INTRODUCTION

Background :

The major problem that faces livestock owners is the high mortality rate in their herds either due to malnutrition or due to diseases, either bacterial , viral or parasitic .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(Walker, et al., 2008).

Infection by members of the genus *Fasciola*, commonly known as liver flukes, may be responsible for morbidity and mortality in most mammal species, but are of particular importance in sheep and cattle to livestock producers.

Fasciolosis is a worldwide zoonotic disease an infection due to the food- and water-borne trematodes *Fasciola hepatica* and *Fasciola gigantica*, are among the most neglected tropical diseases. Among the estimated 91.1 million humans at risk for infection worldwide, as many as 17 million may be infected. Certain areas of the world bear the burden of the highest prevalence of infection. There, school-age children are the most likely to be infected (Robert, 2010).

Humans become accidentally infected by ingesting contaminated drinking water or plants in endemic area. After ingestion of the metacerceriae, the larvae migrates through the intestinal wall, pass into the peritoneal cavity, penetrate the liver capsule and finally matures in the bile ducts.

The definitive diagnosis is made by demonstration of eggs in stool samples, bile or duodenal aspirates or the discovery of worms at surgery (Roya, et al ., 2013).

Human Fasciolosis:

An univariate analysis found an association between four variables and fascioliasis, drinking water supply from canals, eating raw vegetable salads, drinking alfalfa juice and defecating in silos. Since this population has no municipal running potabilized water, it is likely that water from irrigation canals could be a significant risk factor. Viable metacercariae have been found in natural water supplies of endemic areas (Luis,et al.,2005).

In humans the parasite cause biliary obstruction resulting in high fever, diarrhea, chills, bile inflammation , liver enlargement and jaundice (Durga and Pabitra., 2009) .

The status of the disease in Sudan:

Sudan is considered as one of the main countries for exporting meat and leather. This comprises about 20% of the country foreign trade . *Fasciola gigantica* infection causes great economic losses due to total or partial condemnation of livers. The disease is highly enzootic in many areas of the Sudan (Eisa, et al., 2011). Most of the previous studies on the prevalence of fasciolosis in the Sudan were based on slaughter houses records (Amna, et al .,2011).

One study was done in White Nile state to assess the prevalence rate of *F. gigantica* infection in slaughtered cattle and sheep and stressed on the need to implement a control program in the White Nile

State, Sudan during the period 1998-2007, (Amna, et al .,2011). In this study they estimated the prevalence of *fasciola gigantica* as 10.41%. The reports for different seasons in the years 2005, 2006 and 2007 were done , the highest prevalence rate was shown in the wet season for the year 2005 and 2007, but the same season reported the lowest rate in 2006 ($p \geq 0.05$) for cattle infection (Amna;et al., 2011).

Another study in EL Gezira state appeared that the liver fluke is still prevalent in Sudanese goat (12.5%) , but this prevalence was associated significantly with inner irrigation location of animals rather than animals grazing in peripheral areas of the Gezira , because the disease strongly associated with the distribution of the intermediate host(lymnaenatalensis – intermediate host of *F.gigantica*) , (Koko , et al., 2003).

In a study in Abyei area showed that the prevalence of *F. gigantica* is 5%. (Idriss, et al., 2012).

The objectives of this study were :

1. To determine the prevalence of fasciolosis in cattle slaughtered at north Kordofan state abattoir.
2. To assess the risk factors associated with fasciolosis .

Chapter One

Literature Review

1.1Classification:

Class: Trematoda

Order: Monogenea

Family: Fasciolidae

Genus: *Fasciola*

Species: *F.heptica*

F.gigantica

(David L.B, et al., 1969)

1. 2Morphology of *fasciola* :

Fasciola gigantica is common liver fluke in African countries , it occurs in bile ducts and gall bladder, rarely under the peritoneum and in the lungs . The definitive host are sheep, cattle, and other ruminant rarely in rabbits and equines. Generally *Fasciola* is measuring from 2.0 to 3.0 cm in length by .08 to 1.3 cm in breadth . A cephalic cone , the integument is covered by scale , but the posterior surface may be smooth . Hemispheric oral and vertical suckers of equal size are present at the apex and base of the cephalic cone . The intestinal tract has a wall, developed pharynx , a short esophagus and two divergent intestinal ceca with numerous lateral diverticula , there is a posteriorlongate bladder with lateral branches. The highly dendritic testes are situated one behind the other in the middle of the body (David L.B, et al., 1969) .

F. gigantica is larger than *F. hepatica*, and eggs is larger in size, the anterior cone is smaller than *F. hepatica* , the ventral sucker is larger than the *F. hepatica* .The inner intestinal branches take Y or T shape. *Fasciola hepatica* is common liver fluke in Europe and other countries, it is flat, brownish , leaf shaped treamatode.

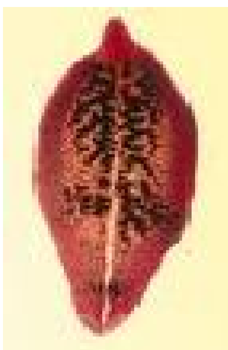

Figure 1: *F. hepatica*

Figure 2: *F. gigantica*

(Mahrukh, et al.,2011)

1.3 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) .

1.4 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 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. This is accompanied by increased contact of the habitats by livestock due to scarcity of pasture and increased grazing around 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).

Figure 3: Lymnaea Snail

(Moayad, et al.,2011)

1.5 Life cycle of the parasite:

Eggs are passed in the faeces of the mammalian host, hatch and release motile miracidia. Hatching may take nine days and the optimal temperatures (22-26° C). Miracidia have a short life span and must locate a suitable snail within three hours. In infected snails sporocysts, redial stages and cercaria develop. Snails pass the motile cercaria which then attach themselves to plant material, where they encyst and become the infective metacercariae. A minimum of 6-7 weeks is required for miracidia to form metacercaria. Under unfavourable circumstances this may take several months. Following on infection of one snail with one miracidium over 600 metacercariae may be produced. Final hosts ingest the metacercaria and these excyst in the small intestine, followed by migration through the intestinal wall, crossing of the peritoneum and penetration of the liver capsule. The immature flukes migrate through the parenchyma (6-8 weeks period), entering the small bile ducts and finally migrating to the larger bile ducts (and occasionally the gall bladder).

Generally the life cycle of both fluke species is the same. The prepatent period of *F. hepatica* is10-12 weeks and one entire life cycle of *F. hepatica* may be completed in a minimum of 17-18weeks. *F.hepatica* may live for years in untreated sheep but in cattle it is usually less than one year. For *F.gigantica* most phases of development take longer and the prepatent period is13 –16 weeks. These parasites are hermatophrodytes , and one adult *F.hepatica* in a bile duct may produce 20 000 eggs per day establishing patent infestation(Robert ,et al., 2010).

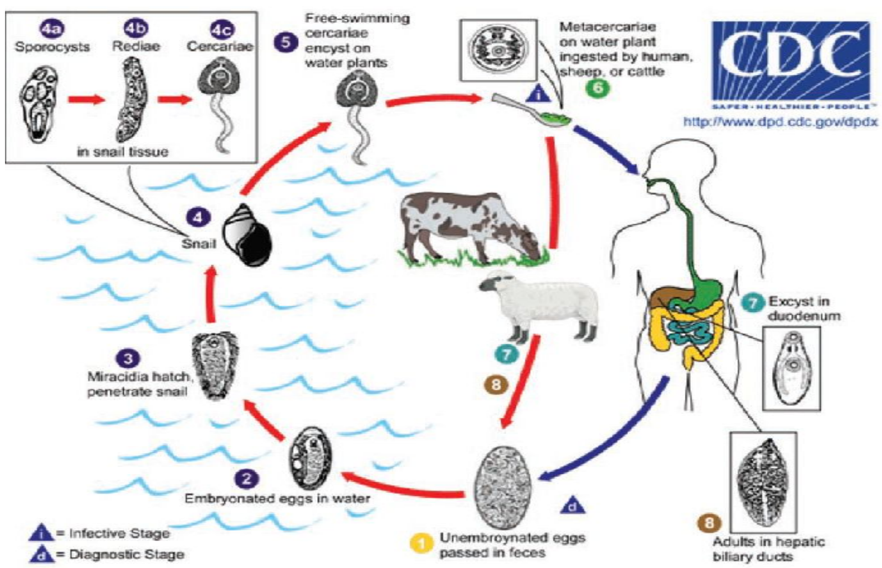

Figure 4:_Life cycle of *Fasciola*—(Robert ,et al.,2010)

1.6 Clinical signs :

Pathogenesis depends primarily on two different stages of development of the parasite in the liver of the host, the level of parasitaemia, and if it is an acute, subacute or chronic infection. Acute and subacute disease is seen in sheep, and occasionally occurs under conditions of heavy challenge in young calves. The chronic form of the disease is by far the most important in sheep, and specifically in cattle.The clinical signs of acute disease are characterised by sudden acute deaths, weakness, anaemia and dyspnoea. Sub acute and chronic fasciolosis is characterized by progressive loss of condition, anaemia, hypoalbuminaemia, emaciation, pallor of the mucous membranes, submandibular oedema. Anaemia is hypochromic and macrocytic and an accompanying eosinophilia is usually present. In milder infections clinical signs may or may not be readily observed, however, adecreased appetite and interference with post-absorptive metabolism of protein, carbohydrates and minerals, may have a significant effect on production ((Dechasa, et al., 2012).

Acute disease is associated with mostly immature flukes, and usually seen in autumn and early winter, 2-6 weeks after ingestion of metacercariae in large numbers (> 2000). Immature flukes migrate through the liver parenchyma and create migratory tracts, which results from direct trauma, coagulative necrosis and release of toxic excretions from the flukes. Lesions may vary from mild (low infestations) to severe in heavy, or repeat infestations. The liver may been larged and haemorrhagic with fibrinous to fibrous exudates on the capsular surface (usually the ventral lobes). The migratory tracts may be visible as dark acute haemorrhagic streaks to, more yellowish white streaks typical of post necrotic scarring and granulation. Sometimes flukes may be seen in the migratory tunnels. If severe haemorrhages are present it may result in large subcapsula rhaemorrhages, which in turn may rupture with severe intra-abdominal haemorrhage and acute haemorrhagic anaemia as consequence. In some heavy and repeat infestations acute lesion of multi focal pinpoint serosal haemorrhages and fibrinous peritonitis, to more chronic fibrous peritonitis may be present (Dechasa, et al., 2012).

Subacute disease is usually seen during late autumn and winter, and 6–10 weeks after ingestion of smaller numbers (500-1500) of metacercariae. At this stage some parasites may have reached the bile ducts whilst others may still be migrating through the parenchyma. Sub capsular haemorrhages may be present but usually these do not rupture ((Dechasa, et al., 2012).

Chronic fasciolosis is associated with mature flukes, and seen mainly in late winter/early spring.It is usually 4-5 months after ingestion of moderate numbers (200-500) of metacercariae. Matureflukes, which are present in the bile ducts, cause necrosis and ulceration of the epithelium givin grise to peribiliary inflammation and severe hyperplasia of the epithelial layer. Mechanical irritation by their scales, and suckers, biliary retention and the production of toxic or irritant products by the flukes may contribute to lesions. Anaemia and hypoalbuminaemia are the most important consequences contributing mostly to the pathogenesis. More than 0.5 ml blood per fluke can be lost per day. Plasma protein may be lost through the bile ducts into the intestine due to the increased permeability of the hyperplastic bile duct epithelium, and loss of plasma proteins trough the fluke's digestive tract. Bile ductular distention in sheep, swine and horses may be more mechanical due to accumulation of parasites and bile, whereas in cattle the inflammatory lesion associated with erosions and granulation seems more prominent ((Dechasa, et al., 2012).

On post mortem the liver may have an irregular outline, and be pale and firm. The ventral lobe is most commonly affected and reduced in size. The liver pathology of chronic disease is characterized by hepatic fibrosis and hyper plastic cholangitis.

Several different types of fibrosis may be present and includes post-necrotic scarring (mainly in the ventral lobe and associated with healing of fluke tracts), is chaemic fibrosis (infarction as consequence of damage and thrombosis of large blood vessels, and peribiliary fibrosis (damage by flukes in the small bile ducts). Fluke eggs may sometimes stimulate agranuloma-like reaction with obliteration of the affected bile ducts as consequence. In bovines calcification of bile ducts, enlargement of the gallbladder and aberrant migration of the flukes is more common. Encapsulated parasites are often seen in the lungs. If adult cows are reinfected, parasitic migration to the foetus and resultant prenatal infection has been reported ((Dechasa, et al., 2012).

1.7 Immunity :

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 immune responses typical of the Th2-type, with eosinophilia, IgE and IgG1 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).

From various vaccine trials with cathepsin L proteases a correlation between antibody responses, and protection has been reported. Naturally-infected animals produce high IgG1 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 titre of IgG2 antibodies seem to be, in general, directly correlated with the fluke burden of animals and it was found that in vaccinated 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 against *F. hepatica* may not necessarily protect animals against *F.gigantica*. This may therefore require presentation of such antigens in different adjuvant formulations, or administration regimes. ELISA has also very successfully been employed to study the serum antibody type response (total IgG, IgG1, IgG2 IgM and IgG in *F.hepatica* and *F.gigantica* infected sheep and cattle (Phiri, et al.,2005).

1.8 Diagnosis:

A part from the presence of typical clinical signs, suggestive haematological and biochemistry findings, typical macroscopic and histological findings the laboratory confirmation may be depend mostly of faecal sedimentation tests, serology tests (ELISA) and possibly in some regions of the world PCR tests. An ELISA test, produced by the Institute Pourquier, employing “F2” antigen purified from *Fasciola* extracts, is currently available for routine diagnostic use in South Africa. It has been validated for the use on ovine and bovine serum, and bovine milk. Pooled and individual serum samples, and milk tank samples may be used, and the tests results/values may be indicative of the level of infection in herds.

Many of the different ELISA's developed will be based on the use of different secretory and excretory products of flukes as antigens (Qureshi, at el., 2012).

In some studies of *F.hepatica* infection in cattle a significant correlation was observed between the intensity of the infection, and the ELISA values obtained. A very recent article published reports on the findings of their quantitative and qualitative evaluation of sediment flotation technique, a copro-antigen ELISA and two indirect serum ELISA's. The sensitivities and specificities of these tests; and the influence of the level of infection and season, on the result were evaluated which makes for very good reading. Many other articles were published on the use of ELISA in other species such as humans, donkeys and water buffaloes (Qureshi, at el., 2012).

Many recent advances were made in the field of molecular diagnostics. Polymerase chain reaction(PCR) tests were developed for the identification of the different snail species, which would be of great advantage in epidemiological investigations. PCR tests, including multiplex PCR and real time PCR, were developed to diagnose/identify flukes and fluke antigens, and used to study many different aspects of the immunology and epidemiology. These findings were published in sever a articles e.g. identification of

F. hepatica in *L.columella* and *L.viatrix* in snails, distinguishing between *F. hepatica* and *F. gigantica*, identification of *F. hepatica* in formalin fixed and waxembedded tissues of *L.viatrix*, and the measurement of interferon-gamma and (INF) interleukin-4(IL-4) expression in *F. gigantica* infection in calves (Qureshi, at el., 2012).

1.9 Control and prevention :

The epidemiological information on trematode parasites of cattle can be used to design appropriate control measures. In principle, control should aim at the reduction of transmission rates. Several control methods,which include cultural, chemical,biological and immunological control.

Cultural and husbandry control methods include practices such as controlling stocking rates, rotational grazing, and the provision of clean grazing. The best way to prevent fasciolosisis to keep cattle away from potentially dangerous water habitats.

Drainage or fencing-off of wet areas pre-vents infection of pastures but is rarely cost effective on grazing land in developed countries and neither is it feasible in developing countries. Complete separation of stock from snail-infested areas is only practical in intensive farming husbandry systems. Hence cultural and husbandry control methods can be applicable in the commercial farming areas. In communal grazing areas animals are communally grazed and there fore practices such as rotational grazing and provision of clean pastures would not be feasible (Pfukenyia, et al-2005).

Habitat management in the form of vegetation clearance is potentially effective both through reducing feed availability of snails and also by enhancing water flow rates during the rainy season. This method of control could probably be possible especially in commercial farming areas where snail habitats are not widespread.

In widespread distribution of the snail habitats, cultural methods are difficult or perhaps impossible in the Highveld region and in communal grazing areas (Pfukenyia, et al-2005).

Long-term snail control can be achieved by drainage of the habitat, but permanent destruction of snail habitats may be expensive and ecologically sensitive, or controversial, especially in widespread habitats. When snail habitats are small and localized fencing of such areas, or annual treatment with a molluscicide, may be more feasible.

The use of chemical molluscicides for the control of the snail hosts of bilharziasis and fascioliasis has been accepted for the present as the most promising and practical means of controlling these diseases, the application of copper sulfate to all water bodies and repeated sprayings of molluscicide at certain points where the human population had close and repeated contact with water bodies.

In 1953, an experiment aiming at the total elimination of snails from an isolated river system was attempted in an African reserve in the Mtoko District, 90 miles (145 km) north of Salisbury. The whole river system was given two successive treatments at a six-week interval, copper sulfate being applied to give a final concentration of 20-30 p.p.m. The application was not repeated again but never theless it was found that snail reinfestation was slow (Clarke, et al.,1961).

Treatment of infected animals will largely depend on the correct use of appropriate and registered anthelminthics. Fasciolosis may be controlled by reducing the populations of the intermediate snail host, or by appropriate anthelminthic treatment.. Registered fluke anthelminthics may be used prophylactically (strategic treatment) to reduce contamination of pastures by fluke eggs at times most suitable for their development; or to remove fluke populations (tactical treatment) at a time of heavy fluke burdens, or at periods of nutritional and pregnancy stress to animal. The best anthelminthics are Oxyclozanide, Nitroxynil, Rafoxanide, Albendazole,Clorsulon and Triclabendazole. All these products are given by the oral route.

Control by means of vaccination has also been extensively investigated. In human, diseases is mostly associated with local endemic animal fasciolosis, and the spread of drug resistant liver flukes. In some parts there may be overlap, and concurrent *Shistosomas* p infections may be seen. Some efforts are therefore also directed at producing vaccines, which could produce cross reaction between *Shistosomas p* and *F. hepatica*.

During the past few years a number of proteins have been identified and investigated as potential candidates for vaccine production. These were tested in various different combinations and trials were conducted in many species of animals including mice, rats, rabbits, cattle and sheep. In some of these trails fairly high levels of protection was seen in sheep and cattle. These proteins are fatty acid binding proteins (nFh12, rFh15, Sm 14), cysteine peptidase (cathepsin L1 and cathepsin L2),leucine amino peptidase, glutahathione- S –transferase. More recent candidates are thioredoxinperoxidase, NK-lysin like molecule, cathepsin L3, cathepsin B cysteine proteases, thioredoxin reductase and enolase, which are under investigation (Parr,et al., 2000) .

It was found that many of these vaccines not only provided protection against the parasites but also resulted in reduction of the faecal egg counts, or production of non or poorly embryonating eggs. Egg production seems vulnerable to the immunological response induced by vaccination. It is notclearly established to what extent reduced egg production would have on the transmission of eggs and this still needs to be mathematically investigated. No articles on field studies of an effective vaccine have been reported so far (Parr,et al., 2000).

Treat young heifers and dry cows with a drug effective against immature fluke – triclabendazole – and follow the above plan for beef cattle.

The two drugs registered for use in lactating cows (oxyclozanide plus levamisole, and clorsulon plus ivermectin) are only effective against adult fluke aged 12-14 weeks or older. If your property is heavily contaminated, you may have to treat lactating cows monthly during summer and autumn, using this product, which also controls gastrointestinal nematode and lungworm infections.

You will improve fasciolosis control with a triclabendazole treatment a month before calving, and immediately after drying off. Mixed grazing Be careful if sheep and cattle are grazing on the same pasture, whether together or alternately: you may need to treat your cattle every time you treat your sheep, to reduce or eliminate contamination of pastures and thus infection. For best results use a drug highly effective against early immature fluke, i.e. triclabendazole, or against advanced immature fluke, nitroxnil (Joseph, et al., 2007).

1.10 Economic impact:

1.10.1 Weight gain:

Monitoring weight gain of young animals is a use full 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 systems that are producing young growing animals for sale (Copeman,et al., 2012).

1.10.2 Draught performance:-

Anaemia 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 in production systems where hand tractors are replacing animals as sources of draught power (Copeman ,et al ., 2012).

1.10.3 Fertility:-

A link has also been observed between infection with *F.gigantica*, anaemia and fertility. There were significantly longer intercalving intervals and a lower packed cell volume in On gole 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).

1.11 Bacteria associated with Fasciolosis:

Type of bacteria found in the carcasses may be of non specific group which comprises that are non pathogenic or only potentially pathogens as *Clostridia*, *Streptococci*, *Bacillus subtitis* , *Enterococci* and *E.coli* in adult animals .These species are present naturally in the intestinal flora pathogen include heamylotic S*taphylcocci* , *Pasterulla* , *Salmonella* and *Listeria monocytogenes*(Ahmed .,2001) and (**Sohair,** et al.,2009).

1.12 The epidemiology of fasciolosis:

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).

F. hepatica is mostly encountered in temperate areas, and in cooler areas of high altitude in the tropics and subtropics, whilst *F. gigantica* predominates in tropical areas. Snails are their intermediate hosts. Amphibious snails of the genus *Lymnaeas p* are widely distributed throughout the world and *L. trunculata* is the most common of them all. In South Africa the most common intermediate hosts are *L. trunculata* (*F. hepatica*), *L. natalenis* (*F.gigantica*) and *L.columella (F.hepatica* and *F.gigantica).*

Other important Lymnaea vectors of *F.hepatica* are *L.tomentosa* (Australia, New Zeeland), L.columella (North America, Australia, New Zeeland), *L.bulimoides*(Southern USA and the Caribbean), *L.humilis* (North America), *L. vector* (Southern America), *L.diaphena* (South America). Other important Lymnaea vectors of *F.gigantica* are *L.auricularis* (Europe, USA.Middle East, Pacific islands), *L.rufescens* and *L. acuminta* (India, Pakistan) and *L. rubiginosa* (Malaysia).

Large numbers of metacercaria will usually be produced when there is optimal availability of suitable snail habitats, optimum temperatures and optimum moisture is present. This frequently results in seasonal patterns of emerging disease in certain parts of the world for e.g. in

Britain metacercaria may appear on pastures from August to October and also in May to June.

Suitable snail habitats will include all areas where snails may survive in clear water or mud such as the edges of streams, ponds, rivers and vleis (permanent natural habitats); or temporary man-made depressions filled with water (tractor tracks etc). A slightly acid environment may be more optimal.Temperature requirements are mean day/night temperatures of 10° C at which both the snails and the flukes will propagate. Below 5° C all activity will stop and above 15° C significant increase in both snails and fluke larval stages may be seen, with the optimum being 22 -26° C. Moisture level are described as optimal when rainfall exceeds transpiration and when field saturation is achieved (Robert ,2010).

1.13 Prevalence of fasciolosis in the world:

Epidemiological analysis of human and animal fascioliasis has been carried out in different parts of the world ,today Fascioliasis is classified as tropical disorder .

A low prevalence of Fascioliasis have been reported from Corsica (0.8%) and China (0.7%), an intermediate prevalence from Porto (3.2%) followed by Egypt (7.3%), and Peru (8.7%) and a high prevalence from Montero valley and Bolivia with prevalence of 34.2 and 66.7 percent respectively (Jahangir,et.al., 2013).

A cross sectional study was carried out from October 2010 to March 2011 with the objectives of determining the prevalence, risk factors and economic importance of bovine fasciolosis in Dessie municipal abattoir

(Ethiopia). Over all prevalence of 25.2% (126 of 500) was observed. Based on origins of animals ,There was no statistically significant difference ($p>0.05$) between the four study areas. Young animals were found with high prevalence (33.33%) followed by old animals (26.11%) and adult animals (24.7%).

However, there was no statistically significant difference between the prevalence in the different age groups of animals. Prevalences of 63.29%, 18.62% and 17.75% were observed in animals of poor body condition, good body condition and medium body condition, respectively. The difference between the prevalence of bovine fasciolosis in animal of different body conditions was statistically significant ($p<0.05$). Of 126 infected livers,65.9%, 18.25%, 9.5% and 6.34% were infected with *F.hepatica*, *F.gigantica*, mixed and immature flukes ,respectively (Ephrem, et al., 2012).

The prevalence of Fasciola species is different in different study areas and the highest prevalence of *F. hepatica* was observed in Kutaber (20.34%) followed by DessieZuria (17.74%) and the highest prevalence of *F.gigantica* was observed in Worehimmenu (9.88%) followed by Kutaber (2.54%). The direct and indirect losses incurred due to fasciolosis in Dessie municipal abattoir were estimated about 2,495,346.13 ETH Birr. Itis concluded that fasciolosis is prevalent in cattle in the study area. Hence, this disease disserves serious attention by the various stake holders in order to promote the beef industry in the study area in particular an (Ephrem, et al., 2012).

Qualitative examination of *Fasciola. gigantica* eggs in faeces and bile were compared with the detection of precipitating antibodies in sera by Agar Gel Precipitation Test (AGPT) in 1000 cattle slaughtered at the Bodija municipal abattoir in Ibadan, Nigeria (Oyeduntan,etal.,2008). Faecal and bile examination methods detected (196) 33.5% and (389)38.9% of the animals as positive for fasciolosis, while (474) 47.4% were positive by AGPT. Both direct bile examination and faecal egg detection methods have high specificity and positive predictive value (100%) when compared with AGPT. However, lower values for sensitivity and negative predictive value were observed for both faecal egg examination (66.5% and 67.9% respectively) and bile examination (81.0% and 78.9%respectively). Fecal and bile examination failed to detect 33.5% and 19.0% of the cases detected by AGPT.

The results of the mentioned study revealed that the AGPT could become a better test for the herd diagnosis of bovine fasciolosis for veterinarians and other investigators in Nigeria (Oyeduntan, et al.,2008).

In Denmark, the prevalence of the liver fluke *Fasciola hepatica* in cattle has increased markedly during the last 3-5 years. Some studies were aimed to identify potentially high risk areas for fasciolosis in cattle in order to advice on appropriate control measures to be taken. The risk factors evaluated for a high prevalence of *F. hepatica* in cattle were environmental, determinants and biotic factors including meteorological data and topography on municipality level (Ersbøll,et al., 2006).

The prevalence of *F. hepatica* in municipalities was based on condemned F. hepatica infected livers of a total of approximately 1.4 million heads of cattle at slaughter during the period 2000-2003 as recorded in the Danish Cattle Database (Ersbøll,et al., 2006).

The median prevalence of cattle infected with *F. hepatica* in the municipalities was 2.8% (range: 0-27%) for beef cattle and 0.7% (0-14%) for dairy cattle. Significant risk factors affecting the prevalence of *F. hepatica* in cattle were the cattle density in the municipality, precipitation adjusted by the potential evapotranspiration (mm) and % coverage with wet areas and soil composition (Ersbøll,et al., 2006).

A cross-sectional study was conducted to determine the prevalence of *Fasciola hepatica* and to investigate the related risk factors in cattle from Kayseri, Turkey (Yildirim1,etal.,2007). Faecal 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 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 faecal examination. The mean number of EPG in infected cattle was 42.8. The highest prevalence was observed in ≥6 age group (87.2%) followed by 3-5 (79.5%) and ≤2 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. This results highlight the importance of initiating a control program for fasciolosis based on regular treatment and prophylaxis in Kayseri Province.(Yildirim1,et al.,2007).

Another study in Pakistan to estimate the point prevalence of fascioliasis and its economic impact in terms of increased milk yield after chemotherapy of a bovine population from the district of Toba Tek Singh, Punjab, Pakistan (Khan,et al., 2001). A total of 2400 cattle and buffaloes were examined quantitatively using the McMaster egg counting technique. Infected cattle and buffaloes (50 of each) were randomly selected and each divided into two groups of 25 animals. Groups A (buffaloes) and C (cattle) were treated with oxyclozanide (orally, 16.6mg kg21 body weight).Groups B and D served as negative controls for buffaloes and cattle, respectively. Pre- and post-treatment milk yield was recorded to determine if there were any changes in milk yield after treatment. Of 2400 faecal samples analyzed , 654 (27.25%) were positive for *Fasciola spp*. with a mean number of eggs per gram(EPG) of 503.2 (Khan,et al., 2011).

The point prevalence and worm burden of fascioliasis was significantly higher (OR ¼ 2.13; P , 0.05) in buffaloes (34.58%; 415/1200; mean EPG maximum likelihood ¼ 521.4) as compared to that of cattle (19.92% ; 239/1200; mean EPG maximum likelihood ¼ 415.8). Among the parasite species, *F. gigantica* (19.88%; 477/2400) was predominant (OR ¼ 3.12; P , 0.05) as compared to *F. hepatica*

(7.38%; 177/2400). An average daily increase of 0.67 and0.87 litres of milk, with 0.41% and 0.37% more fat per animal, was observed in oxyclozanide-treated buffaloes and cattle, respectively. The economic value of reduced production of infected animals was estimated as US$0.33 and 0.32 per animal per day for cattle and buffaloes, respectively (Khan,et al., 2011).

Fasciolosis is usually observed in the southern and south eastern regions of Brazil, where it has an endemic nature (Sandra, et al., 2003). In these regions, the infection rate of dairy cattle ranges between 10 and 100%. The actions taken by the State Program for the Control of Bovine Fasciolosis were based upon the information provided by the Brazilian. Meat Inspection System. In Rio Grande do Sul, the infection caused by F. hepatica is endemic, with condemnation of 52.14% (between 1982and 1988) and 27.4% (between 1989 and 1992)of the livers. Although our study was conducted in a smaller meat packing plant, the liver condemnation rate (10.34%) was similar to the rate reported by authors for different regions of the country, showing poor reduction of fasciolosis (Sandra,et al.,2003).

The landscape of Punjab is amongst the most heavily irrigated on earth and canals can be found throughout the province. Besides that Punjab receives abundant rainfall. Temperature and humidity are favourable forthe growth and multiplication of *Fasciola* and snail so incidence is high during autumn followed by spring and lowest during summer. Infection was the highest at Gujranwala followed by Lahore, Sargodha, Sheikhupura, Jhang while the lowest was at Faisalabad. This may be due to the fact that high level of infection was thought to be associated with the extension of the canal system providing additional areas of swamp and marsh where the cattle were exposed to infective larvae and metacercariae of helminths (Walker et al., 2008).

In Pakistan, majority of the slaughtered animals were harbouring mature flukes in their livers while in clinical ill animals the incidence of infection was based on identification of eggs in the faeces. Therefore, the recorded incidence of the infection was mainly due to mature parasites. It was also noticed that during the year 1999rains started during June changing environmental temperature and humidity, thus favouring the emergence of cercariae from snails. Due to this, metacercariae may show their existence in July after ingestion which produces fasciolosis with advancing age. In the present study, higher infection rate was recorded in older animals as in other laboratories (Pfukenyi, et al., 2003).Though the explicit cause of the high incidence of the disease in adult animals can not be explained fully, it seems to be related to faulty management. It is also possible that the higher infection rate in older animals might have resulted due to relaxation of resistance because of environmental factors at parturition or during lactation. A slightly higher incidence was observed in males than females. The reason for which seems to be related more to be social practice of keeping females under better management and feeding conditions in comparison to males which are generally being let loose to graze freely in pastures(**Umbreen.**, 2012).

A cross-sectional study of fasciolosis was conducted on serum samples from 296 autochthonous Rubia Gallega cattle of 36 farms by using an enzyme-linked immunosorbentassay (ELISA) test with a 2.9-kDarecombinant protein. Results were examined on the basis of the age of the cattle (0-2, 3-8and >8 years), farm characteristics (number of cattle, slope, altitude, rainfall) and *Fasciola*-control measures. The cattle-level prevalence was 71% and the highest values were observed in the oldest animals. Ninety percent (86%, 100%) of the farms had cattle positive to the ELISA test, and by estimating the odds ratio values, they observed the highest risk for fasciolosis in the biggest farms(more than 25 cattle), where cattle received treatment against this trematode, located above 1000 m altitude and where rainfall did not achieve more than 1000 mm.

The present study revealed the effect of temperature, meracidia dose , and snails age on some parameters like prevalence (%) , prepatent period , the number of metacercaria produced , and the percentage of floating metacercaria. The study revealed that the prevalence (%) was significantly increased with temperature decreasing and the increase of maracidia dose , as well as the prevalence (%) was significantly highest in largest snails . The prepatent period was significantly decreased with the increase of temperature and the decrease of meracidia dose. Also, the prepatent period was significantly longest in small snails (Moayad,et al.,2011). Concerning the number of metacercariae production, a study reveals that the production of metacercaria from snails increased at (25+1) C while it decreased significantly at (19+1)C and (30+1)C and it also increased significantly with the increase of meracidia dose and the snails size. The floating metacercaria percentage significantly increased with the increase of temperature but there was no significant an effect for meracidia dose and the size of snails (Moayad, et al., 2011).

In another study period, 9880 male cattle were slaughtered in the Ismailia abattoir. Based on seasons, 2502 (25.2%), 1737 (17.5%), 1950 (19.7%) and 3691 (37.6%) male cattle were slaughtered in autumn, winter, spring and summer , respectively . Autumn and summer seasons were the highest season in number of slaughtered animal in Ismailia abattoir. According to the results of the statistical analysis, significant difference ($P < 0.05$) was observed in number of slaughter cattle between summer season and other seasons. This may due to the site of Ismailia as a tourist city that attracts many people during summer season, subsequently increased in their population and meat demand (Ahmed , et al ., 2013)

There was no animal condemned as result of antemortem inspection allover the study period. As a result of postmortem inspections, 8 (0.10%) carcasses were condemned and 1456 (14.7%) organs had pathological lesions. Feeding, breed and management of cattle may be associated with the presence or absence of pathological lesions and could cause carcass condemnation during the inspection process. It was shown that the proportion of cattle showing pathological lesions differ between countries (Raji et al., 2010). The slaughterhouse and its regulations, represents a key control point of livestock production chain. Results of meat inspection of slaughter animal is too importance for animal health control system (Ahmed . et al ., 2013).

Two hundreds animal fecal samples were collected from endemic areas in Kalubia Province (Egypt) in order to determine *fasciolia* infectious rate to identify associated risk factors. The overall prevalence of fascioliasis in animal was 14% . Species isolated was *F. hepatica* (Abdel Razek., 2007).

Chapter Two

Materials and methods

2.1 Description of the Study Area:

North Kordofan lies in arid and semi-arid zones between Latitude 11.15-16.45 N and longitude 27-32.15 E. It also includes desert climate zone on the far northern parts and more humid climate to the south. The northern parts of North Kordofan State lie in the desert and semi-desert. May and June are the hottest months, July to October are the rainy months while December to February are the coldest months. In general two air movements affect the climate of the area. A very-dry air movements from the north reaching its southern limit in mid-winter and a major air flow of maritime origin that carries moisture, enters from the south and brings rains.

The state covers an area of 58.7 million feddans (25 million hectars) out of which 14.5 million hectar are range land . The livestock population, mainly cattle, sheep, goats and camels are about 6894425 head (Goma , 2008).

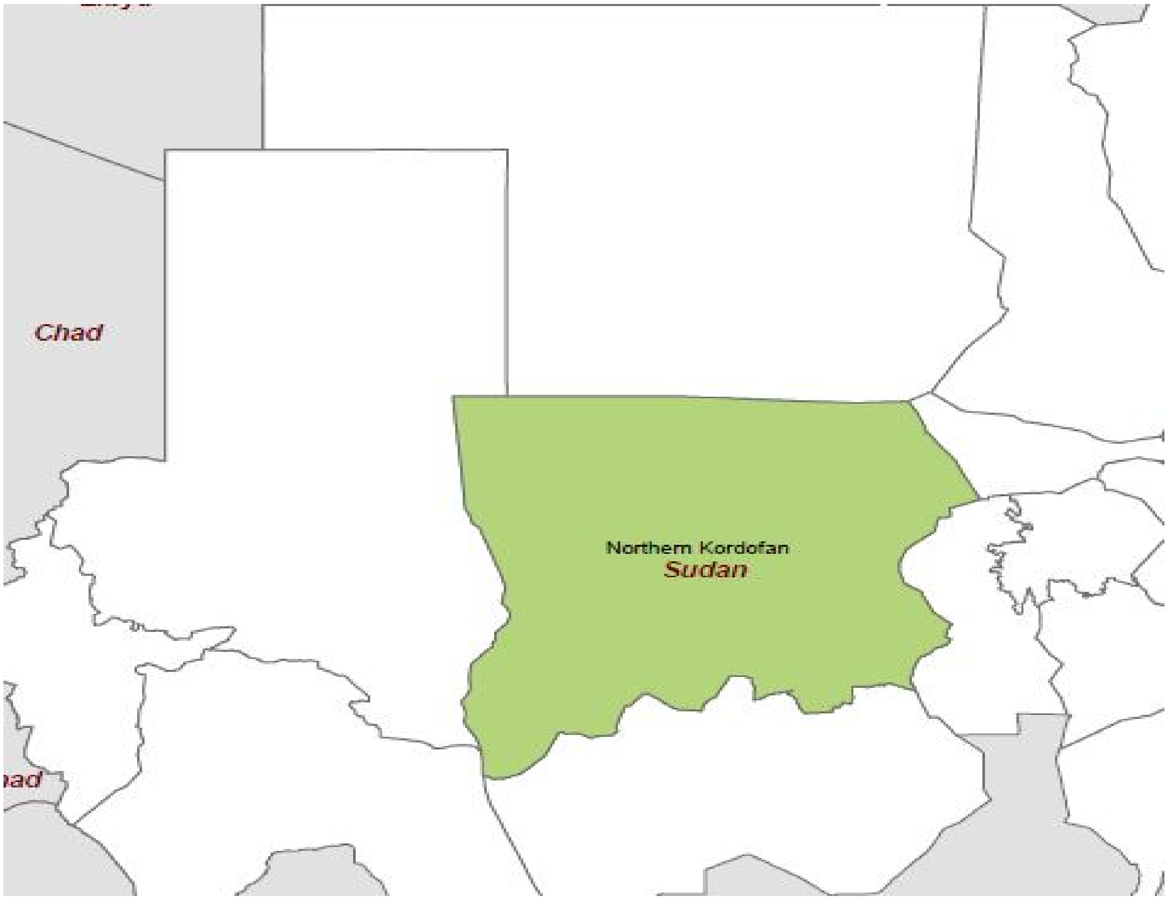

Figure 5: North Kordofan map(MARF, 2011)

2.2 Climate and Rainfall :

Generally the state can be divided into four ecological zones extending from North to South as follows:

- The desert zones with rainfall ranging between 0-75 mm per annum.
- The semi-arid zone with annual rainfall ranging between 75- 300 mm per annum.
- The arid zones with poor savannah in sandy soil (central zone) with rainfall between 300 – 400mmper annum.
- The low rainfall savannah in clay soil zone with rainfall ranging between 400-500mmper annum.

The animal population estimation in state is 3.05 million, of which 64% are rural, 36% urban. Animal population estimated at 13134542 head of animal (MARF, 2012). Water availability, social service and land for agriculture are the main determining factors for population settlements. The main people activity is agriculture, the major crops that are produced in the study area are millet, sorghum and ground nuts. The major cash crops are sesame, karkade, watermelon, groundnut, Gum Arabic and vegetable . Major tribes in North Kordofan are Bederia, Shiweihat, Kababish, Kawahla, Hamar, DarHamid, Jawamaa and Maganeen (Goma., 2008).

The animals which were examined in this study came from two regions in the Sudan. These regions, are, Kordofan, White Nile and South Sudan Country.

2.3 Criterior for selecting the area:-

- Animals come from different States.

- Large numbers of animals.

- Variety of animals.

- Easily accessible.

2.4 . Study Animals :

The total of the livestock population in North Kordofan state is estimated to be as shown in the following table:

The population by head(MARF,2012)

Cattle	Sheep	Good	Camels	Total
9.54880	7.282303	0.365471	1.246187	13.134542

2.5. Study design :

A cross sectional study was used to determine the prevalence and risk factors of fasciolosis in cattle In north Kord0fan state in Sudan from June to august 2013 (martin, et al., 1987)

2.6 .The Procedure :

2.6.1. Elobied slaughter house:

Elobied slaughterhouse is located near a residential area . Thus, it constitutes nuisance and dangers the health of the community in the immediate surrounding environment. It is a small low-walled open-air slaughter house with an impermeable sloped killing floor. Carcasses are cut into parts and hanged on fixed hooks for inspection. Sewage disposal is by collection into pits and then carried away in tanks to be disposed in remote areas of town on open places , because of the water problem in the town , water supply is insufficient specially during summer.

2.6.2. Sample size determination :

The expected prevalence of bovine Fasciolosis for calculation of sample size was taken from the study in South Wollo Zone, Ethiopia in which the prevalence of Fasciolosis in cattle was 25.2% (Ephrem Belay et.al,2012).Sample size was calculated according to the formula by Martin et al., (1987) .

$$n = \frac{(1.96)^2 \, Pexp \, (1 - Pexp)}{d^2}$$

$$n = \frac{4 * 0.252 * 0.748}{0.0025} = 301$$

Where: n = required sample size

Pexp= expected prevalence=25.2% (Ephrem Belay at.el,2012)

d = desired absolute precision = 5%

2.6.3. Sampling methods:

Elobeid abattoir was visited three times per week and inspection for animals was done and simple random sampling technique was used.

2.6.3.1. Ante-mortem Inspection :-

Antemortem inspections was conducted on individual animals, when the animals at rest in fence of the Abattoir shortly prior to slaughter , and selection of animals was done by random sampling method every visited day. Each animal was identified based on the enumerate marks on its body using a pen before slaughter. Attention was given to the factors such as age based on dentition, body condition, breed, sex, and localities of the animals, to determine the impact of these factors on the disease. Fecal samples were collected from the rectum of cattle and preserved in plastic sack , labeled with specific identification number and examined by sedimentation technique for the presence of fluke eggs, through repeated dilution of the fecal suspension and sedimentation of the eggs, which are heavier than most of the fecal particles (Urquhart ,et al., 1996), in the clinic of Elobied veterinary hospital .

2.6.3.2. Post mortem examination:-

Each liver was palpated and inspected by making multiple deep incisions of the lobes and making a deep cut with a number of small sub cuts. Gall bladders duct was opened using a knife and thoroughly investigated for the presence of the adult stage of *Fasciola* . In parallel, the following data were recorded. The size, color of the liver and any lesions in the liver.

2.7. Equipment :

Plastic containers , tea strainer ,measuring cylinder , test tubes , microscope slides , cover slips , pipettes, tea spoon , microscope were used .

2.8. Sedimentation method:

A spoonful of faeces was thoroughly mixed with 30 ml of tap water on container ; suspension was poured through tea strainer on another container and left for 20 min. The supernatant was removed and washed by water this step repeated three times. Finally, small quantity of the deposit was taken using a pipette and put on microscope slide, a cover slip was applied and examined microscopically under the low power, repeated two times for each sample .

2.9. Data analysis:

All the data which were collected about the risk factors and the results, animals were categorized as either positive or negative of fascolosis. For analysis of the data SPSS version 16 was used to calculate:

1. The prevalence of fasciolosis in North Kordofan state.
2. frequency table.
3. cross tabulation table.
4. Univariate analysis (chi-square, degree of freedom ,P. value) table.
5. Multivariate analysis (odds ratio, P. value) investigated the association and its strength between each risk factors and fasciolosis .

Chapter Three

Results

Descriptive statistical analysis frequency tables, cross tabulation and association tables between fasciolosis and risk factors in North Kordofan state(Sudan):

3.1 : Results

From a total of 310 cattle inspected, only 4 (1.3%) animals were positive for liver fluke, while the eggs tests in fecal samples were negative for fasciolosis (table 3.1.1) , thus the overall prevalence of fasciolosis in our study was 1.3%.

Table 3.1.1: Frequency table for distribution of fasciolosis infection among 310 cattle examined in North Kordofan state , Elobied abattoir:

Disease	Frequency	Relative Frequency%	Cumulative Frequency
-ve	306	98.7	98.7
+ve	4	1.3	100.0
Total	310	100	

Negative = - ve

Positive = +ve

3.2 :Interpretation of results :

3.2.1: Age of animals:

During antemortum inspection of animals in Elobeid abattoir , animals were grouped to two groups, less or equal four years was young group and more than four years, old one . Four animals among three hundred ten were found infected with liver flukes (1.3%) (table 3.1.1) .Of these , two animals were young among 208 animals ,the infected rate is (0.9%) , and two animals infected from old group among 102, the infected rate was (1.9%) (table 3.1.2). There was no significant association between fasciolosis and animal age in the univariate analysis (p. value=0.46) (table 3.1.5).

3.2.2: Sex of animals:

Among three hundred ten animals inspected in North Kordofan state abattoir for fasciolosis , 228 were male 73.5% and 82 female 26.5% . The result showed that one male was positive for fasciolosis , the infection rate was (0.4%) (table 3.1.3), and three female, the infection rate was (3.6%) (table 3.1.3). In univeriate analysis there was significance association between the sex and fasciolosis in north Kordofan state (p.value = 0.027). table(3.1.5).

3.2.3: **Body condition**:

Animals which brought to Elobied abattoir to be slaughtered were grouped and examined in to two body condition categories (good body condition, poor body condition). From those categories , the highest fasciolosis prevalence was recorded in poor body condition, the infection rate was (15.3%) (table 3.1.3) compared with prevalence in good body condition (0%) as showed in table (3.1.3). This result revealed that there is statistically significant association (p.value= 0.000) (table 3.1.5) in the occurrence of fasciolosis and body condition

3.2.4:**Breed of animals:**

In this study the breed was categories into three categories which were (Local, Cross ,Foreign), the local animals from any state of the

Sudan, cross animals were breed from Sudanese cattle and foreign breed , foreign animals came from South Sudan country.

The prevalence of fasciolosis was observed in foreign, local, cross breed (2.3% , 1.1% ,0%) (table 3.1.3) ,respectively. In univariate analysis there was no significance association between bovine fluke infections and breed in North Kordofan state,(p. value =0.64) ,table (3.1.4).

3.2.5:Source of animals:

The investigation of animals slaughtered in North Kordofanfor infection of fasciolosis revealed that the infection rate in animals came from White Nile, South Sudan country and Kordofan state were (14% , 8% , .4% table 3.1.3) , respectively. Statistical analysis of the data showed that there was highly significant association between fasciolosis and source of animals, (p.value=0.00),(table 3.1.5).

3.2.6: Size of liver :

In 310 cattle examined there were four (1.3%) livers were large and 306 normal (98.7%). All four large livers were affected with fasciolosis. Thus infection rate was (1.3%) and no one in normal livers was affected with fasciolosis was (0%). In the univariate analysis, there was significant association between liver size and fasciolosis (P-value=0.000), (table 3.1.5).

3.2.7:Color of liver:

In 310 cattle slaughtered at Elobied slaughter house 305 livers in post mortem were brown (98.7%) and 5 livers pale (1.6%), all pale livers were affected (80%) and no brown livers affected (0%). In this study there was significance association between color of liver and fasciolosis (P-value= 0.00), (table 3.1.5).

3.2.8:Lesions in the liver:

Among 310 cows inspected in slaughter house of Elobied abattoir there was no lesions in 300 livers (968%),and 10 livers were found with lesions like calcification , jaundice , hemorrhages ,fibrinous, firm bile duct , in these 10 livers there were 4 livers affected (40%) with fasciolosis and 6 lesions in livers not affected with fasciolosis .In univariate analysis there was significance association between fasciolosis and lesions in livers ,
(P-value=0.00), (table 3.1.5).

Table 3.1.2: Summary of frequency tables for potential risk factors of Fasciolosis in 310 cattle examined at North Kordofan State Slaughterhouse, Sudan :

Risk Factors	Frequency	Relative Frequency%	Cumulative Frequency%
Sex			
Female	82	26.5	**26.5**
Male	228	73.5	**100.0**
Age			
young≤**4 years**	208	67.1	**67.1**
old>**4 years**	102	32.9	**100.0**
Breed			
Local	255	82.3	**82.3**
Cross	12	3.9	**86.2**
Foreign	43	13.8	**100.0**
Body condition			
Good	284	8.4	**8.4**
Poor	26	91.6	**100.0**
Source			
Kordofan	284	91.6	**91.6**
White Nile	14	4.5	**96.1**
Foreign	12	3.9	**100.0**
Size of liver			
Normal	306	98.7	**98.7**
Large	4	1.3	**100.0**
Color of liver			
Brown	305	98.4	**98.4**
Pale	5	1.6	**100.0**
Lesions			
No	300	96.8	**96.8**
Yes	10	3.2	**100.0**

Table 3.1.3: Summary of cross tabulation for potential risk factors of Fasciolosis in 310 cattle examined at North Kordofan State-Elobied slaughterhouse:

Risk factors	No . Inspected	No. Affected (%)
Sex:		
Male	228	1(0.4%)
Female	82	3(3.6%)
Age:		
Young≤4 years	208	2(0.9%)
Old> 4years	102	2(1.9%)
Body condition:		
Good	284	0(*0*%)
Poor	26	4(15.3%)
Breed:		
Local	255	3(1.1%)
Cross	12	0(0%)
Foreign	43	1(2.3%)
Source:		
Kordofan	284	1(0.4%)
White Nile	14	2(14.2%)
South Sudan	12	1(8.3%)
Size		
Normal	**306**	**0(0%)**
Large	**4**	**4(100%)**
Color of liver		
Brown	**305**	**0(0%)**
Pale	**5**	**4(80%)**
Lesions		
No	**300**	**0(0%)**
Yes	**10**	**4(40%)**

Table 3.1.4: Summary of univariate analysis for potential risk factors of Fasciolosis in 310 cattle examined at Elobied slaughterhouse in North Kordofan State – Sudan -using the Chi- square test:

Risk factors	No.Inspected	No. Affected (%)	d.f.	Chi-square value	p- value
Sex: Female Male	 82 228	 3 (3.6%) 1 (0.4%)	1	4.909	0.027
Age: Young≤ 4 years Old> **years**	 208 102	 2 (0.9%) 2 (1.9%)	1	0.537	0.46
Body condition: Poor Good	26 284	4 (15.3%) 0 (0%)	1	44.63	0.000
Breed: Local Cross Foreign	 255 12 43	 3 (1.1%) 0 (0%) 1(2.3%)	2	0.874	o.64
Source: Kordofan White Nile Foreign	 284 14 12	 1(o.3%) 2 (14%) 1 (8.3%)	2	25.19	0.000
Size of liver: Normal Large	 306 4	 0 (0%) 4 (100%)	1	3.1	0.000
Color: Brown Pale	 305 5	 0 (0%) 4 (80%)	1	2.47	0.000
Lesions: No Yes	 300 10	 0 (0%) 4 (40%)	1	1.21	0.000

Table 3.1.5: Multivariate analysis for potential risk factors of Fasciolosis in 310 cattle examined at Elobied slaughterhouse in North Kordofan State – Sudan -using the Logistic Regression:

Risk factors	No. inspected	No. affected (%)	D.f.	Exp(B)	p- value
Sex:			1		0.027
Female	82	3 (3.6%)		1.22	
Male	228	1 (0.4%)		-	
Source:			2		0.000
Kordofan	284	1(o.3%)		-	
Foreign	12	1 (8.3%)		1.80	
White Nile	14	2 (14%)		2.02	
Body condition:			1		0.000
Poor	26	4 (15%)		5.9	
Good	284	0(0%)		-	
Size of liver:			1		0.000
Normal	306	0 (0%)		-	
Large	4	4 (100%)		1.32	
Color:			1		0.000
Brown	305	0 (0%)		-	
Pale	5	4 (80%)		31.17	
Lesions:			1		0.000
No	300	0 (0%)		-	
Yes	10	4 (40%)		6.16	

Chapter Four

Discussion

The result of the present study revealed that the overall prevalence of bovine fasciolosis in Elobied slaughterhouse (North Kordofan state) was 1.4% . The low prevalence of bovine fasciolosis recorded in this study is in agreement with Amna,et al, (2011) .Alison, et al ., (2012) .Idris ,et al., (2012) in White Nile state(10.4%) , Uganda country (1.1%), Abyei state (5%), respectively.

The highest prevalence was observed in animals which originated form White Nile (14%) and lowest prevalence in animals originated from Kordofan and South Sudan country (1%) in both, although previous result in Abyei indicated prevalence (5%). Statistical analysis of result indicate that there was significant difference (p. value < 0.05) ,in prevalence of fasciolosis among the animals brought to the Kordofan slaughter house .This variation may be due to differences in ecological and climatic condition such as rain fall and temperature and livestock management system.

The results of the present study revealed that sex has significant affect on the prevalence of bovine fasciolosis.(p. value < 0.05), where prevalence in females was 3.6% compared with males which was 0.4% This reslut is in agreement with **Kuchai**, et al ., (2011) in Ladakh. However, the work done by Phiri et *al*., (2005) in Zambian cattle and Umbreen et al ., (2012) in Punjab , concluded that sex has no impact on the infection rate and hence both male and female are equally susceptible and exposed to the infection.

Higher prevalence rates in females can be attributed to the fact that most or all the female cattle are kept for breeding and milk productions. Or this could be due to the physiological peculiarities of female animals, which usually constitute stress factors, thus, reducing their resistance to infections.

This study indicated a prevalence in two groups of age ,young (< or= 4 years), old animals (>4 years) as 0.9% and 1.9%, respectively. Statistical analysis , showed the absence of significant variation in the occurrence of fasciolosis among the different age groups of animals . This might be due to similar management systems for all ages.This result was in agreement with reslut done by Ephrem et al., (2012) in Ethiopia. However high prevalence of fasciolosis was seen in old cattle ,compared to young ones and significant statistical differences were observed in other sides in Ethiopia, Shiferam et al., (2011) ,Yemirach et al ., (2012) , Dawit et al., (2011) and Alson., (2007) in Spain.

Infection rate of bovine fasciolsis was statistically analyzed on the base of body condition to study the impact of the disease in debilitating (emaciating) infected animals. The prevalence of fasciolosis in poor body condition was found to be 15.3%, compared with good body condition 0% . Statistical analysis of data showed highly significant association with fasciolosis in cattle slaughtered in North Khordofan state and body condition score (p. value = 0.00) .This finding suggest the import of fasciolosis in causing weight loss and emaciation as characteristic sign of the disease . Also *fasciola* worms are known to suck blood and tissue fluid and even damage the parenchyma of the liver due to the migrating immature worms, Dechasa , et al ., (2012). This result was in agreement with Ephrem, et al ., (2012) , Yemisrash, et al., (2012) , Mihreteab, et al ., (2010) and Dchasa , et al ., (2012) all these studies in Ethiopia . However our results disagree with Shiferaw, (2011), around Assela in Ethiopia.

The prevalence of fasciolosis was observed in foreign, local, and cross breed as 2.3% , 1.1% and 0% , respectively. In the univariate analysis there was no significant association between bovine fluke infections and breed in North Kordofan state,(p. value =0.64) . Most local animals came from Kordofan . Where the climatic condition such as rainfall and persistent water sources is scare . These factors are important for snail vector to develop . The higher prevalence was observed in foreign breed which came from South Sudan country where the most important factors that influence occurrence of fasciolosis are exist, the ideal moisture , heavy rain fall , low temperature and permanent water sources . The low prevalence observed in cross breed may be due to good management to the cross in Sudan to avoid any loss from diseases and to obtain high production to gain high income. This result was in agreement with result obtained in Ethiopia by Ggebret ,et al.,(2010) . However, these results disagreed with observation reported by Asresca ,et al., (2011) and Yildinim ,et al.,(2007) in Andassain , north west Ethopia and Trukey , respectively.

Different lesions of infectious and non-infectious livers causes like abscess, calcification, hemorrhages , fibrosis and firm bile duct, were found in livers which inspected in north Kordofan slaughter house state and all infected livers were large in size and pale in color . All these observations were encountered in infected liver by fasciolosis. These results were in agreement with Ahmed, et al., (2013) , Borij, et al.,

(2010) and Caicedo, et al., (2011) in Egypt , Iran and Mexico, respectively.

According to the results of present study, the prevalence of fasciolosis in North Kordofan state was not high(1.4%), because most animals in study were from Kordofan , compare with studies in EL Gezira state12.5% , Abyei area 5% and White Nile State 10.4%, Koko , et al.,(2003) , Idriss , et al., (2012) , Amna, et al, (2011), respectively . This may be due to the ecological condition of the study area , scare of water sources or low rainfall which make low infestation of cattle to *fascola* due to absent of intermediate host(snails).

Conclusion

Fasciolosis is an important helminth disease and one of the major obstacles for livestock development in Sudan causing remarkable, direct and indirect losses at different parts of the country where its occurrence closely linked to the presence of biotypes suitable for the surviving of snail intermediate host . The study confirmed that there was significant association in the prevalence of fasciolosis with different factors such as sex , animals origin, body condition . In examined cattle showed that *fasciola* parasite was more prevalent in cattle which came from White Nile state compare to animals came from Kordofan and South Sudan country .

Recommendations

The following recommendations are for warded :

- Application of good drainage and building of dams at appropriate sites in marshy and low laying area may reduce the snails existence .
- Mollucicidal activity along streams should be given specially for animals which are grazing near dams or water bodies site .
- Keeping the animals off from marshy areas or by fencing these hazardous zone.
- Finally , the farmers should be aware of and informed about the importance of the disease control programs .

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Appendix 1

Frequency tables for distribution of infection among 310 cattle examined at Elobied abattoir according to potential risk factors:

Appendix 1.1: Sex

Valid	Frequency	Percent	Valid Percent	Cumulative Percent
Female	82	26.5	26.5	26.5
Male	228	73.5	73.5	100
Total	310	100	100	

Appendix 1.2: Age

Valid	Frequency	Percent	Valid Percent	Cumulative Percent
Young	208	67.1	67.1	67.1
Old	102	32.9	32.9	100
Total	310	100	100	

Appendix 1.3: Breed

Valid	Frequency	Percent	Valid Percent	Cumulative Percent
Local	255	82.3	82.3	82.3
Cross	12	3.9	3.9	86.2
Foreign	43	13.8	13.8	100
Total	310	100	100	

Appendix 1.4 : Body condition

Valid	Frequency	Percent	Valid Percent	Cumulative Percent
Good	284	91.6	91.6	91.6
Poor	26	8.4	8.4	100
Total	310	100	100	

Appendix 1.5: Source of animals

Valid	Frequency	Percent	Valid Percent	Cumulative Percent
Kordofan	284	91.6	91.6	91.6
White Nile	14	4.5	4.5	96.1
Foreign	12	3.9	3.9	100
Total	310	100	100	

Appendix 1.6: Size of liver

Valid	Frequency	Percent	Valid Percent	Cumulative Percent
Normal	306	98.7	98.7	98.7
Large	4	1.3	1.3	100
Total	310	100	100	

Appendix 1.7 : Color of liver

Valid	Frequency	Percent	Valid Percent	Cumulative Percent
Brown	305	98.4	98.4	98.4
Pale	5	1.6	1.6	100
Total	310	100	100	

Appendix 1.8: Lesions in the liver

Valid	Frequency	Percent	Valid Percent	Cumulative Percent
No	300	96.8	96.8	96.8
Yes	10	3.2	3.2	100
Total	310	100	100	

Appendix 2

Distribution and prevalence of infection among 310 cattle examined at Elobied abattoir according to potential risk factors:

Appendix 2.1 : Sex

	Sex of animal		Total
	Female	**Male**	
Results - ve	79 96.3%	227 99.5%	306 98.7%
+ ve	3 3.6%	1 0.4%	4 1.3%
Total	**82**	**228**	**310**

Appendix 2.2: Age

	Age of animals		Total
	Young	**Old**	
Results - ve	206 99%	100 98%	306 98.7%
+ ve	2 0.9%	2 1.9%	4 1.3%
Total	**208**	**102**	**310**

Appendix 2.3: Breed of animals

Results	Breed of animals			Total
	Local	Cross	Foreign	
-ve	251 98.8%	12 100%	43 97.7	306 98.7%
+ve	3 1.2%	0 0%	1 2.3%	4 1.3%
Total	254	12	44	310

Appendix 2.4: Body condition of animals

Results	**Body condition**		Total
	Good	Poor	
-ve	284 100%	22 84.6%	306 98.7%
+ve	0 0%	4 15.3%	4 1.3%
Total	284	26	310

Appendix 2.5 : Source of animals

Results	Source of animals			Total
	Korodofan	White Nile	Foreign	
-ve	283 99.6%	12 85.7%	11 91.6%	306 98.7%
+ve	1 0.3%	2 14.2%	1 8.3%	4 1.3%
Total	284	14	12	310

Appendix 2.6 : Size of liver

Results	**Size of** liver		Total
	Normal	Larg	
-ve	306 100%	0 0%	306 98.7
+ve	0 0%	4 100%	4 1.3%
Total	306	4	310

Appendix 2. 7 : Color of liver

Results	**Color of** liver		Total
	Brown	Pale	
-ve	305 100%	1 20%	306 98.7%
+ve	0 0%	4 80%	4 1.3%
Total	305	5	310

Appendix 2.8 : Lesions in the liver

Results	**Lesions in the** liver		Total
	No	Yes	
-ve	300 100%	6 60%	306 98.7%
+ve	0 0%	4 40%	4 1.3%
Total	300	10	310

Appendix 3

Association between fasciolosis infection and potential risk factors using the Chi- square test:

Appendix 3.1: Sex

	Value	Df	Asymp.sig (2-sided)
Pearson chi- square	4.90	1	0.027
Likelihood Ratio	4.15	1	0.100
Linear by Linear Association	4.89	1	0.027
N of Valid Cases	310		

Appendix 3.2: Age

	Value	Df	Asymp.sig (2-sided)
Pearson chi- square	0.537	1	0.464
Likelihood Ratio	0.504	1	0.478
Linear by Linear Association	0.535	1	0.465
N of Valid Cases	310		

Appendix 3.3 : Breed

	Value	Df	Asymp.sig (2-sided)
Pearson chi- square	0.874	2	0.646
Likelihood Ratio	1.574	2	0.455
Linear by Linear Association	0.818	1	0.366
N of Valid Cases	310		

Appendix 3. 4: Body condition

	Value	Df	Asymp.sig (2-sided)
Pearson chi- square	**0.371**	**1**	**0.542**
Likelihood Ratio	**0.706**	**1**	**0.401**
Linear by Linear Association	**0.370**	**1**	**0.543**
N of Valid Cases	**310**		

Appendix 3. 5: Source of animal

	Value	Df	Asymp.sig (2-sided)
Pearson chi- square	**25.19**	**2**	**0.000**
Likelihood Ratio	**11.089**	**2**	**0.000**
Linear by Linear Association	**16.811**	**1**	**0.000**
N of Valid Cases	**310**		

Appendix 3.6: Size of liver

	Value	Df	Asymp.sig (2-sided)
Pearson chi- square	**3.100**	**1**	**0.000**
Likelihood Ratio	**42.75**	**1**	**0.000**
Linear by Linear Association	**125.78**	**1**	**0.000**
N of Valid Cases	**310**		

Appendix 3.7: Color of liver

	Value	Df	Asymp.sig (2-sided)
Pearson chi- square	**2.472**	**1**	**0.000**
Likelihood Ratio	**37.74**	**1**	**0.000**
Linear by Linear Association	**246.391**	**1**	**0.000**
N of Valid Cases	**310**		

Appendix 3.8: Lesions in the liver

	Value	Df	Asymp.sig (2-sided)
Pearson chi- square	**1.21**	**1**	**0.000**
Likelihood Ratio	**29.29**	**1**	**0.000**
Linear by Linear Association	**121.176**	**1**	**0.000**
N of Valid Cases	**310**		

Appendix 4

Questionnaire to get information about potential risk factors associated with fasciolosis disease in North Kordofan

- Animal NO( )

1. Breed of animal

   0-Local( ) 1-Cross( ) 2- Foreign ( )

2. Age of animal

   0- Less or equal four years(young) 1- More than four years (old)

3. Sex

   0- Female ( ) 1- male ( )

4. Body condition

   0- Good ( ) 1- Poor ( )

5. Source of animal

   0- Kordofan ( ) 1-white Nile ( ) 2- Foreign( )

6. Lesions in the liver

   0. No ( ) 1. Yes ( )

7. Color of the liver

   0. Brown ( ) 1. Pale ( )

8. Size of the liver

   0. Normal ( ) 1. Large ( )

9. Result of fasciolosis

   0. Negative ( ) 1. Positive

Appendix 5

استبيان لجمع بيانات عن نسبة الاصابة وعوامل الخطر لمرض

الدودة الكبدية بولاية شمال كردفان

- رقم الحيوان ( )

1- سلالة الحيوان

0- محلى 1- هجين 2- سلالة اجنبية

2-عمر الحيوان

0- اقل من او يساوى اربعة سنوات

1- اكثر من اربعة سنوات

3-جنس الحيوات

0- انثى 1- ذكر

4- البنية الجسمية للحيوان

0- جيد 1- هزيل

5- مصدر الحيوان

0- ولايات كردفان 1- النيل الابيض 2-خارج السودان

6- وجود افات بالكبد

0- لا 1- نعم

7- لون الكبد

0- بنى 1- شاحب

8- حجم الكبد

0- طبيعى 1- كبير

9- نتيجة الفحص لابوكبيدة

0- لا 1- نعم