Introduction

Background

Fasciolosis is an economically important parasitic disease which is 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 that are responsible for wide spread of morbidity and mortality in cattle characterized by weight loss, anemia and hypo proteinemia. 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 **(**Mugugeta *et al*., 2011).

Fascioliosis caused by *Fasciola hepatica* and *Fasciola gigantica*is are regarded as one of the most important parasitic diseases in the world. Fasciolosis is an important parasitic food borne disease, 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 lymnae .Infestation with fasciolosis is usually associated with grazing wet land and drinking from the snail infested watering places**.** (Dechasa *et al*., 2012).

Fasciola gigantica is a parasite of cattle, sheep and wild animal in the tropic and sub -tropics, and is more pathogenic than *Fasciola hepatica* . *F.gigantica* 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 increasing wide 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, adequate 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**)**.

The incidence of fascioliosis in the Sudan has probably increased during recent years as result of agricultural extension and introduction of canalization and pump. Also fascioliosis in Sudan have economic losses a result of livestock mortality and more often due to loss in production and condemnation of infected tissue in the slaughter house ,besides, large amount of money is spent annually on treating infected cases (Altahir.,1975).

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 sheep and cattle**,** through mortality, liver condemnation, reduced production of meat, milk, wool and cost of anthelmintics. Through the world fasciolosis has recently been shown to be an emerging and widespread zoonosis affecting many people **(**Belay *et al.,* 2012**)**

Considering the worldwide spread, occurrence and zoonotic nature, fasciolosis has emerged as a major global and regional concern affecting all domestic animals and infection is most prevalent in regions with intensive sheep and cattle production (WHO, 2007)

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

Several reports exist on how variable climatic factors and patterns determine 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 (Adodokum *et al*., 2008).

Severe out- break with heavy mortalities were encountered in the White Nile .Province among cattle, sheep and goat. In 1959 an outbreak occured in Bahr El ghazal province in which 60 cows perished (Saeed.,1992). In 1973 a high number of sheep near Kosti died from acute fascioliasis. *Fasciola gigantica* was first mentioned in 1914 in the annual report of the Sudan veterinary service (S.V.S).In 1938 fascioliasis was again reported in Malakal.The report of 1953-1954 stated that the incidence of fascioliosis increased in Kosti and Eldueim. Annual reports since 1955 indicate that highest percentages of liver condemnation due fasciolosis are as follows: Upper Nile province (27-58%), Baher El gazal province (16-18%), Equatorial province (13-40%).in all area in Sudan fascioliasis has been reported in several place with high prevalence (Saeed.,1992).

Objectives:

The objective of the study were

(1) To Estimate the prevalence of bovine fasciolosis in Khartoum state.

(2) To investigate the potential risk factors which could be associated with bovine fasciolosis .

Chapter One

1. LITERATURE REVIEW

1.1. Definition:

Liver fluke is a common trematode (flat worm) that causes a parasitic disease called fasciolosis 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).

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.*F.hepatica is transmitted by Lymnea truncatula* and the infection is usually associated with herds and flocks grazing wet marshy land. On the other hand, infection with *Fasciola gigantica* which is transmitted by *L.natalensis* is associated with livestock drinking from snail's infected watering places as well as with grazing wet land **(**Mugugeta *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:

A total of 208 cattle were randomly selected among slaughter houses, household and livestock farms to determine the prevalence of fascioliasis. Epidemiological studies on *Fasciola hepatica* in cattle were undertaken in such localities under different climatic conditions existing in Ladakh region of Jammu and Kashmir State. Infection rate was 51.42%, 27.69% and 21.91% in slaughtered, livestock farm and household cattle, respectively. Significant variations were observed in the prevalence with respect to various host factors and the climate of the study area. Overall, the highest seasonal prevalence (45.19%) in all types of cattle was recorded during wet season while as only (24.40%) was recorded during the dry season. It was noticed that a higher infection rate was recorded in young cattle ages (0- 2 years) (40.02%) than in adult ones (28.04%) (3- 8 years). Moreover, the prevalence of infection in females was more (38.07%) than males (29.09%). It was also observed that the infection rate of *F.hepatica* was high in low land areas (37.14%) as compared to high altitudes (30.09%). This study will provide necessary information regarding fascioliasis in cattle of Ladakh for their effective control and hence for a better production which will be beneficial resource to *poor* people where livestock rearing is one of the important sources of livelihood (*Kuchai et al., 2011).*

Also a cross-sectional study was carried out from November, 2011 to March, 2012 at Nekemte municipal abattoir in Ethiopia, to assess prevalence and economic significance of bovine fasciolosis. Out of 384 cattle examined at post mortem, 21.9% (84) were positive for fasciolosis. The prevalence of bovine fasciolosis was found to be significantly affected ($P < 0.05$) by the age of animal, in which young animals were more affected than adult animals. The prevalence of bovine fasciolosis was also higher ($P < 0.05$) in poor body conditioned animals than medium and good body conditioned animals. Sex of the animal was not found as a significant factor ($p > 0.05$) affecting the prevalence of disease. The prevalence of *Fasciola hepatica* was 14.1% (54), which was predominant among *Fasciola* species, causing bovine fasciolosis in the study areas. Whereas, the prevalence of *Fasciola gigantica* was 5.2% (20), and 2.6% (10) animals were mixed infected. The economic significance of bovine fasciolosis was also assessed based on condemned livers. Thus, based on retail value of bovine liver, the direct economic loss from fasciolosis during the study time was estimated to be 63072 ETB annually**.** (Petros *et al*., 2013)

Another study was conducted to evaluate the economic losses and temporal distribution of the prevalence of liver condemnation due to bovine fasciolosis. The abattoir in Atilio Vivacqua, in the South of the State of Espirito Santo in Brazil, which is under state inspection by the Veterinary Service of the Livestock and Forest Protection Institute of Espirito Santo, was used as the data source. The prevalence of liver condemnation due to fasciolosis over the period 2006-2009 was calculated. The $\chi 2$ test, simple linear regression analysis and $\chi 2$ for trend were used, with significance level of $p \leq 0.05$. Over the period analyzed, 110,956 cattle were slaughtered and the prevalence of liver condemnation due to *Fasciola hepatica* was 15.24% in 2006, 23.93% in 2007, 28.57% in 2008 and 28.24% in 2009. The historical trend of liver condemnation is an increasing trend, thus indicating that this parasitism has become established in the herd as a problem in this region, with prevalence similar to that of traditionally endemic regions. Condemnations occurred throughout the year, with the highest prevalence in April and May and with significant differences between the dry and wet seasons. The economic losses from liver condemnation can be considered high. (Bernardo *et al*., 2011).

Also a total of 239 cattle were sampled across eight locations ranging in elevation from 1112-2072 m. Fecal material was examined for presence of *Fasciola* eggs and sera were tested by ELISA for antibodies against *Fasciola* antigens. Bolstering this, 38 cattle at slaughter from 2 abattoir sites at 1150 m and 1947 m above sea level were inspected; in addition, wild buffalo stool (n = 10) opportunistically picked within Mount Elgon National Park (MENP) at 3640 m was examined. By fecal egg detection, prevalence of *Fasciola gigantica* at low (<1500 m) and high (>1500 m) altitude sites was 43.7% (95% CI 35.4-52.2) and 1.1% (95% CI 0.0-6.0), respectively, while by ELISA was much higher, low altitude - 77.9% (95% CI 69.7-85.4) and high altitude - 64.5% (95% CI 51.3-76.3). The decline in prevalence with increasing altitude was corroborated by abattoir sampling. Thirty seven aquatic habitats, ranging from 1139-3937 m in altitude were inspected for freshwater snails, 12 of which were within MENP. At lower altitudes, *Lymnaea* (Radix) n*atalensis* was common, and often abundant, but at higher altitudes became much rarer ceasing to be found above 1800 m. On the other hand, *Lymnaea (*Galba) *truncatula* was found only at altitudes above 3000 m and within MENP alone. The snail identifications were confirmed by DNA analysis of the ribosomal 18S gene. **(**Howell et *al*., 2012).

In the winter of 1998/1999, sheep on a farm in the province of North Holland, The Netherlands, died from subacute and chronic liver fluke disease despite four previous treatments with triclabendazole (TCBZ). Fecal examinations of sheep and cattle on the farm showed high number of liver fluke eggs. In a randomized clinical trial, the fluke egg output was monitored weekly for 3 weeks in sheep which were treated with TCBZ or with closantel in dairy cows treated with TCBZ or with clorsulon and in heifers treated with TCBZ or clorsulon. The results showed a significant reduction of 99.7, 98%.1% and 99.2%, respectively, in fluke egg output at 21 days in all non-TCBZ treated animals. TCBZ treatment produced percentage decreases of 15.3, 4.3 and 36.6%, respectively. These results are highly indicative of the presence of TCBZ-resistant *Fasciola hepatica* in sheep and cattle on this farm. (Lammertm *et al.*, 2000).

Another report on the species of *Fasciola* present in the Nile Delta, Egypt, appears controversial. Some authors reported the presence of both *Fasciola gigantica* and *Fasciola hepatica*; others reported *F. gigantica* only and mentioned that *F. hepatica* was found only in imported animals. This study was an attempt to identify the species of *Fasciola* flukes collected from locally bred animals. Morphologic, morphanatomic, morphmetric, and chemotaxonomic criteria of the fluke isolates were studied. Speciation based on morphologic and morphometric data was not decisive due to overlap in the values of most measurements. Morph-anatomic data proved the presence of both species, and isoelectric focusing (IEF) of fluke soluble protein confirmed the presence of both *F. gigantica* and *F. hepatica* in Egypt (Lotfy *et al.,* 2002).

Also another study was conducted in the northeastern areas of Punjab province (Pakistan) to analyze the monthly and seasonal pattern of fasciolosis in buffaloes and its relation to some climatic factors (temperature, humidity, rainfall and pan-evaporation) . The fecal samples of buffaloes were collected from April 2003 to March 2005 on monthly basis from randomly selected areas and analyzed for the presence of *Fasciola* egg. From 7200 samples, 1058 (14.69%) were found positive. Seasonal data showed the highest prevalence and egg count (EPG) in autumn and the lowest in spring. Monthly results showed the highest prevalence in September (32.33%) and the lowest in May (4.83%), while mean EPG was highest in October (567 ± 95.5) and the lowest in June (3.2 ± 0.48). Statistically, significant difference ($P<0.05$) was noted within seasonal and monthly prevalence's. Impact of humidity was found significant ($P<0.05$) on disease as compared to other climatic factors. **(**Qureshi *et al*., 2012).

In a cross-sectional study conducted to determine the prevalence of *Fasciola hepatica* and to investigate the related risk factors in cattle from Kayseri, Turkey, fecal and blood samples were collected from 282 cattle from May 2004 to April 2005 and were examined by modified McMaster sedimentation and ELISA techniques to detect *Fasciola* sp. eggs and anti-*F. hepatica* antibodies, respectively. Of the total of 282 examined cattle, 184 were seropositive for *F. hepatica* with a prevalence of 65.2%. In addition 24.5% of seropositive cattle had fluke eggs in the fecal examination. The mean number of EPG in infected cattle was 42.8%, 4.4%. The highest prevalence was observed in ≥6 years age group

(87.2%) followed by 3-5 (79.5%) and ≤ 2 years age groups (51.6%). The differences between ≤ 2 and other age groups were found significant ($p<0.001$), whereas no statistically significant difference ($p>0.05$) was observed between 3-5 and ≥ 6 age groups. The infection was more prevalent in females (70.7%) than males (47.8%) and in cattle from the traditional farms (76.5%) than the small-scale dairy farms (37.2%). No statistically significant difference ($p>0.05$) was observed related to breed**.** (Yildrim *et al* ., 2007).

The aim of previous study was to determine the prevalence and economic significance of fasciolosis in cattle slaughtered at Arusha abattoir in Tanzania. A 3-year database (2005-2008) from the abattoir was retrieved and analyzed. In addition, meat inspection was carried out for one month (July 2008) with focus on *Fasciola* infection and its associated economic loss due to liver condemnation. Results from retrospective study revealed that 8302 (6.7%) livers out of 123790 examined were condemned due to fasciolosis. Analysis of primary data (meat inspection) showed that 150 of 469 cattle livers condemned were due to fasciolosis, a relative condemnation rate of 32.0% per month. Based on the current local price of liver, the economic loss per month due to liver condemnation was estimated at Tanzania shillings (TZS) 1,800,000/- (approximately US $1,500), which summed to TZS 21,600,000/- (US $18,000) per annum. The specific cause of liver fasciolosis was *Fasciola gigantica*. These results indicate that *F. gigantica* infection is an important condition that leads to high liver condemnation rates in cattle slaughtered, resulting into high financial loss. These merits for more extensive epidemiological investigations to better determination of the prevalence, economic impact and public .(health importance of the disease (Mwabonimana *et al.*, 2009

Another study was conducted to determine the prevalence of bovine fasciolosis and to assess the effectiveness of commonly used anthelmenthics in Ginner district south-eastern, Ethiopia from September to December 2011. For the determination of the prevalence, 384 fecal samples were collected and examined by sedimentation technique. The result revealed that 121 (31.51%) animals were positive for *Fasciola* eggs. The infection rate was 30.81 and 32.16% in animals less than four years and greater or equal to 4 years of age, respectively while it was 29.70 and 33.51% in male and female animals, respectively. However, the differences either in age or sex groups were not statistically significant (Fekadu *et al.*, 2012).

Also in another study aimed at determining the prevalence of Fascioliasis in abattoirs located within some selected Local Government Areas of Benin City, Nigeria.A total was done of 180 cattle found within the confines of 3 abattoirs in the respective LGAs were examined. Consequently, 9 abattoirs were investigated for liver flukes (*Fasciola hepatica* and *Fasciola gigantica*), bringing the overall number of cattle examined to 540. The screening exercise was carried out between the 15th of August and 2nd of December, 2012, using standard histo chemical techniques. The results showed that of the 540 cattle examined, 11.5% were infected. The distribution shows that cattle slaughtered at Ikpoba Okha LGA abattoirs had the highest infection rate of 5.74%, followed by abattoirs from Egor (3.33%) and Oredo (2.44%), suggesting that there exist differences in the hygienic status of abattoirs, as well as the mode of water supply.(Odigie,*et al.*,2013).

Another study was conducted during the period between January 1999 and December 2000,to determine the distribution and seasonal patterns of *Fasciola gigantica* infections in cattle in the highveld and lowveld communal grazing areas of Zimbabwe was determined through monthly coprological examination. Cattle fecal samples were collected from 12 and nine dipping sites in the Highveld and lowveld communal grazing areas respectively. Patterns of distribution and seasonal fluctuations of the intermediate host-snail populations and the climatic factors influencing the distribution were also determined by sampling at monthly intervals for a period of 24 months (November 1998 to October 2000) in six dams and six streams in the Highveld and in nine dams in the lowveld communal grazing areas. Each site was sampled for relative snail density and the vegetation cover and properties of water, and mean monthly rainfall and temperature were recorded. Aquatic vegetation and grass samples 0–1 m from the edges of the snail habitats were collected monthly to determine the presence or absence of *F. gigantica* metacercariae. Snails collected at the same time were individually checked for the emergence of larval stages of *F. gigantica.* A total of 16, 264 (calves 5, 418; wieners 5, 461 and adults 5, 385) fecal samples were collected during the entire period of the study 2, 500 (15.4 %) of the samples were positive for *F. gigantica* eggs. Significantly higher prevalence were found in the Highveld compared to the lowveld ($P < 0.001$), for adult cattle than calves ($P < 0.01$) and in the wet season over the dry season ($P < 0.01$). Fecal egg output peaked from August/September to March/April for both years of the study. (Pfukeny *et al.,* 2006).

Another cross sectional study to determine the prevalence of *Fasciola gigantica* in cattle in Nigeria,that was carried out in 9 randomly selected farms and 1 slaughter house between February and May 2012. Fecal and blood samples were collected from 186 cattle in the farms and 200 cattle at slaughter. The fecal samples were analyzed using the formol ether sedimentation technique and the blood by Indirect ELISA kit (Bio-X-Diagnostic, ID VET Jemelle-Belgium) to detect *F. gigantica* eggs and antibodies to *F. Gigantica* antigens respectively. Of the 200 fecal samples collected at slaughter, 39(19.5%) had *F. gigantica* eggs; as compared to 27 (14.5%) positives out of the 186 samples collected from the farms; giving an overall prevalence rate of 66 (17.1%). There was no significant difference (P>0.05) between prevalence of infection of cattle sampled in the farms and slaughter house. 23 (11.5%) of the sera prepared from the 200 blood samples obtained at slaughter had antibodies to *F. hepatica* antigens, as against 5(2.6%) for sera from 186 blood samples collected in the farms; giving an overall sero prevalence of 28(7.3%). There was significant difference (P< 0.05) between infection at slaughter and on farms. Out Of the 200 cattle from slaughter, 20(10.0%) had *F. gigantica* eggs and also were sero positive for *F. hepatica* antigens, and of the 186 cattle from farms only 5(2.7%) that had *Fasciola* eggs and were also seropositive for *F. hepatica* antigens. Both at slaughter and on farms, infection was more prevalent in females than in males. The overall prevalence for Females using coprology and ELISA were 19.3% (41/212) and 7.5 %(16/212) respectively. The respective values for males were 13.7% (24/174) and 6.89% (12/174). However, the difference in the prevalence of females and males obtained was not statistically significant (P>0.05). No statistical difference was observed in breed prevalence. This study has established *F. gigantica* prevalence of 17.1% and 7.3% by coprological and serological examinations of faces and blood of cattle in Zaria in Nigeria (Aliyu *et al*., 2014).

Also another study was designed with the aims of determining the prevalence and risk factors of fasciolosis in cattle, sheep and goats slaughtered from October, 2010 to April, 2011 at Hashim Nur's Ethiopian Livestock and Meat Export industrialized abattoir in Debre Zeit, Ethiopia. One thousand one hundred fifty two ruminants comprising of cattle, sheep and goats (n=384 each) were subjected to routine post mortem examination for fasciolosis. The overall prevalence of fasciolosis in the study was determined to be 21% (242/1152). The prevalence of fasciolosis in adult cattle, sheep and goats were confirmed to be 39.8%, 28.7% and 13.9%, respectively and the prevalence of fasciolosis in young cattle, sheep and goats were proved to be 23.3%, 12.7% and 7.0%, respectively. Significantly higher ($p<0.05$) prevalence of fasciolosis was seen in adult cattle, sheep and goats when compared to young ones. The prevalence of fasciolosis in poor body conditioned cattle, sheep and goats were found to be 38.1%, 28.8% and 13.6%, respectively and prevalence of fasciolosis in medium body conditioned cattle, sheep and goats were found to be 30.0%, 20.5% and 11%, respectively. The prevalence of fasciolosis in good body conditioned cattle, sheep and goats were found to be 24.2%, 14.3% and 7.2%, respectively. Statistical analysis of the data showed the presence of significant difference ($p<0.05$) on the prevalence of fasciolosis in cattle, sheep and goats on the basis of body condition score. The high level of fasciolosis in cattle, sheep and goats in the present study represents high rate of infection and immense economic losses to the country, Ethiopia. In line with this finding it is recommended that farmers who rear cattle, sheep and goats should improve provision of feeds to their animals so that the animal can have good body condition that confers some level of resistance against fasciolosis. Besides, they should be able to regularly treat their animals with the appropriate anthelementics and awareness should be created on the prevention and control methods of fasciolosis. (Abdulhakim *et al*., 2012**).**

1.3. Taxonomy and classification:-

Kingdom: Animalia

Phylum: Platyhelminthes

Class: Trematoda

Order: Digenea

Family: Fasciolae

Genus: Fasciola

Species: *Fasciola hepatica* and *Fasciola gigantica* (Saria. 2011)

1.4. Morphology:-

The fluke has an elongated anterior end known as a cephalic con that contains the oral and ventral sucker. The intestines are highly branched and present throughout the body. The flukes are hermaphrodite worms with both male and female reproductive organs present near the posterior sucker in the center of the body. The female reproductive tract is adense ovary located just above the testes. The size range is 25 to 30 mm and 8 to 15mm in length and width respectively, depending upon species. The adult inhabits the bile duct in the liver or gallbladder of the final host. The short convoluted uterus opens to genital pore above the ventral suckers. The vitellaria are highly dispersed and divided in the lateral and posterior region of the body.

Fasciola hepatica and *F.gigantica are* very similar to each other, varying in length and width. In addition, the cephalic cone of *Fasciola hepatica* is shorter than *Fasciola gigantica*. The shape of the eggs of the two flukes is also very similar with the measuremeant of the *Fasciola hepatica* and *Fasciola* gigantica being approximately 150 um x 90um and 200um x 100um, respectively (Saria., 2011).

1.5. 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 infested watering places (Dechasa et al., 2012).There are many ecological factors affecting snail population including temperature, light, hydrogen ion concentration (PH), vegetation, depth of water, chemical composition of soil and snail population competition. The most important intermediate hosts of *F.gigantica* in Sudan are 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 intermediate 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)

1.6. Life cycle:-

For 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 hot and dry pasture conditions during the summer, metacercariae rapidly die; 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 a newly 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).

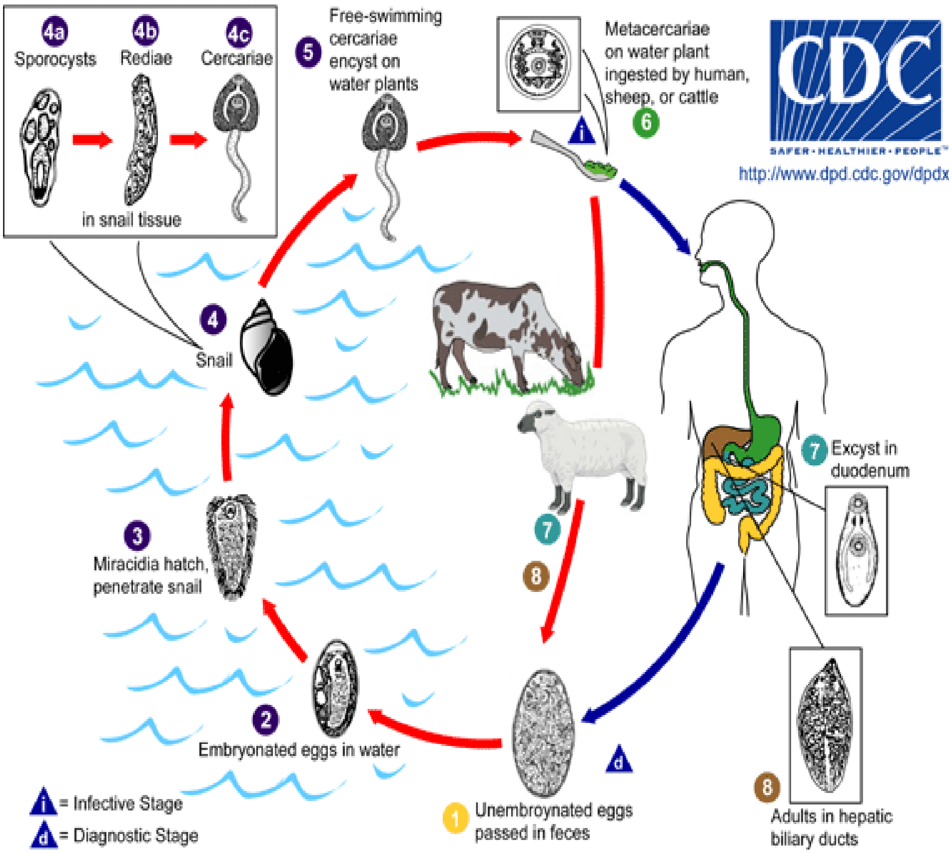


Figure1: Life cycle of *Fasciola* (http://www.dpd.cdc.gov/dpd)

1.7. 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 (Michal, 2004).

Immature wandering flukes destroy liver tissue and cause hemorrhage. In acute fasciolosis the damage is extensive.The liver become enlarged and friable with fibrinus deposit on the capsule .The fluke's migratory tract can be seen, and the surface has an uneven appearance. In chronic cases cirrhosis develops. Mature fluke damage the bile duct which become enlarged or even cystic, and have thickened, fibrotic wall. In acute cases the duct wall becomes greatly thickened and often calcified .Fluke may be found in aberrant sites e.g. lung (Veterinary Manual, 2005).

The principal pathogenic effects of flukes are anemia and hypo albuminaemia. More than 0.5 ml blood per fluke per day can be lost within the bile duct. In acute form, massive invasion of immature flukes into the liver can cause sudden death while in chronic from, there is liver cirrhosis caused by the wandering flukes 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 lesions can predispose cattle to black disease (infectious necrotic hepatitis) and bacillary hemoglobinuria due to *Clostridium novyi* and *C.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).

1.8. Clinical finding:-

Acute fascioliasis is common in sheep and goats while the chronic form is found mostly in cattle. Symptoms of fascioliosis 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 liver. (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 hemorrhae 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 move.

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

1.9. Diagnosis:-

Fasciolosis should be considered when there are deaths or ill animals. Diagnosis in dead animals relies on seeing mature or immature fluke in the liver. (Boray., 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 is carried out using the sedimentation method described by Bile is 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 is examined under the microscope. (Adodokum *et al*., 2008).

Another method is a 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 individual animals .There are evidences to show that sero diagnosis can detect the presence of infection as early as 2 weeks after infection. Furthermore, serological methods like (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).

-:Public health significant .1.10

Fascioliosis is a parasitic disease caused by the fluke *Fasciola hepatica* in humans. The disease is acquired for the most part by eating watercress, other vegetables or by drinking water contaminated with metacercariae. Human fascioliosis 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 fascioliosis 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 symptoms1n *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 by infected by ingestion of contaminated .(vegetation. (Elhaj., 2001

Man is seldom infected by the common liver fluke usually infects himself by eating water grass grown in water in which the snail are living and which is contaminated by the feces of the fluke infested cattle or sheep. During migration of immature fluke, eosinophilia is stage which may aid in diagnosis of human fasciolosis .And the symptoms in human patient include urticaria, jaundice and enlarged tender liver and eosinophilia. .((Elhaj., 2001

The prevention of human fascioliosis 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 (Mascoma *et al*., 2005

1.11. Economic importance:-

Fasciolosis also known as fascioliasis, distomatosis and liver rot, is an important helminthes disease caused by trematode species, *Fasciola hepatica* (the common liver fluke) and *Fasciola gigantica. F. Hepatica* infects more than 300 million cattle and 250 million sheep worldwide and together with *F. gigantica*, causes significant economic losses to global agriculture; estimated at more than US$3 billion annually (Aliyu *et al.*,2014*).*

Economic losses from liver flukes may result directly from increased liver condemnations at slaughter and indirectly from decreased livestock productivity. More economically important, beef producers are affected by increased culling of cows, reduced sale weights of culled cows, lowered reproductive performance in the brood cow herd, reduced calf weaning weights, and reduced rates of growth in stockers; fluke-infected dairy cows produce less milk. Also liver flukes reduce animal fertility in addition liver flukes do affect sex hormone balance and metabolism (Kaplan *et al.,* 2001).

Fasciolosis causes a substantial economic loss which includes; death, loss in carcass weight, reduction in milk yield, predisposes animal to other disease and cost of treatment expenses (Mult *et al*., 2012)

1.12. Prevention and control:

The methods to control fasciolosis generally include strategic application of anthelmintics 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 .Another 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. (Rapsch *et al*., 2008).

Destruction of intermediate host snail host by suitable molluscicides is adopted in many part of the world defined water bodies such as dam, stream and lakes, is often very expensive in term of labor and material. The regular application of molluscicides in three month sufficiently controls the disease since snail is killed before emitting cercaries. (Altahir., 1975.)

The anthelminthic drugs recommended by the World Health Organization (WHO) as essential drugs to treat these diseases, are 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 fascioliosis 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. 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, 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 may 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 (Petros *et al*., 2000)

Chapter Two

MATERIALS AND METHODS

:Study area 2.1

The study was conducted from April to June 2014 in Khartoum State, which is located in North Eastern part of the centre of Sudan. The state is located between 21 o, 25-24 o, 45 East and 15 o, 9-16 o, 45 North. The Khartoum State is bounded by North Kordofan in the west and in the north by Nile River State and in the North West by the Northern State and .by the White Nile State in the South and Gazeera State in the east

.Figure2: Map of Khartoum State

:Study Population 2.2

The study population consists of cattle at different ages, sex, origins .and breeds categories in the study area

2.3 Sampling methods: - *cluster* random sampling techniques were used. The prevalence was calculated using formula described by (Martin *et al*., 1987) as follow:

Prevalence rate = <u>No. of Cattle with fasciolosis</u> x 100

Total no. of cattle at a particular point in time

Sample size determination:-

-:The sample size was calculated by the formula

N= 4**<u>xPxQ</u>**

L2

N= sample size

P= expected prevalence

L= desired absolute precision

Q= (1-P) . (Martin *et al.*,1987)

The expected prevalence was estimated according to the study prevalence of bovine fasciolosis and related risk factors in Dessie Municipal abattoir , south Wollo Zone, Ethiopia (Belay et al ., 2012) .The sample size was calculated as followS:

N= **4X(,252)X(0.788)** **=** 302 animals

(***0.0025***)

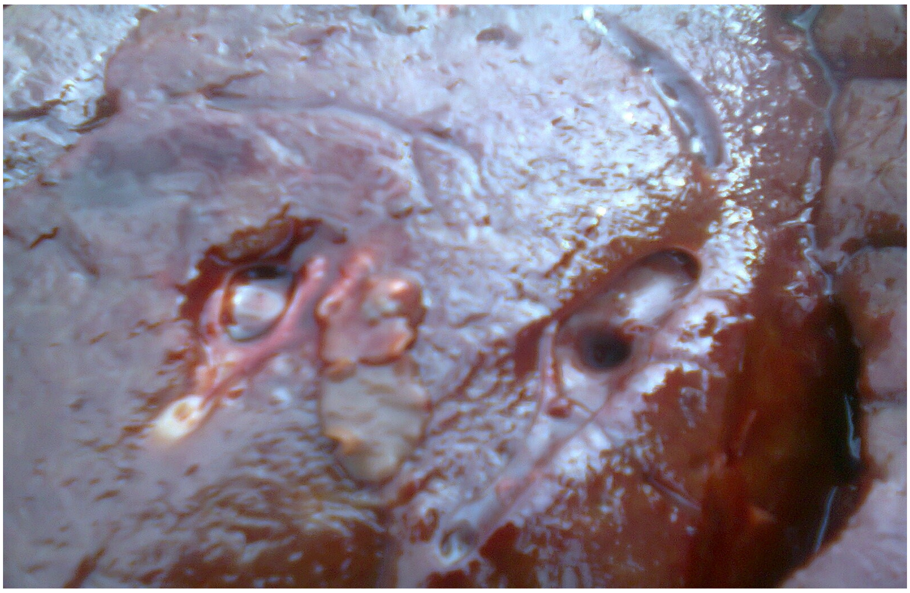
-:The study design 2.4

The study design was a cross sectional study which provides snapshot information on occurrence of a disease (Martin *et al* , 1987). A Cross-sectional study was conducted at Elkadaro slaughterhouse on three randomly selected days, the animals in .these days selected by cluster random sampling method

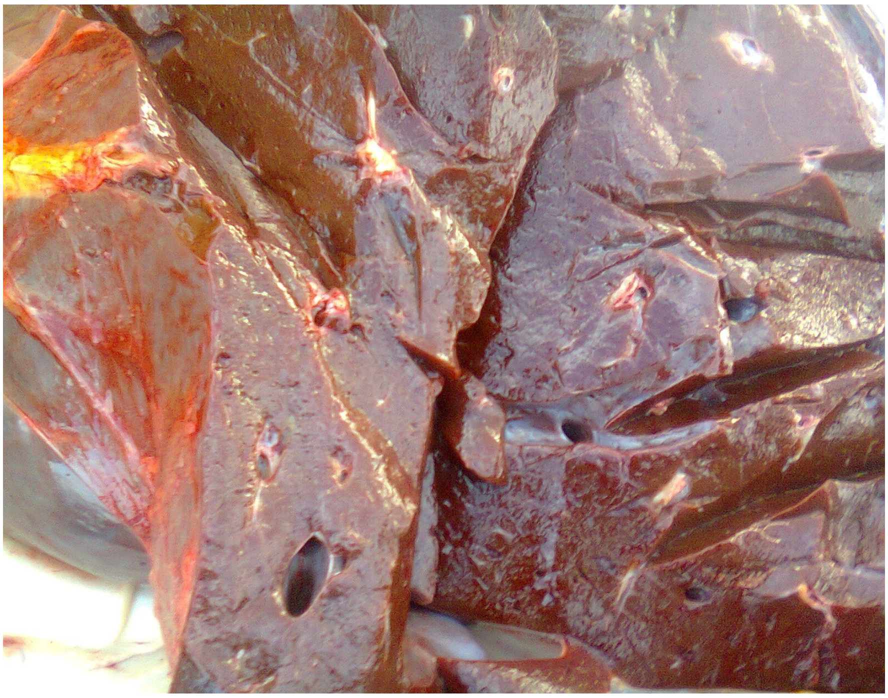
2.4.1. Ante-mortem and postmortem inspection:-

Regular visits were made by the investigator to conduct ante-mortem examination of slaughter animals. The cattle were selected by cluster random method. Total of 302 cattle were examined in Elkadaro abattoir, during the survey period which extended from April 2014 to June 2014 which questionnaire was designed. The information include: animal grazing (indoor/outdoor), source of animal and using drug. During the ante-mortem inspection the ages, sex, breed and body condition of each individual animal were assessed and recorded. The age was divided into two categories: ≤ 3 years, and >3years; sex to male and female; the cattle breed into three categories: local, cross and breed.

Postmortem examinations of liver and associated bile duct were carefully performed by visualization and palpation of entire organ followed by transverse incision of the organ across the thin left lobe.



.Figure3: The adult parasite in the liver surface



.Figure 4: Infected liver of fasciolosis

Fecal samples collection and examination 2.4.2

Fecal sample examination was carried out at the laboratory of the Sudan University of Science and Technology, Khartoum State. Fecal samples were collected directly from the rectum during ante-mortem examination using plastic gloves. Specimens were carried in a plastic container and transported to the laboratory for microscopic examination using sedimentation technique to detect the presence of fasciola *spp*. Eggs. The identification of eggs was done on the basis of morphology. Water was added to feces into a container, mixed thoroughly then filtered through a tea strainer , the filtered material was poured into 15 ml centrifuge tube and centrifuged at 3000 rpm for 3minuts and then the supernatant was discarded. Mixing of water and centrifuging is continued until clear supernatant was obtained. A small drop of the sediment was transferred to slide microscope, covered with cover slip and examined under the microscope at 10x10 magnifications.

2.5 statistical analyses

Results of the study were analyzed using statistical package of social science (SPSS) version 22. At first, descriptive statistical analysis was displayed in frequency distribution and cross tabulation tables. Then, univariate analysis using the Chi-square for qualitative data was carried out. P-value of 0.25 was considered as significant association and the risk factor was then selected to enter the multivariate analysis. Logistic regression was used to analyze the data and to investigate association between potential risk factors and the occurrence of fasciolosis. A p-value of 0.05 indicated significant association between fasciolosis and risk factors.

Chapter Three

Results

Descriptive statistical analysis frequency tables, cross tabulation and association tables between the disease and risk factors: Out of 302 cattle inspected, only 10 (3.3%) animals were positive, for .(bovine fasciolosis (table 3.1

Table 3.1: Prevalence of fasciolosis in 302 cattle examined through .postmortem examination in khartoum State

	Frequency	Percent	Valid Percent	Cumulative Percent

Valid	Negative	292	96.7	96.7	96.7
	Positive	10	3.3	3.3	100.0
	Total	302	100.0	100.0	

Table 3.2: Prevalence of fasciolosis in 302 cattle examined through . fecal examination

	Frequency	Percent	Valid Percent	Cumulative Percent
Negative	297	98.3	98.3	98.3
Positive	5	1.7	1.7	100.0
Total	302	100.0	100.0	

Postmortem results

Age of animals:

The presence of liver fasciolosis in three hundred and two cattle of various age was investigated according to the age distribution of cattle 268 of the cattle were less than or equal 3 years (young) and 34 of cattle were more than 3 years (old), (table 3.3). Among young animals, no animal was found infected. However among adults 10 animals were found infected. Rate of infection within adults was 29.4 % (10/34)

(table 3.4).

The Chi- square test showed significant association between fasciolosis infection and age of animal (p-value = 0.000),(table 3.6).

Sex of animals:

The results of this study showed the distribution of 302 cattle examined for fasciolosis according to sex. Total number of male examined was 293 animals, while the total number of female examined was 9 (table 3.3). Among males, 9 animals were found infected. Rate of infection within males was 3.0% (9/293). While among females, one animal was found infected. The rate of infection within females was 11.1% (1/9) (table 3.4).

The Chi-square test showed no significant association between fasciolosis infection and sex of animal (p-value = 0.184), (table 3.6).

Breed:

The results of study showed distribution of fasciolosis infection in Elkadaro slaughterhouse according to breed. Total number of local breed was 289 animal. Among these 289 animals, 7 were found infected. The rate of infection was 2.4% (7/289). Total number of cross breed examined was 10. Among these, there was no infection. Total number of foreign breed examined was 3. Among these, 3 were found infected .The rate of infection was 100% (3/3) (table 3.4).

The chi- square test showed there was significant association between the infection and breed (p-value = 0.000), (table 3.6).

Body condition:

The body condition of animals and the presence of infection were investigated. Three hundred of cattle were found to be in good condition, while 2 of cattle were found to be in poor condition (table 3.3). Among good healthy animals, 10 were found infected. The rate of infection within good healthy condition animals was 3.3% (10/300). However no animal was found infected among poor animals. The rate of infection within poor animals was 0.0% (table3.4).

The chi- square test showed no significant association between the infection and body condition (p-value = 0.793), (table 3.6).

Source of animals:

Of the total 302 cattle inspected, 198 animals were from nyala, 7 animals were from Elfashir , 3 animals from Ethiopia , 46 animals were from White Nile and 48 animals were from Eddien . (table 3.3). All infected animals found in this study (10 animals) were from nyala ,Ethiopia and White Nile. The rate of infection in nyala , Ethiopia and White Nile was 0.5% (1/198) ,100% (3/3) ,13.0 % (6/46) respectively . (table 3.4).

The chi- square test showed there was significant association between the infection and source of animal (p-value = 0.000), (table 3.6).

Grazing type:

The grazing type of animals and the presence of fasciolosis infection were investigated. Two hundred ninety eight of cattle were found to be in open grazing type, while 4 of cattle were found to be in close grazing type (table 3.3). Among open grazing type animals, 10 were found infected. The rate of infection within open grazing type animals was 3.4% (10/298). However no animal was found infected among close grazing type animals (table 3.4).

The chi- square test showed no significant association between the infection and grazing type (p-value = 0.709), (table 3.6).

Fecal examination results

Age of animals:

Among young animals examined, no animal was found infected. Rate of infection within young animals was 0.0% (0/268). However among adults 5 animals were found infected. Rate of infection within adults was 14.7(5/34), (table3.5).

The Chi- square test showed significant association between fasciolosis infection and age of animal (p-value = 0.000),(table 3.7).

Sex of animals:

Among examined males, 5 animals were found infected. Rate of infection within males was 1.7% (5/293). While among females, no animal was found infected. The rate of infection within females was 0% (0/9) (table3.5).

The Chi-square test showed no significant association between fasciolosis infection and sex of animal (p-value = 0.693), (table 3.7).

Breed:

Among local breed, the rate of infection was 1% while it was 66.6% in foreign breed and 0% in cross breed (table 3.5)

The chi- square test showed there was significant association between the infection and breed (p-value = 0.000), (table 3.7).

Body condition:

Among good animals, 5 animals were found infected. The rate of infection within good healthly condition animals was 1.7% (5/300). However no animal was found infected among poor animals. The rate of infection within poor animals was 0.0% (table 3.5).

The chi- square test showed no significant association between the infection and body condition (p-value = 0.85), (table 3.7).

Source of animals:

All animals from Ethiopia and White Nile province were found to be infected with fasciola. The rate of infection in Ethiopia and White Nile were 0.5% 66.7% (2/3), 6.5 % (3/46) respectively (table 3.5). The chi- square test showed there was significant association between the infection and source of animal (p-value = 0.000), (table 3.7). Grazing type:

Among open grazing type animals, 5 animals were found infected. The rate of infection within open grazing type animals was 1.7% (5/298). However no animal was found infected among close grazing type animals (table 3.5).

The chi- square test showed no significant association between the infection and grazing type (p-value = 0.794), (table 3.7).

Table 3.3: Summary of frequency tables between potential risk factors .and bovine fasciolosis in 302 cattle examined at Elkadaro slaughterhouse

Risk factor	Frequency	Percent	Cumulative percent
Age			
Young≤ 3years	268	88.7%	88.7%
Old > 3 years	34	11.3%	100.0%
sex			
Female	9	3.0%	3.0%
Male	293	97.0%	100.0%
Breed			
Local	289	95.7%	95.7%
Cross	10	3.3%	99.0%
Foreign	3	1.0%	100%
Body condition			

Poor Good	2 300	0.7% 99.3	7%. 100.0%
Grazing Open Close	 298 4	 98.7% 1.3%	 98.7% 100.0%
Origin Nyala Elfashir Ethiopia White Nile Eldien	 198 7 3 46 48	 65.6% 2.3% 1.0% 15.2% 15.9%	 65.6% 67.9% 68.9% 84.1% 100%

Table 3.4: Summary of cross tabulation between potential risk factors and bovine fasciolosis in 302 cattle examined at Elkadaro slaughterhouse

Risk factor	No. tested	No. +ve	Percent
Age Young≤ 3years Old > 3 years	 268 34	 0.0 10.0	 0.0% 29.4%
Sex Female Male	 9 293	 1.0 9.0	 11.1% 3.0%
Breed Local Cross Foreign	 289 10 3	 7.0 0.0 3.0	 2.4% 0.0% 100%
Body condition Poor	 2	 0	 0.0%

Good	300	10	3.3%
Grazing Open Close	 298 4	 10.0 0.0	 3.4% 0.0%
Origin Nyala Elfashir Ethiopia White Nile Eldien	 198 7 3 46 48	 1.0 0.0 3.0 6.0 0.0	 0.5% 0.0% 100% 13% 0.0%

Table 3.5: Cross tabulation between bovine fasciolosis diagnosed through fecal sedimentation and potential risk factors in 302 cattle slaughtered at .Elkadaro abattoir, khartoum State

Risk factor	No. tested	No. +ve	Percent
Age Young≤ 3years Old > 3 years	 268 34	 0.0 5.0	 0.0% 14.7%
Sex Female Male	 9 293	 0.0 5.0	 0.0% 1.7%
Breed Local Cross Foreign	 289 10 3	 3.0 0.0 2.0	 1% 0.0% 66.6%
Body condition Poor Good	 2 300	 0.0 5.0	 0.0% 1.7%
Grazing Open	 298	 5.0	 100%

Close	4	0.0	0.0%
Origin			
Nyala	198	0.0	0.0%
Elfashir	7	0.0	0.0%
Ethiopia	3	2.0	66.7%
White Nile	46	3.0	6.5%
Eldien	48	0.0	0.0%

Table 3.6: Univariate analysis for the association between fasciolosis diagnosed through postmortem examination and potential risk factors in 302 cattle examined in khartoum State using the Chi-square test

Risk factor	Total No	No. positive	(%) Percent	df	X^2	p-value
Age				1	81.5	0.000
(Young(≤3years	268	0	0%			
(Old(>3years	34	10	29.4%			
Sex				1	1.8	0.184
Female	9	1	11.1%			
Male	293	9	3.0%			
Breed				2	88.6	0.000
Local	289	10	3.6%			
Cross	10	0	0%			
Foreign	3	3	100%			

Body condition				1	0.069	0.793
Poor	2	0	0.0%			
Good	300	10	3.3%			
Grazing				1	0.139	0.709
open	298	10	3.6%			
close	4	0	0.0%			
Origin				4	107.9	0.000
Nyala	198	1	0.5%			
Elfashir	7	0	0.0%			
Ethiopia	3	3	100%			
White Nile	46	6	13.0%			
Eldien	48	0	0.0%			

Table 3.7: Univariate analysis for the association between fasciolosis diagnosed through fecal examination and potential risk factors in 302 cattle examined in khartoum State using the Chi-square test

Risk factor	Total No	No. positive	(%) Percent	Df	X^2	p-value
Age				1	40	0.000
(Young(≤3years	268	0	0%			
(Old(>3years	34	5	14.7%			
Sex				1	0.156	0.693
Female	9	0	0.0%			
Male	293	5	1.7%			
Breed				2	78.7	0.000
Local	289	3	1.0%			
Cross	10	0	0.0%			
Foreign	3	2	66.6%			
Grazing				1	0.068	0.794
Open	294	10	3.4%			

close	4	0	0.0%			
Body condition				1	0.034	0.854
Poor	2	0	0.0%			
Good	300	5	1.7%			
Origin				4	88.8	0.000
Nyala	198	0	0.0%			
Elfashir	7	0	0.0%			
Ethiopia	3	2	66.7%			
White Nile	46	3	6.5%			
Eldien	48	0	0.0%			

3.9. Results of multivariate analysis:

3.9.1Postmortem examination: Potential risk factors found to be significantly(p-value < 0.25)associated with fasciolosis in the univariate analysis(table3.7) were entered to logistic regression model .Four risk factors were found to be significantly associated(p-value<0.05) with fasciolosis in the multivariate analysis. These risk factors included Age (p-value 0.000), breed (p-value=0 .000), source of animal (p-value =0.000) and grazing (p-value=0 .005) (table 3.8).

3.9.2. Fecal examination:

Potential risk factors found to be significantly associated (p-value ≤ 0.25) with fasciolosis in the univariate analysis (table3.8) were entered to logistic regression. In the final model four risk factors were found significantly associated (p-value≤0.05) with fasciolosis in the multivariate analysis. These risk factors included age (p-value =0.000), breed (p-value= 0.000) source of animal (p-value =0.000) , grazing (p-value=.000) (table 3.9) .

Table 3.8: Multivariate analysis for the association between fasciolosis diagnosed through postmortem examination and potential risk factors in .302 cattle examined in Khartoum State using logistic regression

Risk factor	No. positive	No. positive (%)	(Exp(B	p-value	CI 95%
Age				0.000	0.000-0.296
(Young(≤ 3years	268	(0%)0	.Ref		
(Old(> 6years	34	(29.4%)10	11.0		
Sex				0.184	0.296-161.6
Male	293	(3.0%)9	ref		
Female	9	(11.1)1	6.9		
Breed				0.000	0.000
Cross	0	(0%)	.Ref		
Local	7	(2.4%)	0.040		
foreign	3	(100%)	9.0		
Grazing				0.000	0.000-0.005
Open	298	(3.4)10	2.46		
Close	4	(0.0%)0	ref		

Origin				0.000	
Nyala	198	(0.5%)1			
Elfashir	7	(0.0%)0	.Ref		570.1- 1.8
Ethiopia	3	(100%)3	32.0		
White Nile	46	(13.0%)6	4.0		
Eldien	48	(0.0%)0	000.		

Table 3.9: Multivariate analysis for the association between fasciolosis diagnosed through fecal examination and potential risk factors in 302 .cattle examined in Khartoum State using logistic regression

Risk factor	No. positive	No. positive (%)	(Exp(B	p-value	CI 95%
Age				0.000	0.000-0.296
(Young(≤ 3years	268	(0%)0	.Ref		
(Old(> 6years	34	(14.7%)5	15.0		
Sex				0.693	0.000-0.000
Male	293	(1.7%)5	26.0		
Female	9	(0.0%)0	.Ref		
Breed				0.000	0.000
Cross	10	(0%) 0	.Ref		
Local	289	(2.4%)7	0.090		
Foreign	3	(100%)3	65.0		
Grazing				0.000	0.000-0.005
Open	298	(3.4)10	2.06		
Close	4	(0.0%)0	.Ref		

Origin				0.000	0.000-0.000
Nyala	198	(0.0%)0			
Elfashir	7	(0.0%)0	.Ref		
Ethiopia	3	(66.7%)2	32.0		
White Nile	46	(6.5%)3	32.0		
Eldien	48	(0.0%)0	1.00		

Chapter four

Discussion

Fasciolosis is aparasitic disease of mammals,especially ungulates .The bodies of trematodes or flukes are dorsoventrally flattened and are unsegmented leaf like.The fluke is cosmopolitan in it's distribution and is the cause of fasciolosis (liver fluke disease,liver rot),especially in sheep and cattle (Soulsby,1986).

Previous studies on animal fasciolosis revealed that the disease is prevalent in the Sudan, in area such as Darfur, Upper Nile, Khartoum, Bahr ElGazal, Equatorial Blue Nile, Kassala as well as North State . (Koko et *al* .,2003). The annual reports since1955 indicate that highest percentages of liver condemnation due fasciolosis are follows:Upper Nile Province (27-58%), Baher El Gazal Province (16-18%),Equatorial Province(13-40%) .In all area in Sudan fasciolosis has been reported in several places with high prevalence .

Our study was carried out in Khartoum State to investigate risk factors and to estimate prevalence of bovine fasciolosis in Khartoum State The prevalence rate was 3.3% by postmortem examination and1.7% by fecal examination using sedimentation techniques.

The prevalence rate of postmortem examination obtained in this study is 3.3% which is higher than prevalence rate by fecal examination (1.7%), because some infected animal might have been misdiagnosed by sedimentation technique which is characteristically poor in detection of fluke (Pfukeny *et al.*,2006).

Also this variation could probably be due to the fact that in abattoir study the liver is usually damaged by immature flukes which cannot be detected through fecal examination. In addition, most cattle that are infected with flukes shed relatively few eggs and only eggs are found from 8 week after infection (Pfukeny *et al.*, 2006).

However the prevalence rate in our study (3.3%) was lower than many other studies from different abattoirs in Sudan in Gezira state, Central Sudan (Koko *et al.*, 2003) where the prevalence was12.5% or in Africa, such as from Nekemte municipal abattoirs the prevalence rate was 21.9% (Petros et al., 2013) and from Jimma municipality abattoirs in Ethiopia, where the prevalence rate was 48.19% (Fromsa *et al.*, 2001).

This variation could be due to the geographical location and ecological condition such as altitude, rain fall and temperature. *Fasciola spp* prevalence has been reported to vary over the years mainly due to variation in amount pattern of rain fall. Bahr el Ghazal Province of south Sudan was also reported to have a high prevalence of fasciolosis than the more open and dry savannah, Darfur provinces (Pfukeny *et al.*, 2006).

In our study, the age was found significantly associated with fasciolosis the p-value is (0.000) in post mortem examination. The prevalence rate is high in old animals than young animals. (29.4%, 0%) respectively. This result is in agreement with previous study in Debre Zait town, Ethiopia by (Abdulhakim *et al.*, 2012).

However, our result does not agree with another study in Ladakh in India by (Kuchai *et al*., 2011) who found higher prevalence in young animals.

In our study, the age was found significantly associated with fasciolosis the (p-value is 0.000) in fecal examination. The prevalence rate is high in old animals than young animals. The prevalence rate of fecal examination was14.7% in old animals and 0%in young animals. These results are in agreement with previous study in Zimbabwe by(pfukeny *et al*., 2006) who found high infection rate in old animal ,the(p-value is.<0.001) , The higher infection rate in older animal was reported to be probably due to longer exposure time, or due to management system with longer exposure of old animals outdoor while young animals are kept indoor (Pfukeny *et al*., 2006).

Also our study is in agreement with another study in Kayseri Province, Turkey by (Yildrim *et al*., 2007) who found high infection rate in old animals. However, our results do not agree with another study in Ginnir District by (Fekadu *et al*., 2012), where a significant difference was not observed between age groups (p-value > 0.05).

In our study, sex was found no significantly associated with fasciolosis in postmortem examination (p- value =0.184). This is agrees with a previous study in Nekemte Municipal abattoir by (Petros *et al*., 2013) who did not find significant association of sex with fasciolosis (p-value > 0.05). However, our study does not agree with study in Assela, Ethiopia by (Mugugeta *et al.*,2011) who found significant association of sex with fasciolosis (p-value <0.05).

Also in our study, the sex was not found significantly associated with fasciolosis (p-value=0.693), in fecal examination .This finding is not in agreement with study carried out around Assela by (Mugugeta *et al*., 2011) who found significant association between sex and fasciolosis .

Also in our study, source of animal was found significantly associated with fasciolosis (p-value=0.000), in postmortem examination .This finding is in agreement with study carried out in southern Espirato Santo by (Bernardo *et al*., 2011). However, our study does not agree with a previous study in Sowth Wollo zone, Ethiopia by (Belay *et al*., 2012).

Our study revealed that grazing type was significantly asciated with fasciolosis the p-value =0.005), in postmortem examination. A higher prevalence of fasciolosis was reported in outdoor (1.7%) as compared with animals in indoor (0%) .This variation IS in our opinion may be due to the fact that animal grazes outdoor is more exposed to the disease from pasture, dams, ponds than animal kept indoor.

In this study there were four risk factors were found to be significantly associated (p-value <o.o5) with bovine fasciolosis in the multivariate analysis. These four risk factors included: age, source of animal, grazing and breed.

Conclusion .4.1

In view of our findings, fasciolosiss is prevalent at Elkadaro abattoir in Khartoum State. Our study further confirmed that fasciolosis diagnosed through postmortem examination is more prevalent than by fecal sedimentation. The overall prevalence was 5%. Old Animals were more affected compared to young animals. The prevalence of fasciolosis is higher in foreign breed and local breed compared to cross breed. The prevalence of fasciolosis is higher in animals which graze outdoor than animal which graze indoor.

4.2. Recommendations:

- More elaborate studies on bovine fasciolosis to reveal the prevalence in other states.

-Economic importance of the disease should be evaluated.

- Knowledge of the disease epidemiology in the country; which vary from place to another according to the agricultural and ecological variation.

- Awareness of animal owner's about the disease, treatment and the control strategy.

-Vegetables grown in fields should be thoroughly cooked to reduce infection of fasciolosis in humans.

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