	Localized Lesions	Generalized
Sheep and goats	4560	10	8	2
		0.17	0.04	0.22%

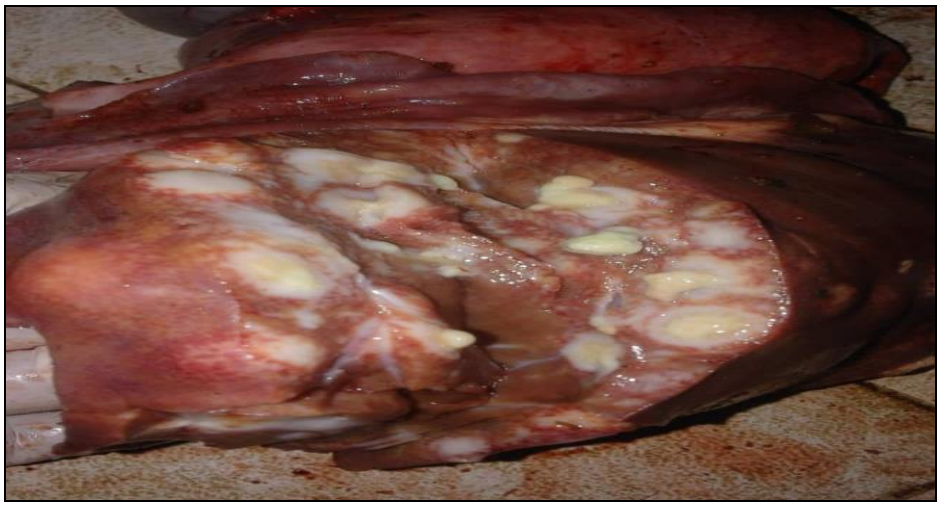

Fig. 3. Bovine tuberculosis in sheep thick fibrous encapsulated yellowish central sticky mucoid pus in the liver

Fig.4. Lesion of tuberculosis in surface of liver in the sheep

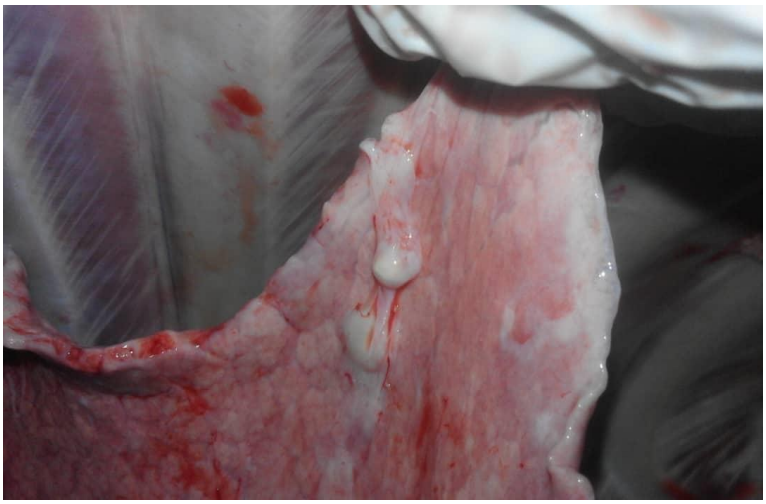

Fig. 5. Granulomatous lesions in the lungs (white color) in the sheep

Fig. 6. Embedded yellow lesions of tuberculosis in the small ruminants.

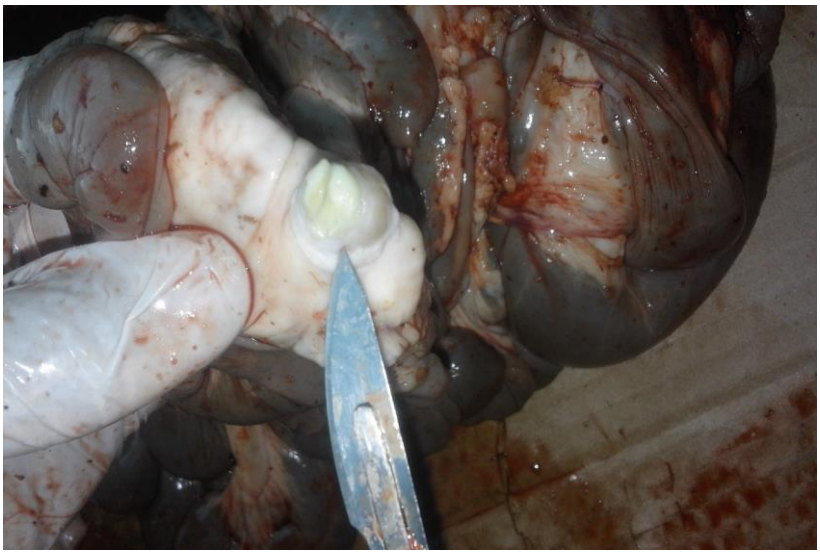

Fig. 7. Mesenteric lymph node with yellowish caseous material in the sheep

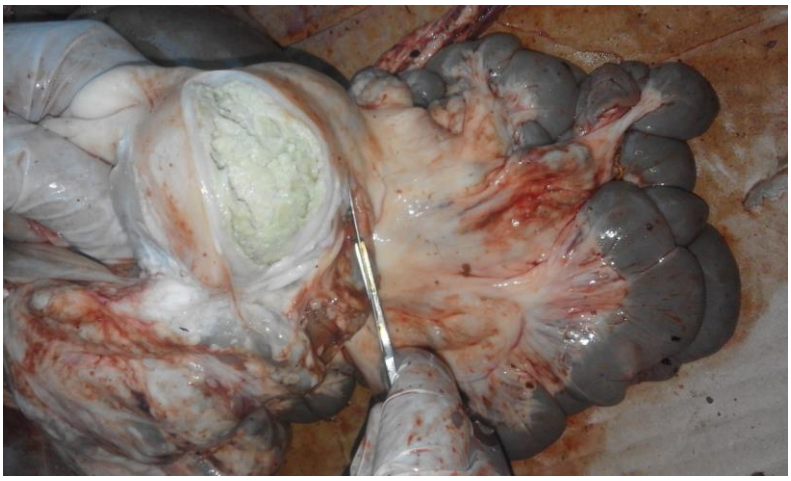

Fig.8. Enlargement mesenteric lymph node with yellowish caseous material in the sheep

CHAPTER FIVE

DISCUSSION

The present study showed low incidence of TB in the sheep and goats, and individual cases are usually detected during routine postmortem inspection at the slaughterhouse (Boukary *et al.,* 2012; Marianelli *et al.,* 2010). Small ruminant's carcasses typically undergo a less detailed post mortem examination than that performed in cattle, and this could explain the fewer reports of TB infection (van der Burgt *et al.,* 2010).

Boukary *et al.,* (2012); Kassa *et al.,* (2012); Ereqat *et al.,* (2013) reported that the practices of farming and behaviour of small ruminants and the close physical contact of pastoralists with their animals may represent a potential risk factor for transmission of M. tuberculosis complex members from animals to human or vice versa.

The infection has been described with TB lesions were mostly confined to the respiratory tract, demonstrating that the transmission of the disease in sheep can occur through aerosols. The TB lesions affected the gastrointestinal tract and the extent and the severity of the lesions observed in the lungs likely suggest that sheep were shedding high amounts of mycobacteria through nasal discharge and hence were able to transmit the disease. Nevertheless, cases of generalization of the infection have been also reported (Marianelli *et al.,* 2010). The pathological findings in small ruminants infected with TB suggest that sheep and goats could act as a reservoir of infection. The presence of lesions in the respiratory tract, suggest that they have the potential to act as domestic reservoir for TB (Bezos *et al.,* 2012; Napp *et al.,* 2013; Zanardi *et al.,* 2013).

Postmortem examination of small ruminants infected with M. bovis frequently reveals circumscribed pale yellow, white, caseous or caseocalcareus lesions of various sizes, often encapsulated, especially in the lungs and mediastinal lymph nodes, or in the mesenteric lymph nodes. Similar gross lesions have been

described in goats infected with M. caprae (Alvarez *et al.*, 2008; Bezos *et al.*, 2010).

In some European countries, including Greece, Italy, Spain, and Portugal, which have high small ruminants census figures and are not officially TB free (OTF), there may be a risk of spread of TB between cattle and small ruminants, especially when animals share pastures (EFSA, 2009). Surveillance of TB in goats in non-OTF countries is therefore important, and given its zoonotic potential, goats used for raw milk production living in mixed cattle-goat herds must be tested for TB (Regulation (EC) 853/2004). However, most non-OTF countries lack an active ante-mortem TB surveillance programme in caprine flocks that are not in close contact with cattle.

TB cases are therefore usually detected in the postmortem examination at the slaughterhouse, though TB in small ruminants is more rarely detected at the abattoir due to a lower quality of the meat inspection than that commonly carried out in cattle.

Gross lesions observed in sheep were similar to those described earlier in the same species by other researchers (Sharp 2000; Neill *et al.*, 2005). There appears to be agreement that lesions are mostly caseous and well encapsulated. In the present study, three of the infected sheep showed extensive and multiple lesions (in both thoracic and abdominal cavities), making the determination of the route of entry difficult to ascertain. It was indicated that the route of transmission of M. bovis within the same species or between different species can be deduced by the pattern of lesions observed in slaughtered animals (Pollock and Neill, 2002). Animals with lesions restricted to the thoracic cavity are presumed to have been infected by the inhalation of aerosols, while those with lesions restricted to mesenteric lymph nodes are thought to have acquired the infection by ingestion (Deresa *et al.*, 2013).

CONCLUSION

The present study concluded that TB lesions were detected among slaughtered goats and sheep in Gadarif State eastern Sudan. Meat inspection procedures at abattoir is good tool to detect the prevalence of diseases. Finally, a continuous monitoring process is required to maximize the effectiveness of bovine TB lesions detection during slaughter surveillance.

Recommendations

Further research on the importance of bovine tuberculosis in Sudan need be carried out through a jointly planned veterinary and medical programme. Also, the Veterinary and Medical institutions in Sudan and in the neighbouring countries should collaborate and design a feasible bovine tuberculosis control programme to reduce zoonotic threat of the disease regionally since slaughter animals move freely across the porous international border.

CHAPTER FIVE

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