

Sudan University of Science and Technology College of Graduate Studies



Prevalence of Bovine Fasciolosis in Hilla Kuku, Khartoum State, Sudan

معدل إنتشار داء الديدان الكبدية في الأبقار في حلة كوكو، ولاية الخرطوم، السودان

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B.V.Sc University of Nyala 2012

A thesis Submitted to the College of Graduate Studies In Partial Fulfillment of The Requirement for Degree of Master of Preventive Veterinary Medicine (MPVM)

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Dedication

To:

My Family;

My Supervisor;

To anyone who contributed

In conducting this Study

Acknowledgemennt

First and foremost, praise to Allah for giving me the chance to finish this work.

I wish to express my deepest sincere gratitude to my supervisor Dr.Mubarak Mustafa Abdelrahman, from Tropical Medicine Reasearch Institute for encouragement and valuable, advice, suggestions and kind supervision. Also I wish to thank Dr. Nagla Abdel Hakeem Abbas for her support. Finally, I would like to express my blessing, thanks, and sincere gratitude to family of Faculty of Veterinary Medicine, Sudan University of Sciences and Technology for their great support.

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Abstract

A cross-sectional epidemiological study was conducted from November

2020 to February 2021 to detrermine the prevalence of Bovine Fasciolosis in cattle at Hilla Kuku Farms. A total of 246 fecal samples from cattle selected randomly for presence of Fasciola species aggs.

Result showed that 13 (5%) cattle were infected with Fasciola species using sedimentation technique. The prevalence distribution of Fasciolosis according to the sex was: 9% in Males, and 4% in Females. The prevalence of fasciolosis according to the age of animal was followes young animal (>1year) 0%, (1-4) 4%, (<4)7%. The prevalence distribution according body condition was 2% in good body condition of animals, 12% in poor body condition animal. The prevalence of fasciolosis according to the uses of anthelmentics was 3% in treated with anthehelmentics drugs 11% in animal that were not treated with asthelmentics drug.

Statistically significant variation was observed in the prevalence of bovine fasciolosis among animals, The prevalence of bovine fasciolosis by fecal examination (5.3%) was found to be significantly associated ($p \le 0.05$) with body condition and using drug. However, sex. and age. were not found significantly associated (p>0.05) with fasciolosis infection in this study.

ملخص البحث

اجريت دراسة مقطعية وبائية في الفترة من نوفمبر 2020م حتى فبراير 2021 لتحديد معدل انتشار مرض الفاشيولا في الابقار بحلة كوكو ولاية الخرطوم. تم إختيار 246 رأس من الأبقار عشوائيا تم فحصهم بواسطة فحص البراز لوجود بيض الفاشيولا بطريقة الترسيب،

وضحت النتائج أن 13 رأس من الأبقار مصابة بطفيل الفاشيولا بمعدل 5.3%. وقد وجد أن معدل إنتشار المرض وفقا لجنس الحيوان كالاتى: 9% فى الذكور ، اما 4% فى الإناث. معدل إنتشار الطفيل وفقا للعمر فقد كان كالأتي: أقل من سنة صفر %، من سنة إلى اربعة سنة 4% أما الأكبر من اربعة سنة 7%. وكان معدل إنتشار المرض وفقا للحالة الجسدية للحيوان كالأتى: 2% فى جيدة الحالة الجسدية، 12% فى سيئة الحالة الجسدية. أما معدل إنتشار المرض إعتمادا على معالجتها بمضادات الديدان كالأتى: 3% فى الأبقار التى تلقت العلاج، 11% فى الأبقار التى لم تتلقى العلاج

أظهرت الدراسات الإحصائية أن نسبة إنتشار مرض الفاشيولا له علاقة معنوية تحت قيم معنوية أقل من او يساوي 0.05 مع عوامل الخطر التالية: الحالة الجسدية للحيوان وإستخدام الدواء، أما عومل الخطر من جنس الحيوان وعمر الحيوان ليس لهم علاقة معنوية مع حدوث المرض تحت قيم معنوية أكبر من0.05.

INTRODUCTION

Background:

Liver fluke is a common trematode (flat worm) that causes a parasitic disease called fascioliosis or fluke disease. It has a pathogenic effect on ruminants (cattle, sheep and goats) as well as other farm animals like horses, donkeys, pigs and to a lesser extent poultry. Species of liver fluke include *Fasciola gigantiga* and F. *hepatica*, with the former being more prevalent in cattle and the latter sheep and goats (Ozung *et al.*, 2011)

Fascioliosis caused by *Fasciola hepatica* and *Fasciola gigantica* regarded as one of the most important parasitic diseases in the world and as meat consumption, responsible for significant public health problems and substantial economic losses to the livestock industry (Odigie *et al.*, 2013).

Both *F.hepatica* and *F.gigantica* are transmitted by the snails of the family *L.ymnaesida* Infestation with fasciolosis is usually associated with grazing wet land and drinking from the snail infesting watering places. (Dechasa *et al.*, 2012).

Fasciola gigantica is a parasite of cattle, sheep and wild animal in the tropic and sub-tropics, and are more pathogenic than fasciola hepatica this is very similar parasite to fasciola hepatica and it is found more commonly in tropical regions of the world. Areas affected include Africa, Asia, many pacific island including Hawaii, the Middle East, Southern Europe, and south of USA. Fasciola hepatica has become increasingwide spread in New Zeland in recent years following the colonization of a large area of the country y by the exotic snail. (Elhaj., 2001).

The development of fasciolosis involves the presence of an intermediate host (*Lymnaea sp.*), suitable habitats for mollusks and environmental factors such as high humidity, optimum temperature and rainfall. Furthermore, when infecting the definitive host, mature flukes lay eggs that spread in the environment and cause pasture recontamination (Silva*et al.*, 2007).

Fascioliosis or liver fluke is worldwide distributed. In the Sudan the disease is highly endemic and reported in many areas of the country such as Upper Nile, Blue Nile, White Nile, as well as Northern states (Koko *et al.*, 2003). Liver fluke infections caused by *Fasciola hepatica*, Fasciola gigantica, are major public health problems in East Asia, east Europe, Africa and Latin America. Currently, more than 780 million people are at risk of infection with fasciolosis. (Marcos *et al.*, 2008). Among many parasitic problems of farm animals, fasciolosis is a major disease, which imposes direct and indirect economic

impact on livestock production, particularly of sheep and cattle, through mortality, liver condemnation, reduced production of meat, milk, wool and cost for anthelmintics and apart from its veterinary and economic importance Through the worm fasciolosis has recently been shown to be are emerging and widespread zoonosis affecting many people (Belay *et al.*, 2012).

Surveys in some Asian countries have shown that among domestic animals, cattle are the most suffering animals from fasciolosis (Kuchi, 2011).

Several reports exist on how variable climatic factors and patterns determine major period and level of fasciolosis transmission in the divergent agro -ecologic zone in the world. Apart from climatic factors, other factors including the sex of the animals have been suggested as a variable that could influence the prevalence of fasciolosis in cattle (Abedokun *et al.*, 2008).

Fasciola hepatica has succeeded in expanding from its European original geographical area to colonize five continents, despite theoretical restrictions related to its biology and in turn dependent upon environmental and human activities (Mas-Coma, 2005). The fluke species are hermaphroditic, have similar life cycles, and cause similar clinical manifestations in animals. Climatic diversities are particularly important to the development of the snails that act as intermediate hosts for fasciolias is in areas with differing environmental characteristics. Techniques have identified two fasciolias, including F. hepatica and F. gigantica. It is established that areas with only one Fasciola spp. are distinct from local and zonal areas where both Fasciolids co-exist (Amor et al., 2011).

Justification:

Fascioliasis is a zoonosis, a disease of animals that can be transmitted to humans. Susceptible animal reservoir hosts for *Fasciola species* include:

- The main domestic animals: cattle, sheep, pigs, buffaloes and donkeys.
- Other domestic animals: horses, goats, and camels.
- Sylvatic animals (hares, rabbits and rodents).

Sheep and cattle are the most important definitive hosts of *Fasciola hepatica*; goats, buffalo, horses, camels, hogs, deer, and rabbits can also be infected (Kaplan., 2001). Fascioliasis is a major constraint in development of livestock industry causing huge economic losses. It causes reduction in productivity of animal in terms of lowered growth rate, meat and milk production, fertility, feed efficiency and draught power (Asrat, 2004).

Condemnation of infected livers and cost of control measures are other sources of economic loss. It has been estimated that economic losses due to Fascioliasis reached up to US\$ 2 billion per year worldwide (Mas-Coma., 2009). Animal resources in the Sudan are considered as one of the largest in the Arab and African countries. Cattle played a significant role in the economic cycle in rural and urban areas.

Intensive and semi-intensive production system of Sudan distributed either within aggregation sites in different locations or in small herds located

The objectives of the study:

Main Objective:

To investigate the presence of bovine fasciolosis at Hilla Kuku, Khartoum State.

Specific Objectives:

- 1. To determine the prevalence of bovine Fasciolosis at Hilla Kuku, Khartoum.
- 2. To investigate potential risk factors which associated with bovine Fasciolosis in the study area.

CHAPTER I

LITERATURE REVIEW

1.1 Definition:

Fasciolosis is an economically important parasitic disease, which caused by trematodes of the genus *Fasciola* that migrate in the hepatic parenchyma, and establish and develop in the bile ducts *.Fasciola* is commonly recognized as liver flukes and they are responsible for wide spread of morbidity and mortality in cattle characterized by weight loss, anaemia and hypo proteinaemia. The two most important species, *Fasciola hepatica* found in temperate area and in cooler areas of high altitude in the tropics and subtropics and *Fasciola gigantica*, which predominates in tropical area (Mulugeta *et al.*, 2011).

The distribution of fasciolosis is linked to climatic factors, management of reservoir animals, topographic factors and presence in the environment of molluscs of the genus *Lymnaea*, which are needed for the parasite's life cycle to be completed (Bernardo *et al.*, 2011).

The snail of the genus *Lymnae natalensis* and *Lymnae truncatula* are known as intermediate host in life cycle of fasciolosis. Infection with *Lymnea truncatula* is usually associated with herds and flocks grazing wet marshy land. On the other hand, *Fasciola gigantica* is a fresh water snail and infection with this species is associated with livestock drinking from snails infected watering places as well as with grazing wetland (Mulugeta *et al.*, 2011).

It is estimated that 17 million people are infected in the world and 91.1 million are at risk of infection, both species overlap in many areas of Africa and Asia, whereas *F. hepatica* is a major concern in the Americas, Europe and Oceania.

Geographical pattern of these flukes is not uniform. The rural areas of the Andean Region of Peru and Bolivia are the most constantly affected regions in the world with prevalence rates between 6 and 68%. The proximity of these rural areas to big industrialized cities creates a potential source of infection to non endemic area (Marcos *et al.*, 2008).

In tropical regions, fasciolosis is considered as the single most important helminthes infection of cattle with prevalence rates of 30-90% in Africa, 25-100% in India and 25-90% in Indonesia (Fekadu *et al.*, 2012).

1.2 Epidemiology:

The epidemiology of fasciolosis depends on the grazing habitat preference of the animal. Metacercariae can survive up to 3 months after harvesting in hay from endemic high land areas that are consumed by ruminants in arid and low land areas (Dechasa, 2012).

Fasciola. Hepatica is mostly encountered in temperate areas, and in cooler areas of high altitude in tropics and subtropics, whilst *F. Gigantica* predominates in tropical areas. Snails are their intermediate host. Amphibious snails of the genus Lymnaea species are widely distributed throughout the world.

1.3 Geographic distribution and prevalence of Fasciolosis in the world:

Fascioliasis has the widest geographic spread of any emerging vector-borne zoonotic disease occurring in more than 51 countries worldwide (Mas-Coma; 2009), while 91 million are at risk worldwide (Keiser and Utzinger, 2009). The cercariae of liver flukes were observed from a pond first time by OttoMuller in 1773 (Andrews, 1999). According to Wikipedia Free Encyclopedia (2010), countries where Fascioliasis of ruminants was repeatedly reported are:

- Europe: UK, Ireland, France, Portugal, Spain, Switzerland, Italy, Netherlands, Turkey, Germany,
 Poland.
- Asia: Russia, Thailand, Iraq, Iran, China, Vietnam, India, Nepal, Japan, Korea, Philippines.
- Africa: Kenya, Zimbabwe, Nigeria, Egypt, Gambia, Morocco.
- Australia and the Oceania: Australia, New Zealand.
- Americas: United States, Mexico, Cuba, Peru, Chile, Uruguay, Argentina, Jamaica, Brazil. The prevalence of *Fasciola* infection depends on several factors related to the biology of the vectors, biology of the parasite and the management of flocks and herds. A lot of prevalence studies have been carried out to investigate the level of spread of *Fasciola* species infections.

2.4 Geographic distribution and prevalence of Fasciolosis in Sudan:

Bovine Fasciolosis in the Sudan is endemic in White Nile, and South Sudan in Upper Nile, Bahr El Ghazal and Equatoria. Some foci of Fasciolosis also exist in Southern Kordofan and Southern Darfur province. Fasciolosis undoubtedly accounts for serious eeconomical losses in the Sudan as a result of mortality, reduced productivity, expenses of treatment and particularly condemnation of infected livers (Haroun; 1975).

1.5 Taxonomy and classification:

Kingdom: Animalia

Phylum: Platyhelminthes

Class: Trematoda

Order: Digenea

Family: Fasciolidae

Genus: Fasciola

Species Fasciola hepatica and Fasciola gigantica (Saria, 2011)

1.6 Etiology:

Fasciolasis is caused by *Fasciola gigantica and Fasciola hepatica*. Adult flukes of both species (*Fasciola hepatica* and *Fasciola gigantica*) live in the bile ducts and immature live in the parenchyma of the liver (Vercruysse *et al.*, 2001).

1.7 Morphology of Fasciola:

Fasciola gigantica measures 4 to 10 cm in length (Figure 1), and the distribution of the species is limited to the tropics and has been recorded in Africa, the Middle East, Eastern Europe and south and eastern Asia (Torgerson et al,. 1999). Fasciola hepatica measures 2cm to 3 cm in length by 1.3cm in width (Figure 2), and has a cosmopolitan distribution. Fasciola hepatica is one of the largest fluke in the world. The adult worm has a very characteristic leaf shape with the anterior end being broader than the posterior end and anterior cone and a ventral sucker at the base of the cone which allow it to attach to the lining of the biliary ducts. Each worm possesses ovaries and testes which are highly branched and allow for individual flukes to produce eggs independently (Usip et al., 2012).



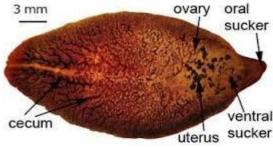


Figure (1.2): Morphology of mature adult worm adult worm of *F. gigentica*

Figure (1.2): Morphology of mature of *F* .hepatica

Source: http://www.parasitesinhumans.org/fasciola- hepatica-liver-fluke, 2021

1.8 Morphology of Fasciola Eggs:

The *Fasciola* egg is yellow-brown in color, large and oval in shape. It has an indistinct operculum (lid). It contains an unregimented ovum surrounded by many yolk cells (Monica, 1987). The shape of the eggs (Figure 3) of the two flukes is also very similar with the measurements of *F. hepatica* and *F. gigantica* being approximately $150\mu m \times 90\mu m$ and $200\mu m \times 100\mu m$, respectively (Saira , 2011).

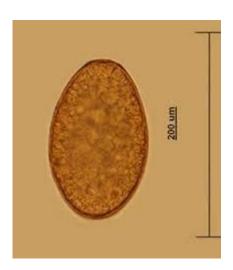


Figure (1.3): Morphology of *Fasciola* Egge **Source** http://upload.wikimedia.org/wikipedia/commons, 2021.

1.9 Host of fasciola:

The adult fluke is a natural parasite of sheep and cattle but also may infect the horse, goat, camel, Lama, elephant, buffalo, dog, rabbit, guinea pig, monkey and man, (Mahrukh *et al.*, 2011).

1.10 Intermediate Host:

Information from the studies indicates that the snail intermediate host, populations undergo marked seasonal variations in density with generally low densities during the rainy period and high densities in the post-rainy periods. The snail host(fig3) of *F. gigantica* in tropical Africa is *Lymnaea Natalensis* (Moayad *et al.*, 2011). The numerical size of the population is dependent on several climatic factors, such as flooding, desiccation and temperature and on the natural rate of increase of the snail species following catastrophes. Information gained from the observations indicates that the transmission of trematodes by the intermediate hosts is high during the dry season. The increase in transmission during the dry season is attributed to decreased water volume observed in the habitats during the dry season leading to high focal concentration of the intermediate hosts (Pfukenyia *et al.*, 2005).

This is accompanied by increased contact of the habitats by livestock due to scarcity of pasture and increased grazing a round water bodies, There by favoring accumulation of trematode eggs in close proximity to snail habitats. these factors result in increased frequency of contact between miracidia and snail intermediate hosts thereby increasing the prevalence of infection in the latter (Pfukenyia*et al.*, 2005).



Fig. (1.4) *Lymnaea* **Snail** DPDx- Laboratory identification of parasites of Public Health Concern

1.11 Transmission:-

Both *F. hepatica* and *F. gigantica* are transmitted by the snails of the family *lymnasidae*. Infection with fasciolosis is usually associated with grazing wet land and drinking from the snail infesting watering places (Dechasa *et al.*, 2012). There are many ecology factors affecting snail population include temperature, light, hydrogen ion concentration (PH), vegetation, depth of water, chemical composition of soil and snail population competition. The most important intermediate hosts of Egigantica in Sudan is *L. natalensis*, *L. aurcularia*; however *L. rufescens* and *L. acuminate* are the host snails in the Indian subcontinent; *L. rubigiosa* and *L. natalensis* are the hosts in Malaysia and in Africa respectively. The most important and widespread (Europe, Asia, and North America) intermediate host of *F. hepatica* is *L. truncatula*. (Soliman., 2008).

1.12 Life cycle:-

The life cycle of *Fasciola* species occurring in any particular area the following conditions must be satisfied. There must be an initial presence of infected final hosts, the intermediate snail host must be present and there must be an opportunity for transmission of the parasite from the final host to the snail habitat (Mahato *et al.*, 2000).

Adult liver flukes reside in the bile ducts of host animals, and eggs are passed onto the pasture in the feces. After a short period of development (usually 2 to 3 weeks), a miracidium hatches from the egg and attempts to find and penetrate a snail intermediate host. The parasite develops and replicates asexually in the snail over many weeks. Under optimal conditions, parasite maturation within the snail to the cercarial stage takes approximately 5 to 7 weeks, and a single miracidium can develop into several hundred cercariae. Under wet conditions, cercariae emerge from the snail and swim until they find and attach to vegetation. The cercariae then shed their tails and secrete a protective coat, forming the encysted infective stage called metacercariae. Cattle become infected primarily by ingesting the metacercarial cysts on forage, but they also can become infected by ingesting cysts suspended on soil and detritus while drinking contaminated water. The length of time that metacercariae survive on pasture primarily depends on available moisture. Under the hot and dry pasture conditions of coastal Texas during the summer, metacercariae were rapidly killed however, under conditions of high humidity, during the summer, metacercariae may survive for extended periods. Once ingested by a ruminant host, the metacercariae excyst, releasing juvenile flukes. The juvenile flukes penetrate the wall of the small intestine, migrate through the peritoneal cavity over a week's time, and then penetrate through the liver capsule. Juvenile flukes migrate through the hepatic parenchyma for approximately 6 to 8 weeks before entering the bile ducts

where they mature. Egg production can begin as early as 8 weeks after infection however, most infections do not become patent until after approximately 11 to 12 weeks. Thus, completion of the entire parasite life cycle, from the time an egg is shed on to pasture until anewly infected animal re infects the pasture with the next generation of fluke eggs, generally requires 18 to 24 weeks (4.5 to 6 months) (Kaplan., 2001).

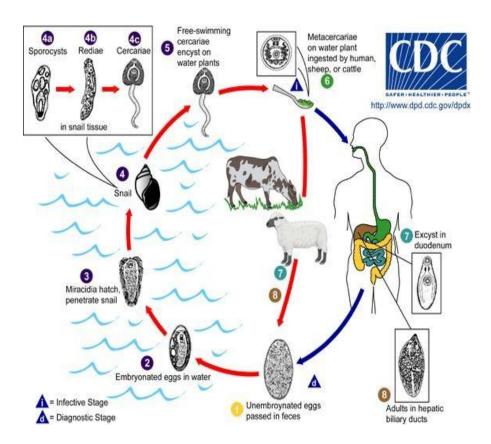


Figure (1.5): Life cycle of *Fasciola* (http://www.dpd.cdc.gov/dpd) 2021.

1.13 Pathogenesis and pathology:

Pathology associated with disease are caused by the inflammation of the bile duct which causes thickening of the lining and eventually leads to fibrosis that results in reduced flow of the bile and back pressure builds leading to atrophy and necrosis of the liver parenchyma, causing liver abscesses (Michael, 2004).

Immature wandering flukes destroy liver tissue and cause hemorrhage. In acute fasciolosis damage is extensive the liver Is enlarged and friable with fibrinus deposit on the capsule. Migratory tract can be seen, and the surface has an uneven appearance. In chronic cases cirrhosis develops. Mature fluke damage the bile duct witch become enlarged or even cystic, and have thickened, fibrosed wall. In acute the duct wall become grating thickened and often calcified .fluke maybe found in aberrant sites e.g. lung (Veterinary Manual, 2005).

The principal pathogenic effects of flukes are anemia and hyper albuminaemia. More than 0.5 ml blood per fluke per day can be lost within the bile duct. In acute form, there is massive invasion due to immature flukes into the liver which cause sudden death while in chronic from, there is liver cirrhosis caused by the wandering flukes which when mature. Calcification of bile ducts and enlargement of gall bladder has been noticed in chronic cases and sub mandibular edema frequently occurs (Salam et *al.*, 2009).

Traumatic injury caused by the migrating flukes, tracts of coagulative necrosis develop, which result in a diffusely fibrotic hepatic parenchyma containing hemorrhagic streaks and foci. These lesion can predispose cattle to black disease (infection necrotic hepatitis) and bacillary hemoglobinueia due to *Clostridium novii and Cl. haemolyticum* respectively (Kaplan *et al.*, 2001). Also chronic inflammation, chronic injuries and regenerative hyperplasia of the bile duct epithelium may be related to malignant transformation. Recent reports have identified *Fasciola hepatica* as a neoplastic risk agent, primarily in animals, which could cause the genetic damage of the surrounding host tissue (Azra *et al.*, 2004).

1.14 Clinical finding:

Acute fascioliasis is common in sheep and goats while the chronic form is found mostly in cattle. Symptoms of fasciolosis include anemia, emaciation and reproductive dysfunction in animals with the chronic form. While in acute fascioliasis; the animals usually show signs of anorexia, dullness, diarrhea, muscular atrophy, subcutaneous edema and impaired immune systems. Hepatic fascioliasis is often characterized by a swollen. (Ozung *et al.*, 2011).

Sub- acute fasciolosis is characterized by jaundice, some ill thrift and anemia. The burrowing fluke causes extensive tissue damage, leading to hemorrhage and liver damage. The outcome is severe anemia, liver failure and death in 8–10 weeks (Boray, 2007).

Chronic fasciolosis is the most common form of liver fluke infection in sheep, goats and cattle – and particularly in more resistant hosts, such as horses and pigs. It occurs when the parasites reach the bile ducts in the liver.

The fluke ingests blood, which produces severe anemia and chronic inflammation and enlargement of the bile ducts. The clinical signs develop slowly. The animals become increasingly anemic, appetite is lowered, the mucous membranes of the mouth and eyes become pale and some animals develop edema under the jaw (bottle jaw). Affected animals are reluctant to travel.

Fasciolosis may not show any obvious symptoms. Some animals may show abdominal pain and may become jaundiced. Death is usually due to blood loss resulting from hemorrhage in the liver. The liver hemorrhage is the result of the immature fluke burrowing through the liver (Boray, 2007).

1.15 Diagnosis:

Fasciolosis should be considered when there are deaths, anemia. Diagnosis in dead animals relies on seeing mature or immature fluke in the liver (Bory, 2007).

Chronic fasciolosis is indicated by fluke eggs in fecal samples. The sampling technique is generally reliable in sheep but much less so in cattle. Fecal examination for *F. gigantica* egg was carried out using the sedimentation method described by rep was examined for eggs using a modification of the method described. This was carried out by mixing equal volumes of bile and water, straining through a tea strainer before centrifuging at 3000 rpm. After obtaining a clear supernatant by repeated mixing of sediment with water and Centrifuging, the sediment was examined under the microscope (Adodokum *et al.*, 2008).

Serological test (ELISA) is also available for diagnosis of fasciolosis. It detects infection with both immature and adult fluke in a flock or herd, but it is not sensitive enough for diagnosis, in evidences show that sero diagnosis can detect the presence of infection as early as 2 weeks after

infection. Furthermore, serological methods like Enzyme Linked Immune sorbent Assay (ELISA) can detect serum antibodies to specific antigens of *Fasciola sp.* using adult fluke extracts, or excretory/secretory (ES) materials. Also Agar Gel Diffusion Test (AGDT) has also been demonstrated to be simple and valuable for detection of *Fasciola sp.* (Adodkum *et al.*, 2008).

1.16 Public health significant:

Fasciolosis is a parasitic disease caused by the fluke *Fasciola hepatica*the disease is acquired for the most part by eating watercress, other vegetables or by drinking water contaminated with metacercariae. Human fasciolosis is a serious health problem in many countries, the number of reported cases have increased significantly worldwide. One of the remarkable clinical characteristics of human fasciolosis in endemic areas is the relative absence of parasites cause bile duct obstruction or hepatic dysfunction. Early diagnosis and treatment might avoid expensive and risky procedures. The early detection gastrointestinal symptoms 1n *some* cases are diagnosed during surgery (Marcos *et al.*, 2005).

Fasciola may give rise to condition known as "halzoan" This is characterized by an acute irritation of the throat, which is due to the transient attachment of the fluke ingested in row liver. Wide variety of other mammals' could be infected by ingestion of contaminated vegetation (Elhaj, 2001). Man is seldom infected by the common liver fluke and number reported probably amount to not more than 300 cases .Man usually infects himself by eating water grass grown in water in which the snail are living and which is contaminated by the faces of the fluke infested cattle or sheep. And the symptoms in human patient including urticaria, jaundice, enlarged tender liver and eosinophilia. (Elhaj, 2001) The prevention of human fasciolosis may be achieved through strict control of watercress and other metacercariae-carrying aquatic plants for human consumption, especially in endemic zones. Among vegetables incriminated freshwater plant species which may differ according to geographic zones and human dietary habits (Mas coma et al., 2005.

1.17 .Immunity of Fasciola:-

It has been reported that sheep and cattle do not develop strong immunity to infection with F. hepatica, or to re- infections, and this lack of resistance in ruminants is believed to be associated with the inability of their macrophages to produce nitric oxide. (Phiri et al., 2005). Several studies suggest liver flukes to elicit oxide. Immune responses typical of the Th2-type with eosinophilia, IgE and IgGI antibody production. It is thought that helminths are able to with stand the effects of some components of the Th2 arm of the host immune response, and although these responses cause pathological damage to the host it can be tolerated over a long and sustained period. Migrating and adult F. hepatica seem to secrete substances that may include Th2 responses, and products such as cathepsin L proteases appear to actively lessen Th1 responses (Phiri et al., 2005). Adult flukes in bile ducts are thought to be immunologically safe, and can survive for many years at these sites, although they still secrete antigens, which may be responsible for maintaining a Th2- immune response during chronic fasciolosis (Phiri et al., 2005). Naturally infected animals produce high IgGI antibody titres controlled by the Th2 cytokine IL4, but little or no IgG2 at all. In contrast, both IgG1 and IgG2 are produced in vaccinated animals. The titer of IgG2 antibodies seem to be, in general, directly correlated with the fluke burden of animals and it was found that in vaccinate groups animals with the lowest liver fluke numbers had the highest IgG2 levels. This may inactivate parasitic enzyme activation, block parasite migration and feeding. It may also be detrimental to the parasites as they may also activate complement and enhance phagocytosis by macrophages. Sheep macrophages do not produce nitric oxide following binding to sera from infected animals, but they may become activated in the presence of IgG2. Eosinophils and neutrophils may also contribute to killing already damaged flukes . It has been seen, however, that F. hepatica and F. gigantica have different immune modulation and strategies to evade the host immune responses and it seem that in practice F. gigantica homologues of antigens with protective properties again.

1.18 Economic impact:-

1.18.1 Weight gain:

Monitoring weight gain of young animals is a useful way to assess the impacts of infection and interventions to prevent or control infection, improved nutrition or treatment with an anthelmintic. Younger growing animals are more susceptible to infection, less costly to maintain under experimental conditions and any results can be applied to production system that are producing young growing animals for sale (Copeman *et al.*, 2012).

1.18.2. Draught performance:

Anemia resulting from fasciolosis has been shown to reduce work output by 7-15%, combined with a further indirect reduction of 20% in potential work capacity in animals whose growth has been restricted by fluke infection. It can be concluded that liver fluke can seriously lower the work potential of both cattle and buffalo. The economic significance of this may, however, be changing rapidly production systems where hand tractors are replacing animals as sources of draught power (Copeman*et al.*, 2012).

1.18.3. Fertility:

A link has also been observed between infection with *F. gigantica*, anemia and fertility. There were significantly longer inter-calving intervals and lower packed cell volume in Ongole cows in Indonesia infected with *F. gigantica* than in those treated with Triclabendazole each July for two years. Also anther study found that treated cows had a mean inter calving interval of 18.5 months whereas in untreated cows the interval was 31.5 months. It is thus reasonable to conclude that infection with *F. gigantica* is likely to adversely affect reproduction (Copeman *et al.*, 2012). Many studies confirmed that fasciolosis is an important disease entity causing considerable loss of revenue due to condemnation of affected liver and carcass weight reduction (Dechasa *et al.*, 2012).

1.18.4 Bacteria associated with fasciolosis:

Type of bacteria found in the carcasses may be of non specific groups which comprises that are non pathogenic or only potentially pathogens as <u>Clostridia</u>, <u>Streptococci</u>, <u>Bacillus subtitis</u>, Enterococci and <u>E.coli</u> in adult animals. These species are present naturally in the intestinal florapathogens including heamylotic Staphylcocci, Pasterulla, <u>Salmonella</u> and <u>Listeria moncytogenes</u> (Ahmed, 2001)

1.19. Prevention and control:

The methods to control fasciolosis generally include strategic application of anthelementics to eliminate the parasite from the host at the most convenient time for effective prevention of pasture contamination, reduction of the number of intermediate host snails through drainage and other practices and reduction of the chances of infection by efficient farm and grazing management. In fact control of fasciolosis requires intervention of relationships between the environment, ruminant hosts, snail hosts, the parasite life cycle, agricultural cycles and animal husbandry procedures. Therefore, good understanding of the environment-host-parasite inter relationships are essential for

formulating the control measures suitable for an area (Mahato *et al.*, 2000) The types of control measures depend on the setting (such as epidemiologic, ecologic, and cultural factors). Strict control of the growth and sale of watercress and other edible water plants is important (Rapsch*et al.*, 2008). No vaccine is available to protect people against *Fasciola* infection. Individual people can protect themselves by not eating raw watercress and other water plants, especially from endemic grazing areas. As always travelers to areas with poor sanitation should avoid food and water that might be contaminated, Vegetables grown in fields that might have been irrigated with polluted water should be thoroughly cooked. other Suitable control strategies, such as pasture management strategies could help to avoid some of these losses. Geographical information systems such as risk maps could help identify areas where disease monitoring should be established. Since *F. hepatica* transmission is linked to its intermediate host *L. truncatula*, information on suitable environmental conditions can help locate possible areas with enhanced infection risk by means of cartography (Rapsch *et al.*, 2008).

The anthelminthic drugs recommended by the World Health Organization (WHO) as essential drugs to treat these diseases, namely Praziquantel and Triclabendazole (TCZ), WHO recommends the inclusion of FBTs in the group of helminthic diseases whose control relies on the preventive chemotherapy concept, i.e. early administration of anthelminthic drugs, either alone or in combination, to infected individuals to prevent overt morbidity in later stages of life. Life Triclabendazole (TCZ) is the treatment of choice for fasciolosis and is effective at a single dose of 10 mg/kg body weight against the adult parasites in the bile ducts and immature flukes migrating through the liver (WHO, 2006).

Strategic liver fluke treatment of all cattle and buffaloes which are older than 8 months should be carried out once a year. In addition, animals in poor condition should be treated to prevent severe losses, especially in high prevalence areas or where strategic treatment was Problems of liver fluke control include the lack of knowledge about the parasite at farmer's level and the lack of availability of drug supplies at the village level, or both. Which are important to allow strategic treatment and control of animals (Kuchai *et al.*, 2011).

Various drug such as Bithionol and Praziguantel are used for treatment of human fasciolosis, but with variable result. Emetine and Dehydroemetine are used frequently with significant efficacy in the treatment of human fasciolosis, but cause variety of serious and toxic side-effect. Preliminary studies on therapeutic effect of Triclobendazole have demonstrated that this drug is highly effective in human chronic fasciolosis (Pedros *et al.*, 2000).

CHAPTER II

MATERIALS AND METHODS

2.1. Study Design:

A cross-section epidemiological study was conducted to estimate the prevalence of bovine fasciolosis infection in dairy cattle farms and to investigate potential risk factors that could be associated with infection.

2.2 .Study Area:

The present study was conducted in Khartoum North, Khartoum, State, Sudan. Khartoum North Located at latitude 15⁰ 37` 59.99 N and longitude, 32⁰ 37` 59.99 E. Hilla Kuku Which the samples collected form sample frame.

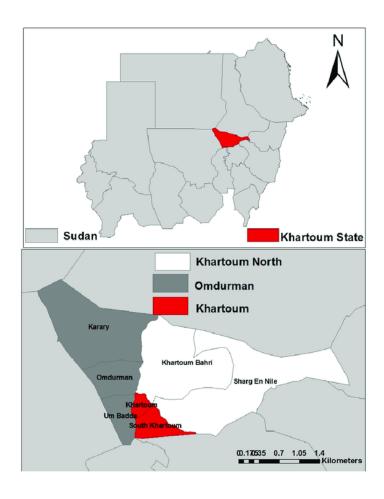


Figure 2.2: Location of Khartoum North, Khartoum, state Sudan including in Study (Hilla Kuku)

2.3. Sample size:

The sample size was calculated using formulaa (Thrusfield, 2005) based on last previous study (20.3%) for prevalence of bovine Fasciolosis in cattle conducted by (Mohammed, Abdel hamid A. Elfadel 2011) at Elkadaro Abattoir, Khartoum State, Sudan.

Multi stage random sample was used to select localities then simple random samples were collected by the last previous prevalence and sample size was 246 cattle (42 males and 204 females).

N = (1.96)2p(1-p)/(d)2

N= Required sample

P= Expected Prevalence

D= Desired absolute precision

N = (1,96)2*.,2*(1-.2)/(.05)2=246.

2.4. Questionnaire:

Questionnaire was filled in farm during the collection of samples from animals which including information:

General information about the herd and farms

Risk factors associated with the disease.

The risk factors including: (age, sex, body condition, uses of the treatment). The sex including two categories male and female. The age categories into three categories less than 1 year, 1-4 years, and over 4 years. The body condition include good and poor. The use of anthlemintics: yes or no

2.5. Methodology

2.5.1 Fecal samples collection and examination

Fecal sample examination was carried out at the laboratory of University of Sudan for science and technology. Fecal samples were collected directly from the rectum using plastic gloves. Specimens carried in a plastic container and transported to the laboratory for microscopic examination using sedimentation technique to detect the presence of *fasciola spp* eggs and identification was done on the basis of morphology of the egg. Water was added to feces into a container, mixed thoroughly then filtered througha tea strainer, the filtered material was poured into 15 ml centrifuge tube and centrifuged at 3000 rpm for 3minuts then removed from the centrifuge the tube had sediment, then the supernatant was discarded. Mixing of water and centrifuging until clear supernatant was obtained then a small drop of the sediment was transferred to slide microscope slide. Covered with cover slip and examined under the microscope at 10 magnification.

2.6. Statistical analysis:

All the data collected during the study period from November 2020 to February 2021. analysis of the data was carried out using SPSS version16. Descriptive statistical analysis was used and illustrated in frequency tables., Unavailable analysis was performed by *Chi* square and illustrated in tables showing the risk factors, number of animal tested, percentage of the positive number, and *p*-value. Risk factors with a *p*-value less than or equal 0.05 were entered in a multi- variate analysis using logistic regression to investigate association between bovine fasciolosis and potential risk factors. Results were illustrated in tables showing Exp B, 95% confidence interval and *p*-value. A *p*-value of 0.05 or less indicated significant association between bovine fasciolosis and the risk factors.

CHATER III

RESULTS

3.1 Fecal examination result:

Out of 246 cattle investigated for Fasciolosis, 13 cattle were found to be positive in fecal examination. The overall prevalence rate was 5.3% (13/246) through fecal examination.

3.2 The effect of Sex on Fasciolosis:

Total number of Male was 42 animals of 4 animals were found infected and the prevalence in males was (9.5)%.

Total number of Females was 204 animals. Among these 9 animals were infected and the prevalence in females was (4.4%), There was no significant association (*p*-value 0.177) between sex and Fasciolosis.

Table 3.1 The effect of Sex on Fasciolosis:

Sex	No examined	Positive (%)
Male	42	4 (9.5%)
Female	204	9 (4.4%)
Total	246	13 (5.3%)

3.3 The effect of Age on fasciolosis:

The age distribution of 246 cattle examined for fasciolosis by age showed that no animal was infected from the first age group (<1 year) 0 (0%).

4 animals were infected out of 90 animals (4%) among the age group 1-4 (years). And 9 animals were found to be infected and the prevalence was 7%. Out of 121 examined from the old animals group >4 (years) Table (3.3).

Table 3.2 The effect of Age on Fasciolosis:

No. examined	Positive (%)
35	0 (0%)
90	4 (4%)
121	9 (7%)
246	13 (5%)
	35 90 121

3.4. The effect of Anthelmentics uses on fasciolosis:

A total of 5 cattle out of 175 (3%) were tested positive from the cattle treated by anthelementics. While 8 cattle were tested positive out of 71 cattle (11%) not treated by anthelementics Figure (3.1). In the Chi-square test there was a significant association p-value (0.008) Between using drug and Fasciolosis.

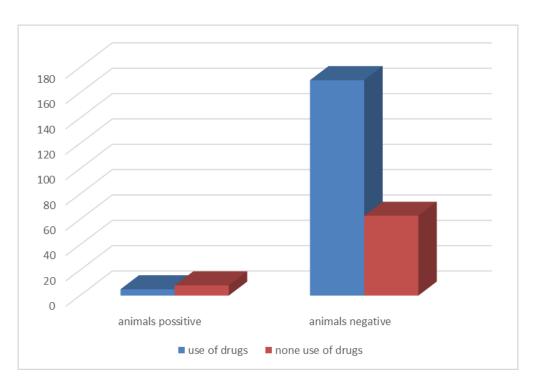


Figure (3.1): The effect of Anthelmentics uses on fasciolosis

3.5. The effect of body condition on fasciolosis:

A total of 5 positive out of 183 (2%) were from the good body condition, While 8 positive out of 63 (12%) of the poor body condition. figure (3.2). In the Chi-square test the result showed significant association p-value (0.002) between body condition and fascilosis.

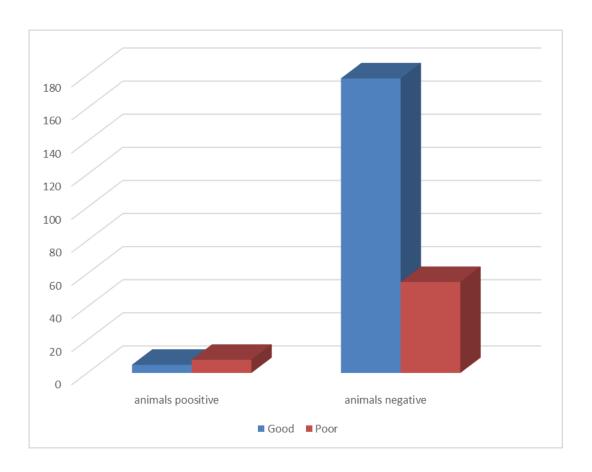


Figure (3.2) the effect of body condition on Fasciolosis

3.6. Result of multivariate analysis:

Potential risk factors found to be significantly associated (p-value<0.05)with Fasciolosis . In the univariate analysis were entered to logistic regression. In the final model two risk factor were found significantly associated (p-value<0.05) With Fasciolosis in the multivariate analysis. These risk factor included body condition (p-value=0.002), Antihelmetics (p-value=0.008).

Table 3.4 :Multivariate analysis for the association between Fasciolosis and Risk factor:

Risk factor	No. positive (%)	В	Sig	EXP	95%CL	
					Lower	Upper
Body condition		-1.45	.016	4.274	.072	.763
Good	183 (2%)					
Poor	63 (12%)					
Anthelmintics		-1.24	.039	3.467	.088	.941
Use drug	175 (3%)					
Non use drug	71 (11%)					
Constant		6.730	.000	837.16		

CHAPTER IV

4.1.DISCUSSION

Liver fluke is a common trematode that causes a parasitic disease called fascioliasis or fluke disease. It has a pathogenic effect on ruminants (cattle, sheep and goats) as well as other farm animals like horses, donkeys, pigs and to a lesser extent poultry Species of liver fluke include *Fasciola gigantiga* and F. *hepatica*, with the former being more prevalent in cattle and the latter in sheep and goats (Ozung *et al.*, 2011).

Previous studies on animal fasciolosis revealed that the disease is prevalent in the Sudan, such as Darfur, Khartoum, Blue Nile, Kassala and south Sudan such as Bahr ElGazal, Upper Nile and Equatorial States (Koko *et al.*, 2003). The annual reports since 1955 indicated that highest percentages of liver condemnation due to fascioliases were follows:

Upper Nile province (27-58%), Baher El Gazal province (16-18%), Equatorial province (13-40%). In all areas in Sudan fascioliasis had been reported in several places with high prevalence (Intisar, 1992).

This study was carried out at Hilla Kuku, North Khartoum, to investigate risk factors and to estimate prevalence of bovine fasciolosis in Khartoum state. The prevalence rate was 5.3% by fecal examination for fasciola eggs using sedimentation techniques.

However the prevalence rate in this study (5.3%) was lower than many other studies, Central Sudan by Koko *et al.*, (2003) where the prevalence was12.5%. This variation in result could be due to the geographical location and ecological condition such as rain fall and temperature, *Fasciola spp* prevalence had been reported to vary over the years mainly due to variation in amount and pattern of rain fall. In this study, the age was not significantly associated with fasciolosis the (*p*-value, 0.202) in fecal examination. The prevalence rate in old animals and middle age than young animals (7%,4%, 0%) respectively, These results aware in agreement with a previous study in Zimbabwe by Pfukeny *et al.*, (2006). Also this study is in agreement with another study in Kayseri province, Turkey by Yildrim *et al.*, (2007). However, current results also agreed with another study in Ginnir District by Fekadu *et al.*, (2012), where no significant differences were observed between age groups.

In this study, the sex was not found to be significantly associated with fasciolosis (*p*-value=0.177), This finding is not in agreement with a study carried out around Assela by Mulugeta *et al.*, (2011) who found significant association between sex and ffasciolosis, this might be due to the different geographical location.

Body condition of animal in the current study was found to be significantly associated with fasciolosis (*p*-value=0.002). This finding is in agreement with a study carried out in southern Espirato Santo by Bernardo *et al.*,(2011). However, it does not agree with a previous study in Sowth Wollo zone,

The prevalence rate of fasciolosis in poor animal condition (12%) was higher than the prevalence rate of good condition (2%). This finding suggest the importance of bovine fasciolosis in causing weight loss and emaciation as a characteristic sign of the disease.

Use of anthelmentics was found significantly association with fasciolosis (*p*-value 0.008), The prevalence is higher in animals whose owner did not used drug (11%) than in animals whose owner used drug (3%). This result is attributed to effectiveness of the drug use, Joseph (2007). Reported that the drugs play an importance role in the control of bovine fasciolosis.

In this study there were two risk factors found to be significantly associated (p-value <0.05) with bovine fasciolosis in the multivariate analysis. These two risk factors included: Body condition of animal and use of drug.

4.2. Conclusion

In view of the findings fasciolosis is prevalent in Hilla Kuku, North Khartoum. the study further confirmed that fasciolosis diagnosed through fecal sedimentation was low because of these animals were kept in door. The prevalence of fasciolosis was higher in poor body condition compared to animals with good body condition. The prevalence of fasciolosis was higher in Males than Females. The Prevalence of fasciolosis was higher in animals whose owners did not use anthelmintics compared to those their owners used anthelmintics.

4.3.Recommendations:

- Increase the awareness of the disease among animal's owners.
- Encourage the use of anthelementics for the farm animals.

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Appendences

Appendix (a)
Questionnaire:

بسم الله الرحمن الرحيم جامعة السودان للعلوم والتكنولوجيا كلية الدراسات العليا ماجستير الطب الوقائي

معدل انتشار داء الديدان الكبدية في الأبقار في حلة كوكو، ولاية الخرطوم ،السودان

محلية	••••••	التاريخ
		_
10		
عوامل الخطر		
عمر الحيوان:		
4-1 ()> 1	()<4 () 4-	
جنس الحيوان		
بسل اسیوان ذکر ()	أنثي ()	
()3		
الحالة الجسدية للحيوان	ان	
ختر () جخ	ضعیف ()	
	•1	
إستخدام مضادات الديدان		
نعم () لا	لا ()	