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A Pathological Study of Lesions in Sheep Liver in Nasr Aldeen Slaughterhouse Khartoum-North

دراسة مرضية لآفات كبد الضآن في مسلخ نصر الدين الخرطوم بحري

By:

Ahmed Alsari Alsondi Hassanin

Areej Abass Elshiekh

Azkar Ibrahim Altayb

Islam Imam Mohammed

Maali Alamin Mohammed

Omar Faraj Jomaa

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Supervisor:

Professor Dr. Amel Omer Bakhiet Department of Pathology Parasitology and Microbiology

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DEDICATION

We humbly dedicate this study to

OUR PARENTS

for their love, patience, encouragement, untiring support.....

for their prayers day and night that made us able to get success

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- In the name of Allah the most merciful and beneficent first and foremost praise is to **ALLAH**, the Almighty, the greatest of all, on whom ultimately we depend for sustenance and guidance. I would like to thank Almighty Allah for giving us opportunity, determination and strength to do this research.
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ABSTRACT

A retrospective study and active slaughterhouse survey were conducted in Nasr Aldeen Slaughterhouse at Alhaj Yousif - Almygoma Khartoum -North during October 2016 to March 2017. The objectives of the present study were to provide data were collected from the records of slaughter house on the lesions of ovine livers, their monthly prevalence rates and to describe lesions grossly and microscopically. Samples of liver were collected during meat inspection with irrespective of age and breed. Samples of liver were collected. Gross study was performed during collection of samples from the slaughterhouses primarily and then during trimming of the samples for histopathology. Livers exhibiting gross alterations were cleaned with normal neutral saline and the changes were recorded. The lesion containing tissue samples were collected and preserved in 10% neutral formalin solution for histopathological study. The tissue pieces were washed in running tap water, dehydrated in ascending grades of alcohol, cleared in benzene and embedded in paraffin wax (60-62°C melting point). Sections of 4-5 micron thickness were cut and stained with haematoxylin and eosin as per the standard procedure. The lesions were frequent and varied with different percentages. The results of this research showed that the percentage of ovine hepatic lesions of condemned livers during the six months to total number of

slaughtered animals was 0.69 %. The total number of slaughtered sheep was 41,052million. The highest percentage of lesions was calcification and abscesses 0.2% and due to parasites such as S. hepatica, fasciola and hydatid 0.1%. The percentage of monthly ovine liver condemnation due to various causes during the study period was in October 0.64%, November 0.83%, December 0.77%, January 0.83%, February 0.5% and March 0.54%. The percentage of various causes of liver condemnation was as follows: Fasciola 0.03%, S. hepatica 0.02%, cysticercosis 0.05%, abscess 0.2%, necrosis 0.06%, adhesions 0.002%, fibrosis 0.02%, Calcification 0.2%, Fatty change 0.02% 0.007% stones 0.007% Degeneration 0.002% jaundice 0.002% and other changes in colour 0.01%. Histopathological examination of livers revealed a number of conditions or lesions such as chronic congestion, haemorrhage, hepatitis, fibrosis, fatty degeneration, calicification and centrilobular necrosis. In conclusion, this study identified the major pathological causes of livers condemnation in Nasreldin Slaughterhouse in Khartoum – North. Also the study clearly suggests that most of the diagnosed and reported parasites were associated with variable degrees of pathological lesions.

Keywords: Ovine, Liver, Lesions, Slaughterhouse, Khartoum-North

المستخلص

أجريت دراسة استعادية ومسح حي في مسلخ نصر الدين في الحاج يوسف - المايقوما الخرطوم بحري خلال الفترة من أكتوبر 2016 إلى مارس 2017. وهدفت هذه الدراسة الى تقديم البيانات التي تم جمعها من سجلات المسلخ عن أفات كبد الضأن ومعدلات انتشارها الشهرية ولوصف الأفات بشكل عياني ومجهري. تم جمع عينات من الكبد خلال تفتيش اللحوم بغض النظر عن العمر والفصيلة فحصت الآفات الكبدية. وقد أجريت الدراسة الإجمالية أثناء جمع عينات من المسلخ ومن خلال الكشف على اللحوم. تم تنظيف الكبد الذي أظهر تغييرات وأفات بمحلول ملحي محايد عادي وتم تسجيل التغييرات. تم جمع الأفات التي تحتوي على عينات الأنسجة والحفاظ عليها في 10٪ محلول الفور مالين محايد للدر اسة النسيجية. ثم غسل قطع الأنسجة في مياه الصنبور، ثم التجفيف في درجات تصاعدية من الكحول، وتم تنقيتها في البنزين ووضعت في شمع البارافين (60-62 درجة مئوية درجة انصهار). تم قطع أجزاء من النسيج سمك 4-5 ميكرون وصبغت بصبغة الهيماتوكسيلين ويوزين وفقا للطرق القياسية. كانت الأفات متكررة ومتنوعة بنسب مختلفة. وأظهرت نتائج هذا البحث أن نسبة الأفات الكبدية في الأكباد المعدمة خلال الستة أشهر إلى إجمالي عدد الحيوانات المذبوحة كانت 0.69٪. وبلغ إجمالي عدد الأغنام المذبوحة 41052 رأس. وكانت أعلى نسبة من الأفات هي النكلس والخراجات 0.2٪ وبسبب الطفيليات مثلا فاشيولا و الشيستوسوما و الأكياس العدارية 0.1%. كان للإعدامات الشهرية للكبد أسباب مختلفة خلال فترة الدراسة في أكتوبر 0.64%،و نوفمبر 0.83%،و ديسمبر 0.77%،و يناير 0.83%،و فبراير 0.5% و مارس 0.54٪. النسبة المئوية لمختلف أسباب إبادة الكبد كانت على النحو التالي: فاشيو لا 0.03٪، و الشيستوسوما 0.02 % وأكياس عدارية 0.05%، و الخراج 0.2%، و نخر 0.06%، الالتصاقات 0.002٪، و التليف 0.02٪، و التكلس 0.2٪، والتغير الدهني 0.007٪ و الحصاوي 0.007٪ واليرقان 0.002٪ والتغيرات الأخرى في اللون 0.01٪. لوحظ من خلال الفحص النسيجي المرضي للكبدعددا من الحالات أو الآفات مثل الإحتقان المزمن والنزف والتهاب الكبد والتليف وانتغيير الدهني والتكلس والنخر المركزي. في الختام، أوضحت هذه الدراسة الأفات المرضية الرئيسية لإعدامات الكبد في مسلخ نصرالدين في الخرطوم بحري. كما أشارت هذه الدراسة بوضوح إلى أن معظم الطفيليات التي تم تشخيصها والإبلاغ عنها كانت مرتبطة بدرجات متفاوتة بالآفات المرضية

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INTRODUCTION

Sudan is the third largest country in Africa and has one of the largest livestock populations on the continent. Livestock in Sudan are mostly raised under nomadic condition with traditional methods of management and natural grazing; this livestock are concentrated in western and central states (Osman *et al*, 2005). Cattle, sheep, goats and camels were estimated to a number about 141,827 million (FAO, 2011). This represents most species diverse livestock populations in the African and Arab region. Sheep population was estimated to be 52,014 million (FAO, 2011; Elbadawi *et al.*, 2013). Several breeds were raised, but the predominant and preferred one was the so-called desert sheep, which had both good weight and good milk yield (Metz, 1991).

Slaughterhouses are used as sources for information about diseases epidemiology (Suhair, 2013). Meat inspection at slaughterhouses, for hygienic quality, involves both ante and postmortem examination. Abattoir meat inspection is necessary to remove gross abnormalities from meat and its products, to prevent distribution of contaminated meat and to assist detecting and eradication of certain livestock diseases. The post mortem inspection is an essential step which meat hygiene revolves since it provides information for evaluation of clinical signs and pathological process that affect the wholesomeness of meat (Mesfin and Mekonnen, 2014).

Liver is one of the most important organs of the body as it is involved in many metabolic disorders and parasitic diseases (Jibat, 2006). It may harbor pathogens which are dangerous for human consumption when passed with localized or mild infection. Liver lesions are common and they indicate the presence of disease in other organs and systems as the liver acts as a catchment for the vast absorptive area of the gut, with all its resident microorganisms (Kelly, 1985). Any observation and information obtained at slaughterhouse can contribute to the understanding of slaughtered animals' diseases.

The Objectives of this study were

- To investigate the occurrences of pathological conditions affecting the liver of slaughtered sheep at Naser Aldeen Abattoir, Khartoum -North City Sudan
- To study the pathology of lesions of the condemned livers

CHAPTER ONE

LITERATURE REVIEW

1.1. Livestock

Livestock plays an important role in Sudanese Economics. According to the report from FAO (2011), Agriculture production constitutes the backbone of Sudan's economy in terms of its contribution to GDP. Livestock form an essential component of the agriculture sector which provides employment and household income in rural areas. Livestock is considered as an indispensable source of income for small producers in Sudan. According to reported information from FAO (2011) that rural Sudan communities own 80% of the livestock. Animals are a source of protein for human diets and can serve to provide income, employment and foreign exchange within a country. For many low income producers, livestock also serves as a store of wealth, provides draught power and organic fertilizer for crop production, acts as a means of transport, and serves as a vital component of social functions and exchange. Livestock play important roles for production of food and represent great socioeconomic and cultural values in various societies around the world (Okomo-Adhiambo, 2002). In Sudan, the rural communities own 80% of the livestock and the nomadic tribes own 90% of the rural holdings with livestock playing a central role in their livelihoods (Lutfi *et al*, 2005; Osman et al., 2005).

Animals face many pathological conditions caused by bacteria, viruses and parasites. The pathological lesions of animals/livestock lead to decrease in

production, emaciation and loss of appetite. Animal also take time to reach the peak of production after recovery (Abusara and Abdelgadir, 2014).

1.2. Ovine

The **sheep** (*Ovis aries*) is a quadrupedal, ruminant mammal typically kept as livestock. Like all ruminants, sheep are members of the order Artiodactyla, the even-toed ungulates. Although the name "sheep" applies to many species in the genus *Ovis*, in everyday usage it almost always refers to *Ovis aries*. Numbering a little over one billion, domestic sheep are also the most numerous species of sheep. An adult female sheep is referred to as a *ewe*, an intact male as a *ram* or occasionally a *tup*, a castrated male as a *wether*, and a younger sheep as a *lamb*. Mature sheep weigh from 35 to 180 kg (<u>https://en.wikipedia.org/wiki/Help:IPA/English</u>, 2017; (https://www.britannica.com/animal/sheep, 2017)

Sheep husbandry is practiced throughout the majority of the inhabited world, and has been fundamental to many civilizations. A group of sheep is called a flock, herd or mob. Many other specific terms for the various life stages of sheep exist, generally related to lambing, shearing, and age. Sheep have a deeply entrenched place in human culture, and find representation in much modern language and symbology. As livestock, sheep are most often associated with pastoral, Arcadian imagery (https://www.britannica.com/animal/sheep).

The Sudanese sheep has been classified on the basis of morphology and distribution into: Sudan Desert and Sudan Arid Upland (McLeory, 1961a,b; and Wilson and Clarke, 1975). Sudan Desert sheep are further classified into tribal sub types, e.g., Hamari, Kabashi, in North and West Kordofan States, Shugor, Dubasi and Watish in the Central States (El-Hag

et al., 2001). More than 82% of sheep in Sudan are of the Sudan Desert type (Sulieman *et al.*, 1990).

Sudan desert sheep is raised by nomadic tribes in the semi-desert zone and the low rainfall wood land savannah belt. Genetic characterization of local Sudanese sheep populations showed that the desert sheep has higher values of heterozygosity compared to the Western and Southern populations, including West African, Arid upland and Nilotics sheep populations (Gornas *et al.*, 2011, El Hassan *et al.*, 2017).

1.3. Slaughterhouses

Slaughterhouses provide a good opportunity for detecting diseases of both economic and public health importance. Surveys of livestock diseases and condemnation rates at slaughter can give a useful guide to the incidence of the less acute, chronic and mild diseases which occur in production animals in regions served by various abattoirs (Chambers 1987). Frequent encounters of bovine pathological lesions in the liver have been constant features in the annual reports of various slaughter houses worldwide (Babalola, 1975; Ogunrinde 1980; Antia, 1982, Raji et al, 2010). Any observation and information obtained at slaughterhouse can contribute to the understanding of slaughtered animals' diseases. The pathological examination represents a useful tool to make a diagnosis within the slaughter line. A wide variation has been recorded in the incidence of abnormalities found over different geographical locations. These findings are affected by various factors such as the degree of veterinary supervision and critical appraisal of abnormalities by the person carrying out the survey (Al-Dahash and David, 1977; Okoli, 2001; Raji et al, 2010). It is necessary

to know the extent to which the public is exposed to zoonotic diseases as observed in abattoirs. In addition, the financial implications of condemnation to the butchers in terms of meat may be substantial (Antia, 1982; Halle, 1998). Records of ante and post-mortem inspections are useful epidemiological data for the evaluation of diseases at farm level and to verify the efficacy of prophylactic and therapeutic interventions (Ogunrinde 1980; Antia, 1982). Numerous abattoir surveys of bovine pathological conditions have been conducted to investigate macroscopic and microscopic abnormalities (Al-Dahash and David, 1977; Ogunrinde 1980; Antia, 1982; Matovelo and Mwamengele, 1993; Raji *et al*, 2010). Therefore, information gathered on animals slaughtered at an abattoir can be a convenient and inexpensive source of information (Roberts and Suhardono, 1996). The data can be used to determine trends in prevalence and significance of the disease(s) especially where the reporting system is reliable (Roberts and Suhardono, 1996).

1.4. The liver

The liver is a vital organ of the body and susceptible to various disease conditions and parasites that affect the total health status of the animal. It is a complex organ that performs many metabolic functions which are dependent upon the integrity and interaction of four anatomic subunits: the hepatic parenchymal cells, the biliary system, the hepatic vascular system, and the Kupffer or reticulo-endothelial system. The liver is one of the main metabolic organs and, as such, is positioned between the digestive tract and the systemic circulation. The majority of compounds absorbed from the gastrointestinal tract reach the liver directly through the portal circulation. The liver is the regulatory site of carbohydrate, protein and lipid metabolism. In addition, the liver is an organ responsible for excretion (bile

for the digestion of fats), a storage organ (glycogen, vitamins, trace elements), an organ where synthesis occurs (albumin, fibrinogen, prothrombin), and it participates in immune regulation (Kupffer's cells) (Runnels *et al*, 1965; Kelly, 1993; Jones *et al.*, 1997).

Liver function is only impaired once more than 80% of the liver has been damaged. However, the liver does possess a unique capacity for maintaining its specific functions and simultaneously repairing and regenerating its own tissue Information was recorded by the livestock officers when liver disease conditions were identified by meat inspectors at post-mortem inspection. Where further diagnosis was required, specimens were submitted to an Animal Health Laboratory. The information recorded included daily cattle kill; number of lines (a line of cattle being a group of animals originating from the same property and owner); farmer's name, address, and farm location; number of cattle in the line; and number of livers condemned and reason for condemnation (Kearns, 1987).

1.5. The pathological conditions of liver

1.5.1. Liver fluke: (liver fluke disease)

It is caused by *F. hepatica, F. gigantica, F. magna* and *Dicrocoelium dendriticum*. It is a sequence of acute parenchymal hepatitis and chronic cholangitis in sheep. Rue Jensen, (1974) stated that in acute disease, a traumatic invasion of liver parenchyma by immature flukes that cause necrosis, hemorrhage, inflammation and later fibrosis. Massive invasion causes hematomas and liver may be ruptured leading to fatal black disease. A biliary cirrhosis from prolonged residence of adult flukes which occupy the bile ducts, known as chronic disease and cause leakage of blood, chronic cholangitis and hypoalbuminemia (Rue Jensen, 1974).

Urguhart (1956) suggested that cirrhosis characteristic of hepatic fascioliasis is due to the healing of the migration tracts, chronic cholangitis and hyperplasia of the connective tissue and bile ducts. He attributed granulomatous lesions in the livers to the presence of fluke eggs. He also stated that obstruction of the biliary tract appear to play part in the pathogenesis of the hepatic cirrhosis in fascioliasis. Fasciola species is common cause of liver condemnations. At Shuman Abattoir in Bulgaria 15% of livers were condemned due to fascioliasis (Stoimenov and Kaloyanov, 1973). In northwest Argentina, out of 2090 livers inspected in large slaughterhouse in Salta; 13% were found to have lesions of fascioliasis (Dwinger *et al.*, 1982). In Rome Meat Centre in 1984, 0.63% of liver condemned were due to fascioliasis (Severini *et al.*, 1985).

In Sudan, Abu-Rigaila (1983) found that the incidence of the naturallyoccurring fascioliasis in sheep increase during the dry season (January – March) when animals were crowded in large numbers around drinking sites. Haroun (1975) reviewed the annual reports of the veterinary services of the provinces of Sudan for the period (1955 – 1974).

Economic losses result from the treatment of the disease and the condemnation of livers among sheep-dependent peoples. The disease also has public health significance, causing human fascioliasis and "halzoun" disease (Saleha, 1991).

The common route man becomes infected with liver fluke is by eating uncooked plants on which cercariae of liver fluke have encysted. Man may also become infected by swallowing cercariae that are living free in shallow pool of fresh water. He is not infected by eating infected liver, which are condemned as unfit for human consumption, because of aesthetical reasons (Thornton, 1973; Musa, 2007).

1.5.2. Cysticercosis: (Cysticercus tenuicollis)

Immature metacestode cysts of the dog tapeworm *Taenia hydatigena* migrate through the liver parenchyma sometimes causing fatal hemorrhage or encouraging the multiplication of *Clostridium novyi* organisms, which cause the black disease. Mature cysts formed in the peritoneal cavity over-lying the liver capsule, eventually collapse and become calcified (Linklater and Smith, 1993).

1.5.3. Echinococcosis: (Hydatidosis)

A chronic two-host zoonosis of sheep and other herbivores is characterized by the formation of variable cysts in the liver and lungs and is caused by the larvae of *Echinococcus granulosus*, a tape-worm of dogs and wolves. Economic losses result from condemnation of infected livers (Rue Jensen, 1974). Hydatid infection of the liver is always associated with a marked connective tissue reaction, which encapsulates the cyst within a fibrous wall up to 13 mm thick. Hydatid cysts that give rise to brood capsules or daughter cysts are described as fertile; other are sterile and characterized by their smooth inner lining, usually with slight turbidity of the contained fluid (Gracey, 1986). Hydatid cysts may at times show degenerative changes, the vesicular fluid disappearing and the cavity contracting and becoming filled with a caseous matter, which may calcify. Degeneration of hydatid cysts occurs early and with particular frequency in the sheep (Gracey, 1986).

1.5.4. Bacterial Necrosis:

Bacterial necrosis is caused by infection with *Fusibacterium necrophorum*, which is frequently found in the intestinal tract. It reaches the liver by the portal vein causing coagulative necrosis (Gracey, 1986).

In Sudan there are reports of liver condemnations due to bacterial hepatitis (Central state – 1990/1991) and necrosis (Eastern state – 1991/1992, Khartoum state – 1991/1992). From the Annual Reports of Animal Researches (1989-1992).

1.5.5. Liver abscesses:

Liver abscess is any encapsulated lesions, which contain necrotic matter or pus, regardless of the agent, which is responsible for these changes (Thornton, 1973). Abscesses occasionally forms in the liver of all species as the result of the entrance of microorganism, mainly pyogenic cocci or other pus-producing bacteria, by several routes. These are the portal vein, the hepatic artery, the umbilical vein in newborn, the duct system, the hepatic vein, and by penetrating foreign bodies in the reticulum (Jones *et al.*, 1997).

Abscesses of the liver may cause signs of toxaemia because of the destruction of hepatic tissue or the liberation of the potent toxins. The toxaemia of traumatic hepatitis is usually due to toxins from *Corynebacterium pyogenes* and *Fusobacterium necrophorum*, which are implanted in the lesions by the perforating foreign body. Many septicemias in lambs are caused by *Haemophilus Agni* (Blood *et al.*, 1986). An increase in the incidence of liver abscesses and in the number of livers condemned was observed in Ukrainia, 1982–1988, in the feedlot system of fattening beef cattle. Thus, Vloizo and Lewtschenko (1992)

found that liver abscesses were most frequent (55.2%) of all lesions observed in 2747 fattening bulls. Liver abscesses are reported in Sudan as a cause of liver condemnation from all states. In 1991/1992, 4.2% of cattle liver condemnations were due to abscesses (Animal resources annual reports, 1991-1992).

In Khartoum state, Obeid (1994) reported that 6.04% of cattle condemned livers during the period (1990 – 1993) were due to abscesses, and he noticed that there was no distinct variation in their seasonal occurrence.

1.5.6. Liver cirrhosis:

Liver cirrhosis or fibrosis is a natural response of the liver tissue to injury. It is the commonest condition which renders the liver unfit for human consumption. Hence the affected organ is totally condemned during inspection (Thornton, 1973 and Kelly, 1993). In general, the commonest cause of liver cirrhosis is parasitic infection such as fascioliasis and schistosomiasis (Thornton, 1973).

1.5.7. Fatty change:

The effect of toxic factors in the liver is often manifested by pathological fatty change (Thornton, 1973). The condition could be result from acute febrile and toxemic conditions, intestinal diseases in cattle, chemical poisoning by phosphorus, chloroform or carbon tetrachloride and may accompany acute bacterial necrosis of the liver (Gracey, 1986).

1.5.8. Calcification:

Tissue calcification may be regarded as part of the defensive mechanism of the body to enclose and immobilize some foreign agent. It usually occurs in necrotic tissues and most commonly encountered in connection with chronic tuberculous lesions, or in parasitic infections in animals, such as trichinosis or *Cysticercus tenuicollis* (Gracey, 1986).

1.5.9. Congestion:

The liver with acute passive congestion is dark red and swollen with rounded edges. In chronic passive congestion (nutmeg liver) the surface of the liver is slightly nodular and rough. It leads to anoxic centrilobular necrosis and the spaces created in places of the destroyed cells become filled by blood (Jones *et al.*, 1997).

1.5.10. Adhesion:

Adhesions commonly occur in fibrinous inflammations, when the fibrinous exudates become organized into bands of collagenous tissue. It is often seen between serous surfaces and may interfere with organ functions. Adhesion of the liver with the diaphragm is a frequent cause of liver condemnation. Superficial liver abscesses may results in local peritonitis and formation of adhesions (Thornton, 1973).

1.5.11. Melanosis:

Non-malignant melanosis of the liver and other tissues occasionally occurs in lambs of heavily pigmented breeds. No illness is associated with the condition but the livers are not passed for human consumption (Linklater and Smith, 1993).

In Sudan there are no reports of cattle or sheep liver condemnation due to melanosis.

1.5.12. Hepatic Tumors

One of the commonest tumors of the bovine liver is carcinoma, which increases the weight of the liver till it reaches up to 80 Ib. the commonest benign tumor is the adenoma of the liver which may be in size of orange (Thornton, 1962).

In Sudan at meat-inspection examination after abattoir slaughter, two Desert sheep aged 3 to 4 years showed hepatoma. There were several tumours in the liver of each but no metastatic lesions in other organs of the body. The macroscopic and microscopic appearances are described as also the chemical changes in the serum constituents (Adam and Ramadan 1974).

Analysis of data of the condemnation records from Khartoum slaughterhouses by Mai Aydarous (2004) during five years periods (1998-2002) showed that sheep liver condemnation was significantly higher in January and August while it was less frequent in September, October, November and December. Her results indicate that 35.1% and 72% of the cattle and sheep + goats livers respectively, were condemned due to non-specific causes. These could be due to the changes caused by parasitic, bacterial, viral or fungal infections or to chemical and toxic factors.

1.4. Diagnosis of liver diseases The liver is embellished with diverse metabolic activities, and evaluation of its functional status is dependent upon its ability to perform a specific metabolic function (Kataria *et al.* 2011). A number of tests have been devised for the detection of alterations in hepatic functions out of which the measurement of serum enzyme activity is very important in clinical cases (Varley, 1988). Generally

enzyme levels in serum increase due to increased membrane permeability, cell necrosis or cytosol leakage in the serum (Alemu *et al.*, 1977), therefore liver cell damage due to toxic agents, injury or diseases has been detected in domestic animals by measuring the activity of liver specific enzymes in the serum. The important enzymes related with hepatic functions are alkaline phosphatase, gamma glutamyl transferase, 5'-nucleotidase, aspartate aminotransferase, alanine aminotransferase, ornithine carbamoyl transferase, glutamate dehydrogenase, sorbitol dehydrogenase, arginase, lactic dehydrogenase, aldolase, isocitric dehydrogenase and glucose -6-phosphatase (Wolf and Williams, 1973).

CHAPTER TWO

MATERIALS AND METHODS

2.1. Location of the study area and time

A retrospective study and active abattoir survey were conducted in Nasr Aldin Slaughterhouse at Alhaj Yousif - Almygoma Khartoum - North during October 2016 to March 2017. All data were obtained from records.

Sheep slaughtered at this slaughterhouse are represented of the various breeds in Sudan. The visits were mainly done at weekly interval. Meat inspection was carried out at the slaughterhouse by the helped of the veterinarian staff.

2.2. Study population

Male sheep of different breeds and ages were presented for slaughter from different localities in Sudan. A total of 41529 sheep were slaughtered over six months and examined following ante-mortem and post-mortem inspection procedure. The data of the gross lesions were collected. It was not possible to get the exact records on breed, gender, weight and age for each slaughtered animals during the study period due to poor recording systems at the abattoir.

2.3. Collection of the samples

Livers from sheep showing pathological lesions or abnormalities were collected and thoroughly examined. All lesions were recorded.

2.4. Pre and post slaughter examination

The records of numbers slaughtered and the organ lesion(s) observed and condemned were noted by the veterinarians. All animals presented for slaughter were physically observed a day before or shortly prior to slaughter. Inspection of the animals was made while at rest or in motion for any obvious sign of disease.

Post slaughter examination involved visual examination of carcasses and organs with keen attention being directed to livers, lungs, hearts, gastrointestinal tract and skin. Through palpation and incision of suspected organs, gross pathological lesion of each diseased organ was established and recorded as described by Gracey (1985).

2.5. Samples

The grossly affected livers from sheep of different ages were collected from the slaughterhouse. The livers were examined externally and internally (the lobes, gall bladder and bile ducts) for the presence of parasites, and other lesions; nodules abscesses, hydatid cysts, areas of necrosis, fibrosis, congestion and/or haemorrhage, calcification, adhesion, tumor and any other pathologic changes.

2.6. Criteria for selection

Liver inspected during postmortem examination and lesion(s) observed and condemned were retrieved from the abattoirs record books. Such records were used to establish the prevalence of liver diseases and lesions affecting the sheep slaughtered. Routinely, meat inspectors carry out antemortem examination of all animals presented for slaughtered day before or shortly prior to slaughter. This is followed by postmortem meat inspection involving visual examination, palpation and systematic incision of carcasses and visceral organs particularly, liver, lung, kidney, heart and spleen according to procedures described by Gracey *et al.* (1999) and the Sudan general guidelines on meat inspection.

Liver diseases and lesions were grossly diagnosed based on pathological changes of organ colour, size, morphology, consistence, presence of lesions and parasites. At the end of meat inspection all partial and total condemned liver were taken to the laboratory for further examination and identification of the lesions and parasites.

2.7. Pathological lesions

The records of the organ lesion(s) that observed and condemned were noted in records. All liver lesions were collected, determined the average weight and imaged by digital camera. This examination has been achieved macroscopically and microscopically.

2.8. Macroscopic examination

The livers were examined externally and internally for the presence of parasites, nodules abscesses, tumors, fatty change, calcifications, necrosis, fibrosis, pigmentation and hepatitis. The post slaughter examination involved visual examination of carcasses and organs with keen attention being directed to livers. Through palpation and incision of suspected liver, gross pathological lesion of each diseased organ was established and recorded as described by Gracey (1985).

2.9. Microscopic examination

Previously found results on some organ's fragments (pieces of liver parenchyma of 1cm depth), were cut and then were fixed in 10 % neutral buffer formalin solution and processed by routine paraffin embedding technique for microscopy. The tissue pieces were washed in running tap water, dehydrated in ascending grades of alcohol, cleared in benzene and embedded in paraffin wax (60-62°C melting point). Sections of 4-5 micron thickness were cut and stained with haematoxylin and eosin as per the standard procedure (Humason, 1972, Bancroft *et al*, 1996). The histopathology technique and examination were done in the Department of Pathology, Central Veterinary Research Laboratories, Soba Khartoum.

2.10. Steps of histopathology technique

- 1. Fixation: By using chemical fixatives as formaldehyde, ethyl alcohol, acetic acid, mercuric chloride to prevent postmortem autolysis.
- 2. Dehydration and clearing: Removing water firstly, after excessive washing of fixatives. Dehydrating agent; as ethanol, isopropanol and butanol. Increasing alcohol concentrations until total dehydration occur with absolute ethanol.
- 3. Embedding: Clear specimens process through high paraffin concentration at melting points 50°-68° C throughout infiltration process and then placed into molds surrounded with paraffin and cooled.
- Sectioning: After paraffin removed, blocks were trimmed to expose the embedding tissue. The block mounted on microtome for 3-5μm, then sectioned and floated in warm water and picked up on slide.

5. Staining and mounting: Tissue for staining were soluble in water or alcohol, the paraffin must be removed before staining. Rehydration and then slides are stain. The specimens were dehydrated, cleared, and covered with mounting medium that miscible with clearing agent. A cover slip was applied to produce a permanent preparation.

2.11. Prevalence of lesions

Prevalence of pathological lesions or conditions was calculated as the number of sheep slaughtered to that has had lesions, expressed as a percentage of the total number of sheep.

2.12. Statistical analysis

Collected post mortem/meat inspection records were entered, edited, validated and performed by basic- statistic tool (statsoft.com). The test statistics used were mean and percentages (%).

CHAPTER THREE RESULTS

3.1. Sheep slaughtered and condemned livers

In the study period, 41529 male sheep were slaughtered in the Nasr Aldeen Slaughterhouse. The results of this research showed that the percentage of ovine hepatic lesions of condemned livers during the six months to total number of slaughtered animals was 0.69 %.

Table (1) shows the rate and causes of ovine livers condemned due to various pathological conditions during the period (6 months).

Table (2) shows the monthly ovine liverS condemnation due to various causes during the study period. It was found that the percentage in October 0.64%, November 0.83%, December 0.77%, January 0.83%, February 0.5% and 0.54% in March.

The highest percentage of lesions was calcification and abscesses 0.2% and due to parasites such as S. hepatica, fasciola and hydatid cyst 0.1%. In details the percentage of various causes of liver condemnation was as follows: Fasciola 0.03%, S. hepatica 0.02%, cysticercosis 0.05%, abscess 0.2%, necrosis 0.06%, adhesions 0.002%, fibrosis 0.02%, Calcification 0.2%, Fatty change 0.02% 0.007% stones 0.007% Degeneration 0.002% jaundice 0.002% and other changes in colour 0.01% Table (3).

Table (1) Causes and rates of ovine livers condemned due to various pathological conditions in during the period (6 months) in Nasr aldeen slaughterhouse

Month	October	November	December	January	February	March	Total no. of Slaughtered animals
No. of slaughtered animals Causes	5906	6941	7010	7298	6902	7472	41529
Fasciola	7	3	-	3	-	1	14
S. shisto	1	2			1	3	7
H. cyst	2	2	3	8	4	4	23
Abscess	16	20	21	17	11	12	97
Necrosis	-	12	6	5	1	1	25
Congestion	-	-	-	-	-	-	-
Adhesion		1					1
Fibrosis		2	3	4			9
Calcification	12	15	17	16	16	12	88
Fatty change					7	3	10
Cirrhosis		1		1		1	3
Gall stones			3				3
Degeneration			1				1
Discoloration						5	5
Undifereniated						1	1
Total lesions	38	58	54	61	35	41	287
Rate of total lesion/month	0.0064	0.0083	0.0077	0.0083	0.0050	0.0054	0.0069

Causes	Percentage
Fasciola	0.03%
Schistoma	0.02%
Cysticercosis	0.05%
Abscess	0.2%
Necrosis	0.06%
Adhesion	0.002%
Fibrosis	0.02%
Calcification	0.2%
Fatty change	0.02%
Cirrhosis	0.007%
Stones	0.007%
Degeneration	0.002%
Jaundice	0.002%
Discoloration	0.01%

Table (2) Percentage of various causes of ovine liver condemnation atNasreldin slaughterhouse during six months

 Table (3) Monthly ovine liver condemnation due to various causes at

 Nasereldin slaughterhouse during six months

October	November	December	January	February	March
0.64%	0.83%	0.77%	0.83%	0.50%	0.54%

3.2. Pathological findings

Macroscopic examination revealed that the most lesions found were abscesses, calcification and parasitic lesions. Gross study was performed during collection of samples from slaughterhouse primarily and then during trimming of the samples for histopathology. Fatty change and calcification appears as grayish spots on liver. Whitish foci of abscess lesions were found on the surface of the liver. Their size varied from 0.5 to 1.5 cm in diameter. In some cases of fascioliasis, the affected liver was slightly swollen and appeared pale in color with round edge, the capsule was thick, rough with whitish or reddish discoloration and fibrosis of the bile ducts which indicated sub acute form of infection. Some affected livers with hydatid cysts showed severe damage of the parenchyma. The Cirrhosis of liver was also observed in the peripheral surface of the livers (Fig. 1-7).

3.3. Microscopic findings

Microscopically, Hepatic degeneration was observed in all infected livers. There were lymphocytic infiltrations with few reticulo-endothelia cells. Considerable fibrous connective tissue proliferations were noted at the portal areas. In chronic cases, the areas of infiltration with lymphocytes and mononuclear cells and proliferation of fibroblasts represented the hemorrhagic tracts. Accumulation of lymphocytes and proliferation of fibrous connective tissues in the portal areas distorted lobular architectures. Multifocal areas of hepatocellular necrosis were seen. Lesions consist of dilated sinusoids as irregular vascular spaces and in some areas atrophic hepatocyte were seen. Dilated sinusoids were filled with erythrocytes and neutrophils leucocytes, desquamated hepatocytes, and some endothelial cells. Considerable proliferation of fibrous connective tissue was marked mainly in the portal areas indicating congestion. Fatty changes were seen in the cytoplasm of hepatic cells as variable sizes and shapes of empty vacuoles. (Fig. 9).

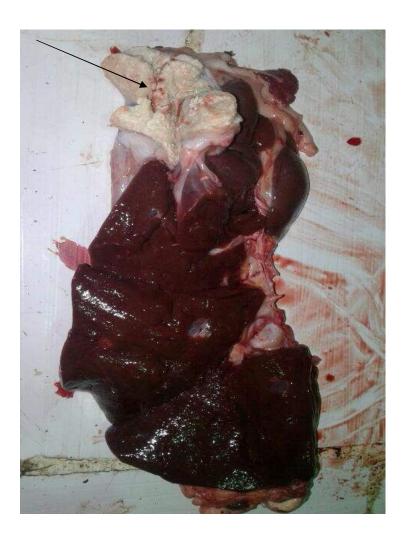


Fig. 1. Liver of sheep showing abscess



Fig. 2. Liver of sheep showing calcification, necrosis, haemorrhage and multifocal abscesses

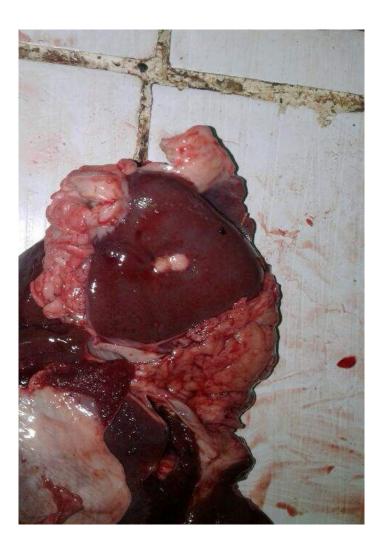


Fig. 3. Liver showing calcification and abscess



Fig. 6. Liver of sheep showing diffuse (extensive) fatty change



Fig. 7. Liver of sheep showing almost diffuse fatty change that is represented as homogenous pale regions

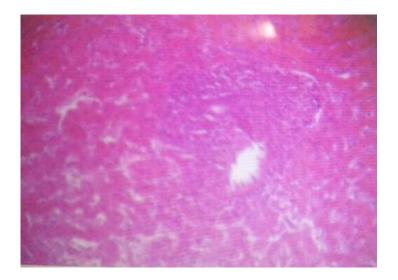


Fig. 8. Liver showing Centri-lobular necrosis H&E x 100.

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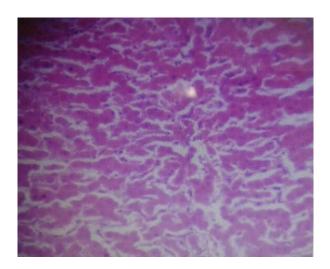


Fig. 9. Fatty change in liver H&E x 100

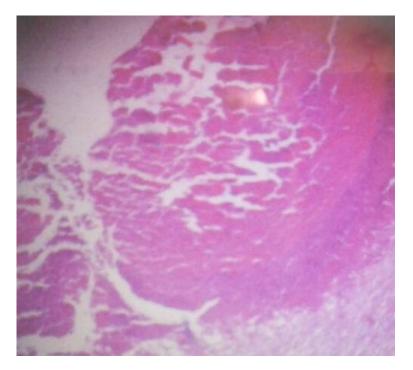


Fig. 10. Liver section showing hepatic necrosis. H&E x 100.

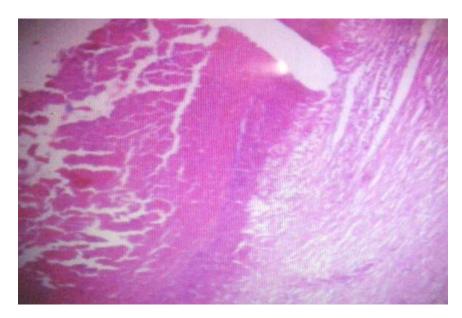


Fig 11. casious necrosis (granuloma and fibrous tissue),

H&E x100

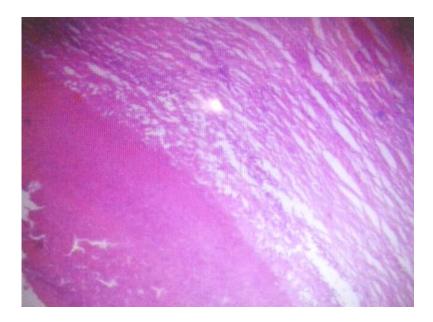


Fig.12. Calcification (necrotic area surrounding by fibrous tissue, infiltration of inflammatory cells) H&E x100

CHAPTER FOUR DISCUSSION

There was no animal condemned as result of antemortem inspection all over the study period. As a result of postmortem inspections, zero (0.00%) carcass was condemned and 287 livers had pathological lesions. The slaughterhouse and its regulations, represents a key control point of livestock production chain (Ogunrinde, 1980; Antia, 1982). Results of meat inspection of slaughtered animal are too important for animal health control system (Fries, 1994).

This study demonstrates that liver diseases and lesions represent a serious problem and may continue to be a drawback to livestock industry and may pose health risks to meat consumers from contrasting zoonotic diseases in Sudan. The number of different liver disease conditions has been recorded as the major cause of condemnations which included fasciolosis, *schistosoma bovis*, abscess, necrosis, fatty degeneration, fibrosis and calcification.

The present study shows the findings of liver pathological conditions or diseases in sheep in Nasereldin slaughterhouse. Calcification, necrosis abscesses and fasciolosis were the most common pathological condition that caused condemnation of the liver. Similar study was conducted by Khan *et al.* (1986) they reported 32.80% in Kovak valley in upland district of Balochistan. Similar findings were reported by Swai and Ulicky

Other important result in this study was the report of hepatic necrosis in causing high rate of condemnation of sheep livers.

Liver cirrhosis which is one of the non-specific causes of sheep liver condemnations was thought to be caused by fasciola infections. Other two non-specific causes of sheep liver condemnations were fatty changes and liver congestions.

In the present study the liver abscesses was found to be (0.2%) and similar results were obtained in Nigeria by Cadmus and Adesokan (2009) and Ahmedulla *et al.*, (2007) in Bangladesh.

Apart from its veterinary and economic importance throughout the world, fasciolosis has recently been shown to be a re-emerging and widespread zoonosis affecting a number of human populations (Mas-Coma *et al.*, 2005; Esteban *et al.*, 2003; Mellau *et al*, 2010). Furthermore, as a zoonotic disease, the World Health Organization (WHO) estimated that 2.4 million people were infected with *Fasciola* in 1995 and a further 180 million were at risk of infection (World Health Organization, 1995).

Hydatid Cysts (HC) in the liver was found low in Naseldin Slaughterhouse. Similar low level of infection rates of HC were reported by Njoroge *et al.* (2002) and Ansari-Lari (2005). The differences in prevalence of HC may arise due to differences in environmental conditions that are conducive to the perpetuation of the parasite, abundance of infected definitive host, livestock husbandry, stocking rate, nature of the pasture and grazing patterns of animals. Calcification was among the lesions which significantly contributed to the liver condemnation similar by Swasi and Ulicky 2009 in Tanzania.

Non-specific causes of liver condemnations in this study went confirming those results gained by Mai Aydarous (2004) who reported that 72% of sheep and goat livers were condemned in Khartoum State due to nonspecific causes, with differences in the number of condemned liver detected between the two studies. In this study other conditions were observed but with lower prevalence included congestion, fatty change, tumor or melanosis, TB and liver fibrosis. These suggest that they are likely to be of minor concern and appear sporadically.

CONCLUSION AND RECOMMENDATIONS

Conclusion

Although the data obtained was for six months, it can be used as a baseline for the proper evaluation of the status of the liver diseases in sheep. This pathological lesions or conditions in the liver of sheep impact negatively on the economic status of the traders and the livestock industry. Nevertheless, the observed frequency of hydatid cysts and liver fasciolosis at slaughterhouse should be considered as a risk for public health since they are zoonotic.

Recommendations

- Large scale studies are recommended for future research to evaluate all pathological conditions in sheep.
- The presence of zoonotic diseases such as fasciolosis and *C. ovis* may pose a health risk to meat consumers and this suggests a need for a rigorous meat inspection procedure, to minimize the chance of consumers acquiring infection through contact with and/or consumption of infected meat.

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APPENDIX

ملحق الصور



Clips



Dehydration and cleaning



Microtom



Sectioning area



Drying



Embeding



TISSUE WATER BATH



PARAFFIN DISPENSER

