INTRODUCTION

Background:

Fasciolosis is an economically important parasitic disease, which is caused by Trematode of the genus *Fasciola* that migrates in the hepatic parenchyma, and establishes and develops in the bile ducts (**Troncy, 1989)**. *Fasciola* is commonly recognized as liver flukes and they are responsible for wide spread of morbidity and mortality in cattle characterized by weight loss, anemia and hypo proteinemia (**Tsegaye *et al*., 2011)**. The parasite is transmitted by ingestion of metacercariae of *Fasciola* species on plants from contaminated fresh water. Fasciolosis is now recognized as an emerging animal disease (WHO, 1995).

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. *Fasciola hepatica* is found in area above 1800 m.a.s.l. In between these altitude limits, both species coexists where ecology is conductive for both snail hosts, and mixed infections prevailed . The snail of the genus *Lymnae natalensis* and *Lymnae truncatula* are known as intermediate host in life cycle of Fasciolosis. Infection with *Lymnae truncatula* is usually associated with herds and flocks grazing wet marshy land. On the other hand, Fasciola gigantica is a fresh water snail and infection with this species is associated with livestock drinking from snails infected watering places as well as with grazing wetland, which may be seasonally in undated (**Tsegaye *et al*., 2011)**. Fasciolosis is an economically important disease of domestic livestock, in particular cattle and sheep and occasionally man . The disease is responsible for considerable economic losses in the cattle industry, mainly through mortality, liver condemnation, reduced production of meat, milk, and wool, and expenditures for anthelmintics (**Hillyer *et al*., 1997**). increased costs for replacement stock In cattle, losses include reduced production and quality of milk, lower growth rates and lower feed conversion rates in fattening cattle (**Boray, 2007**).

The disease causes poor health, reduced growth rate, high mortalities, especially in small ruminants and calves . For example, **Kithuka *et al.* (2002)** reported up to 0.26 million USD annual loss attributable to Fasciolosis-associated liver condemnations in cattle slaughtered. Apart from its veterinary and economic importance throughout the world, Fasciolosis has recently been shown to be a re-emerging and widespread zoonosis affecting a number of human populations . **(Mwabonimana *et al*., 2009).** Human fascioliosis was characterized by the fallowing symptoms due to the migrating larvae causing localised or generalized toxic and allergic reactions lasting 2-4 months; the major symptoms of this phase are fever, abdominal pain, gastrointestinal disturbances, urticaria, and respiratory symptoms; the usual signs are hepatomegaly and splenomegaly, ascites, anemia, chest signs and jaundice. In human endemic areas, the infection is usually repetitive and the acute lesions are superimposed on chronic disease. Thus, the acute phase may be prolonged and overlap on to a latent or an obstructive phase, fascioliosis chronicity and superimposed repetitive infections posing additional pathological complications **(Coma, 2004).**

The parasite lives in liver (*Fasciola*, liver fluke), The life cycle of flukes are always indirect, involving intermediate host before invasion of definitive hosts. The snails such as *Lymnaea spp* for *Fasciola*; dependent of their close environment (nature of the soil), and of the climatic conditions for survival and multiplication of the intermediate hosts and also for the survival and evolution of larval stages (miracidium, sporocyst, redia, cercaria, and metacerceriae). **(Dorchies ., 2006).**

Pathogenesis of Fasciolosis varies according to the phase of parasitic development in the liver and species of host involved, essentially the pathogenesis is twofold; the first phase occurs during migration in the liver parenchyma and is associated with liver damage and hemorrhage. Early infection, during fluke migration, there is hyper proteinemia, hyper globulinemia, and hypo-albuminemia. The hypo-albuminemia is associated with plasma volume expansion caused by liver damage and reduced albumin synthesis. The second phase occurs when the parasite is in the bile ducts, and results from the hematophagic activity of the adult flukes and from the damage to the mucosa, by their cuticles spines (**Yeneneh *et al*., 2012).**

1.1 Justification:

Bovine Fasciolosis is one of the important diseases in the areas from where the animals brought resulting remarkable losses due to liver condemnation. Is one of the zoonosis diseases also the human fascioliosis is at present emerging or re-emerging in our country, including increases of prevalence and intensity and geographical expansion. Research in recent years has justified the inclusion of fascioliosis in the list of important human parasitic diseases. Beside the economic loss caused by the disease. This study is the beginning to develop appropriate solutions to control the disease and reduce the economic loss.

1.2 Objective of the study:

1\ To estimate the prevalence of bovine Fasciolosis in Khartoum State.

2\ To investigate the risk factors associated with bovine Fasciolosis in Khartoum State.

CHAPTER ONE

1. LITERATURE REVIEW

1.1. Definition:

Fasciolosis, a disease of the bile duct of domestic herbivorous animals, contributes to great economic and health losses in the cattle industry in many countries worldwide **(Qureshi *et al*., 2012)** Fasciolosis is an economically important disease of domestic livestock, in particular cattle and sheep. The disease is caused by digenean Trematode of the genus *Fasciola*, commonly referred to as liver flukes. The two species most commonly implicated as the etiological agents of Fasciolosis are *F. hepatica* and *F. gigantica*. *F. hepatica* has a worldwide distribution but predominates in temperate zones while *F. gigantica* is found on most continents, primarily in tropical regions **(Kassaye *et al*., 2012).** Both *F. hepatica* and *F. gigantica* are transmitted by the snails of the family Lymnaesidae **(Terefe *et al*., 2012)**. The differences most frequently highlighted between the two species are the increased length in *F. gigantica* and the general appearance of the body, which is narrower and elongated in *F. gigantica* and shorter, broader and curved in the shape of a lancet in *F. hepatica* **(Kendall, 1965).** It has also been noted that these two species differ in the shape and size of their cuticles scales **(Varma, 1953).** Other authors have differentiated both species on the basis of the ramification patterns of the reproductive organs and intestines **(Bergeon and Laurent, 1970**), but the natural branching shape of these structures make this characteristic impractical. Surprisingly, although many morphometric studies have dealt with F. hepatica, very few have focused on F. gigantica **(Srimuzipo *et al*., 2000).** 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 male and female reproductive organs are present near the posterior sucker in the center of the body. The female reproductive tract is dense ovary and is located just above the testes and is linked the adult mature and gravid fluke is flat with it is body shape like leaf. 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 to short convoluted uterus that opens to genital pore above the ventral suckers. The vitellaria are highly dispersed and divided in the lateral and posterior region of the body **(Saria., 2011).**

Fasciola hepatica is very similar to each other, varying in length and width. In addition, the cephalic con of the *Fasciola hepatica* is shorter than *Fasciola gigantica*. The shape of the eggs of the tow flukes is also very similar with the measure meant of the *Fasciola hepatica* and *Fasciola gigantica* being approximately 150 um*90um and 200um *100um, respectively **(Saria., 2011).** Characteristics of *Fasciola* egg is yellow-brown in colour, large and oval in shape. It has an indistinct operculum (lid). It contains an unsegmented ovum surrounded by many yolk cells. **(Monica, 1987).**

1: 21 Taxanomy Of Bovine Fasciolosis: According to **Dunn (1978)and Soulsby (1982)**

Kingdom: Animalia

Phylum: Platy helminthes

Class: Trematode

Order: Digenean

Family: Fasciola

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

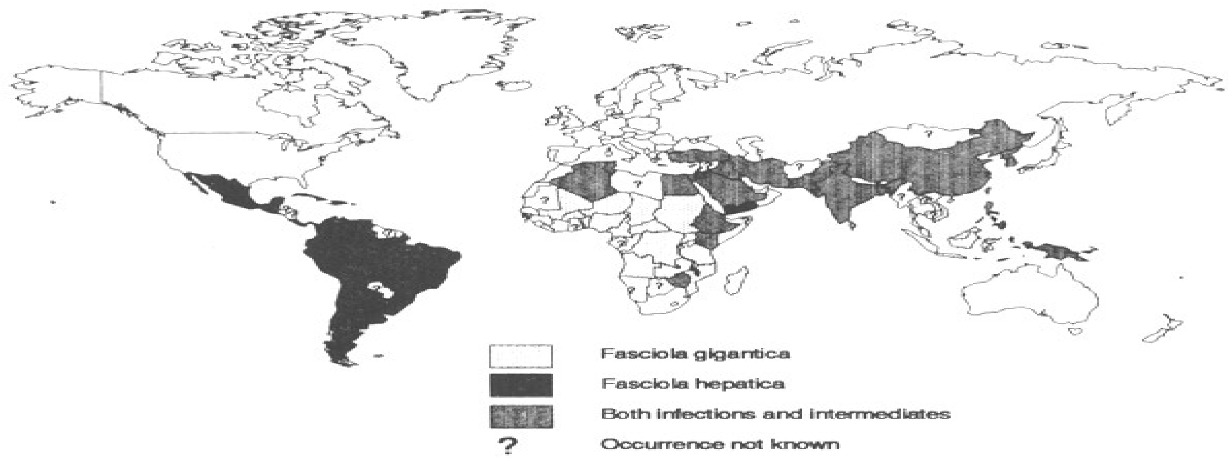
1. 3. Epidemiology:

The epidemiology of Fasciolosis depends on the grazing habitat preference of the animal. metacercariae can survive up to 3 months after harvesting in hay from endemic high land areas that are consumed by ruminants in arid and low land areas **(Malone et al., 1998. Terefe et al., 2012).**

1.4.Distribution:

Fascioliosis also known as (Fasciolosis, , distomatosis and liver rot) is a parasitic disease that primarily strikes livestock animals like sheep and cattle. Fascioliosis is an important Helminth disease caused by two Trematode *Fasciola hepatica* and *Fasciola gigantica*. This disease belongs to Trematode zoonosis, i.e. a disease of animals that can be transmitted to humans. It appears in many parts of the world, but it’s generally more common in South America, parts of Asia, and Africa. In Europe, the Americas and Oceania only F*asciola* hepatica is of concern, but the distributions of both species overlap in many areas of Africa and Asia **(Mas-Coma, *et al,* 2005).**in Africa the regional distribution of liver flukes is complicated by the separate and simultaneous occurrence of F. hepatica and *F. gigantica*. Moreover, on the base of morphology intermediate forms have been described in region 4 particularly **(FAO,1992).**

This review on the geographical distribution of Fasciola species and their host preferences is based on the selection of relevant data from literature published during the last 15 years. This has created a database which compile the most recent information on the distribution patterns of Fasciola gigantica, *F. hepatica* **(FAO,1992).** 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. *Fasciola hepatica* is found in area above 1800 m.a.s.l. In between these altitude limits, both species coexists where ecology is conductive for both snail hosts, and mixed infections prevailed. **Yilma Jobre ; *et al*,(1998**). The snail of the genus *Lymnae natalensis* and Lymnae truncatula are known as intermediate host in life cycle of Fasciolosis .Infection with *Lymnae truncatula* is usually associated with herds and flocks grazing wet marshy land. On the other hand, *Fasciola gigantica* is a fresh water snail and infection with this species is associated with livestock drinking from snails infected watering places as well as with grazing wetland, which may be seasonally in undated **Payne WJA. (1990**).



Figure(1) Distribution of Fasciola spp. in developing countries **(FAO, 1992)**

1.5.Transmission of Fasciolosis:

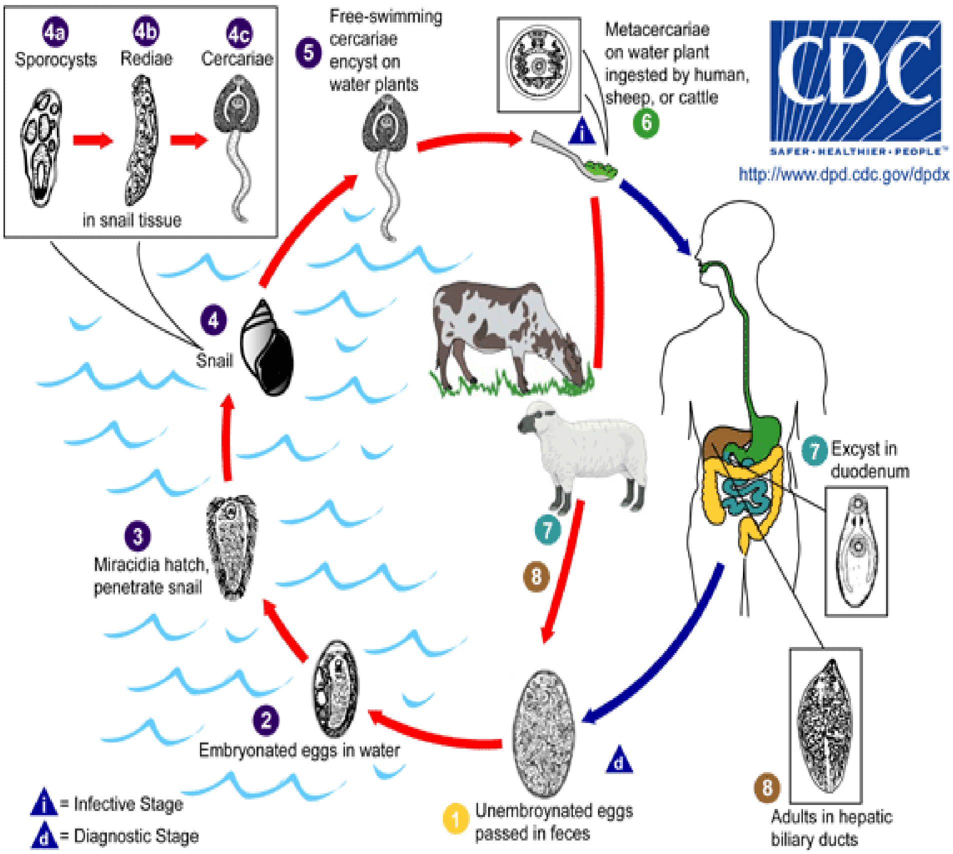
Once an animal is infected with fascioliosis, it will pass eggs from the faces of the animals. On entering the water, the eggs hatch into miracidia. The miracidia locate water snails by chemotaxys. Sporocyst produce rediae and rediae may produce second generation rediae. Cercariae emerge from the snails. Encystment of Cercariae on vegetation occurs at the edge of water. Sheep and cattle become infected by ingesting metacerceriae while grazing. People normally catch the disease more often in situations where livestock live in the same general areas where food is grown. Most humans catch it from underwater food plants like watercress. Infection can potentially be avoided by cooking these plants fully before eating them. In some areas, eating these plants raw is relatively customary, and fasciliasis in humans is more common in those areas.(**Usip *et a*l., 2014).**

1.6.Life Cycle:

Infestation with Fasciolosis is usually associated with grazing wet land and drinking from the snail infesting watering places **(Terefe *et al*., 2012)**. The life cycle of *Fasciola spp.* is a typical of Digenetic Trematode. Eggs laid by the adult parasite in the bile ducts of their hosts pass into the duodenum with the bile. The eggs then leave the host through the feces. At this stage, eggs are still not embryonated, further development to maturation taking approximately two weeks. The eggs then hatch to release the motile miracidium, which will then locates and penetrates the intermediate snail host. The need to find a suitable host to penetrates an urgent one, for those miracidia failing to do so generally die within 24 hours.6After penetrating the snail, the miracidium loses its cilia and becomes a sporocyst. The sporocyst dividing and forming redia (forum with sucker and primitive gut), and a fully mature redia showing redia and cercaria stages. The cercaria of *Fasciola spp.* have a rounded body measuring between 0.25and 0.35mm long, with a long thin unbranched tail measuring approximately 0.5mm long. The mobile cercaria snail generally leaves the snail 4-7 weeks after infection by migrating through the tissues of snails. This is during moist conditions when a critical temperature of 10°C is exceeded. On emerging from the snail the cercaria attaches to submerged blades of grass or other vegetation like watercress; the tail falls away and the cercaria body secretes a four-layered cyst covering from cysto genius glands located on the lateral regions of the body. The formation of the cyst wall may take up to two days. The meta cercaria (encysted, resistant Cercariae) is the infective form to the definitive host. Generally, meta cercaria are infective to ruminants such as cattle and sheep, but also to other mammals including human beings .One miracidium hatching from a fluke egg can produce up to 4,000 infective cysts (meta Cercariae) due to the vegetative multiplication at the spore cyst and redia stages.. The meta cercaria cyst is only moderately resistant, not being able to survive dry conditions. If however they are maintained in conditions of high humidity and cool temperatures ,they may survive for up to a year (**Andrews, 1999**; **Soulsby, 1982; Dunn, 1978**), infection through hay as a vehicle of infection in non-endemic areas. The meta cercaria cysts, when ingested along with the contaminated vegetation by the definitive host enter into the small intestine, releasing the young parasite which penetrates the gut wall, entering the peritoneal cavity. From there, it migrates directly to the liver over a period of approximately seven days, directly to the liver. The juvenile fluke (also referred to as adeloscaria) then penetrates the liver tissues, through which it migrates, feeding mainly on blood, for about six weeks. After this period, the fluke enters the bile ducts, maturing in to a fully adult parasite after about 3 months from initial infection. Egg production then commences and completing the lifecycle. Adult flukes can survive for many years in the livers of infected hosts and lay between 20,000 and50,000 eggs/day. The rate of egg production is responsible for the degree of pasture contamination and thus greatly influences the epidemiology of the disease.. Animals grazing in wet marshy areas, favored the intermediate host, are more likely to become infected. Typically, long and wet seasons are associated with a higher rate of infection. However, sheep are more likely to ingest large numbers of cysts during dry periods following a wet season. This is due to a reduction in available pasture ,forcing the animals to graze in swampy areas or in areas where the water has receded, thus exposing them to vegetation heavily infected with meta Cercariae (**Richter *et al.*, 1999**).

In the past, human Fasciolosis was limited to populations within well-defined watershed boundaries ;however, recent environmental changes and modifications in human behavior are defining new geographical limits and increasing the population sat risk (**WHO, 1999**) .

A tentative diagnosis of Fasciolosis may be established based on prior knowledge of epidemiology of the disease in a given environment, observation of clinical sign **(Terefe *et al*., 2012).** Infected cattle can exhibit poor weight gain and dairy cattle have lower milk yield, and possibly metabolic diseases **(Bekele *et al*., 2010).** Information on grazing history, seasonal occurrence, and identification of snail habitats. Confirmatory diagnosis however, is based on demonstration of *Fasciola* spp. Eggs through standard examination of feces in the laboratory, post mortem examination of infected animals. Even though it is impossible to detect *Fasciola* in live animals, liver examination at slaughter or necropsy was found to be the most direct, reliable, and cost effective technique for the diagnosis of Fasciolosis **(Terefe *et al*., 2012).**



Figure(2). Life cycle of *Fasciola* (ASRAT, 2004)

1.7.Clinical Signs:

The clinical features of Fasciolosis can have acute, sub-acute and chronic forms. Acute Fasciolosis occurs as disease outbreak following a massive, but relatively short-term, intake of metacercariae **(Urquhart *et. al*., 1989).** The high fluke intake is often the result of certain seasonal and climatic conditions combined with a lack of appropriate fluke control measures. It typically occurs when stocks are forced to graze in heavily contaminated wet areas as a result of over stocking and/or drought. Animals suffering from acute Fasciolosis especially sheep and goat, may display no clinical signs prior to death; while some may display abdominal pain and discomfort and may develop jaundice (Soulsby, 1982; Urquhart *et. al.*, 1989). In some cases, the liver capsule may rupture and fluid may lick into the peritoneal cavity causing death due to peritonitis. More commonly, on ingestion of fewer metacerceriae, fever and eosinophilia is seen (**Soulsby, 1982**).

Death usually results from blood loss due to hemorrhage and tissue destruction caused by the migratory juvenile flukes in the liver resulting in traumatic hepatitis. This is more commonly seen in sheep than in other hosts. Sub-acute Fasciolosis is caused by ingestion of a moderate number of metacerceriae and is characterized by anemia, jaundice and ill-thrift. The migrating fluke causes extensive tissue damage, hemorrhage and in particular liver damage. The result is severe anemia, liver failure and death in 8- 10 weeks (**Urquhart *et. al.*, 1989**).

Chronic Fasciolosis is the most common clinical syndrome in sheep and cattle. It occurs when the parasite reaches the hepatic bile duct. The principal effects are bile duct obstruction, destruction of10 liver tissue, hepatic fibrosis and anemia. The onset of clinical signs is slow. Animals become anemic and anorectic, as the adult fluke becomes active within the bile duct and signs may include dependent edema or swelling under the jaw ('bottle jaw'). Affected animals are reluctant to travel. Death eventually occurs when anemia becomes severe. Additional stress upon anemic animals, such as droving, may lead to collapse and death. Cattle typically present with signs of weight loss, anemia and chronic diarrhea (**Mitchell, 2001**).

In addition to these, a condition known as *'black disease'* is a complication, which usually is fatal. Here, a secondary infection due to the bacterium *Clostridium novyi* Type B, proliferating in necrotic lesions produced by the young larvae migrating in the liver is responsible for the fatal outcome (**Radostits, *et. al.*, 1994**). Chronic Fasciolosis provides the right environment in the liver for the germination of the spores of the bacterium. This form of the disease is much more common particularly in man. In humans the presence of the flukes causes a number of non-specific symptoms including malaise, an intermittent fever, mild jaundice, anemia, eosinophilia and frequently pain under the right costal margin. Furthermore, *Fasciola spp.* do not appear to be fully adapted to using man as a definitive host, as the flukes may often give rise to ectopic infections, particularly in the lungs and subcutaneous tissues, where they may be found encysted.

1.8.Pathology and pathophysiology:

Pathogenesis of Fasciolosis varies according to the parasitic development phases: parenchyma and bilary phases. The parenchyma phase occurs during migration of flukes through the liver parenchyma and is associated with liver damage and hemorrhage. The bilary phase coincides with parasite residence in the bile ducts and results from the hematophagic activity of the adult flukes and from the damage to the bile duct mucosa by their cuticles spines (**Urquhart, *et. al.*, 1989**).

In the bile ducts of some permissive hosts, such as the sheep, rabbit, rat and mouse, the bilary stage of the disease is common. In others, such as cattle and humans, few flukes survive beyond the migratory phase and biliary disease is relatively rare (**Behm and Sangster, 1999**). Light infections due to *Fasciola hepatica* may be asymptomatic. However, they may produce hepatic colic with coughing and vomiting; generalized abdominal rigidity, headache and sweating, irregular fever, diarrhea and anemia (**Behm and Sangster, 1999**). In domestic ruminants, an adverse effect of acute or chronic Fasciolosis includes decreased weigh gain and milk production, decreased female fertility, work power and mortality. Hepatic pathology, even when only limited areas of the liver are damaged, results in significant disturbances in mitochondrial bioenergetics metabolism of carbohydrates, proteins, lipids and steroids, as well as bile flow and bile composition (**Calléja, *et. al.*, 2000**). Sheep and goat are very susceptible to acute Fasciolosis and the damage results from the immature flukes tunneling through the liver parenchyma with extensive tissue damage and hemorrhage that culminate in severe clinical disease and high mortality in the grazing sheep in Africa **(Okewleo, 2000)**. During the movement of the immature stages of *Fasciola hepatica,* which may continue for months, symptoms may include abdominal pain, an enlarged liver, fever, and diarrhea. (**Mitchell (2001**) indicated that the pathology associated with diseases are caused by the inflammation of the bile ducts 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 of the liver parenchyma and cirrhosis **(Okewleo, 2000).**

Occasionally the worms penetrate the bile duct wall into the liver parenchyma causing liver abscesses. The complexity arises from several sources. Maturation of flukes involves development and growth for over 12-16 weeks during which time the fluke travels between and within organs. Because an individual fluke may pass the same part of the liver twice (or more) during these peregrinations, fresh and resolving lesions caused by the sequential insults may be found in the same section of tissue; as the migratory fluke grows the size of its track through the liver increases as does the damage and the inflammatory response. Calves are susceptible to Fasciolosis but in excess of 1000 metacercariae are usually required to cause clinical Fasciolosis (**Behm and Sangster,1999**). The disease is characterized in calves by weight loss, anemia, and hypo proteinemia after infection with 10,000 metacerceriae. Resistance develops with age so that adult cattle are quiet resistant to infection (**Behm and Sangster, 1999**).

Even though, the rate of development of human Fasciolosis is similar to that in sheep, as an unnatural host, only few flukes develop sufficiently to reach the bile due. Fasciolosis has a major effect on blood components (plasma proteins). Hypo albumin anemia and Hyper globulin anemia commonly occur in liver fluke infections in all host species. During the parenchyma stage of the infection, liver damage caused by the migrating flukes compromise liver function, which in sheep and calves is reflected in a decline in plasma albumin concentrations, attributed partly to reduced rate of synthesis and partly to an expansion of the plasma volume **(Behm and Sangster, 1999; Urquhart, *et. al.*, 1989).** Nevertheless, during bilary stage of the infection loss of blood from hematophagic and into the intestines is so extensive, causing severe anemia, that synthetic capacity of the liver is insufficient to replace the lost albumin (small molecular size) that oozes through the hyper plastic bile ducts (Chollengitis). Thus, a progressive loss of plasma albumin occurs in all infected host species, starting from around the time the fluke commences blood feeding. This results in disturbance in intravascular and extra vascular on cotic pressure leading to the development of edema, often markedly visible at sumandibular region of ruminants('bottle jaw'). Liver trauma is the abrasion caused by cuticles spines and the prehensile action of the suckers and appears to account for the majority of the damage caused in the liver. Death of the host is a 13 consequence of the hemorrhage induced by this damage. The oral sucker is the route by which liver flukes obtain most of their nutrition. It appears to cause considerable damage to liver tissue and macerated hepatic cells have been observed inside the sucker and pharynx. The oral sucker extends during migration and feeding from the earliest stages is capable of disrupting cells. The muscular pharynx assists in this process and oral sucker is a major organ involved in tissue disrupting (**Behm and Sangster, 1999).** Although the inflammatory process has an important role in protecting the host against severe consequences of liver damage by the flukes, perhaps by retarding the growth of the parasite and contributing to hepatic healing process, there is accumulated evidence, in rats, that the response also contributes to hepatic dysfunction. There is evidence also that the infected rat liver is under oxidative stress during the parenchyma stage of the infection.

The liver plays a central role in the physiology of the body, being responsible for a large proportion of the body's amino acid metabolism, for carbohydrate and lipid balance, urea synthesis, detoxification metabolism, ketogensis, albumin and glutathione synthesis as well as aspects of homeostasis. Therefore, it is to be expecting that many systemic changes will be induced by liver fluke infections that ultimately cause reduced productivity in livestock. Both anorexia (inappetance) and the quality of the diet of infected sheep contribute to hypo albuminemia during the infection **(Behm and Sangster, 1999**).

1.9.Diagnosis of Bovine Fasciolosis:

Diagnosis of Fasciolosis may consist of tentative and confirmatory procedures. A tentative diagnosis of Fasciolosis may be established based on prior knowledge of the epidemiology of the disease in a given environment; observations of clinical signs, information on grazing history , seasonal occurrence and examinations of snails must be considered. Confirmatory diagnosis, however, is based on demonstration of *Fasciola* eggs through standard examination of feces in the laboratory**.** postmortem examination of infected animals and demonstration of immature and mature flukes in the liver. The latter is helpful in deciding the intensity of infection **(As rat *et a*l., 2004)**. In animals, intravital diagnosis is based predominantly on feces examinations and immunological methods. Biochemical and hematological profile **(Torgerson and Claxton, 1999).** Similarly to humans, feces examinations are not reliable. Moreover, the fluke eggs are detectable in feces 8–12 weeks post-infection. In spite of that fact, fecal examination is still the only used diagnostic tool in some countries. While coprological diagnosis of Fasciolosis is possible from 8-12 weeks post-infection (WPI). *Fasciola hepatica* specific antibodies is recognized using ELISA or Western blot since 2 – 4 weeks post-infection **(Zimmerma *et al,* 1982; Duménigo, *et al,* 2000).** Therefore, these methods provide early detection of the infection. Microscopic identification of eggs is useful in the chronic (adult) stage(**Usip *et al*., 2014).**. There are other laboratory tests (enzymatic and/or serological procedures used to qualify the infection mainly for research purposes. Serological assays are often used to detect infections due to immature forms where fecal egg output is often nil. Such tests allow the detection of substance like cathepsin L proteases, excretory secretary products, detection of Ag in milk, and ELISA detection of antibodies against the flukes plasma concentration of Gamma-glutamine transferase (GGT), which are increased within the bile duct damage **(Cornelissen, *et. al*., 2001)** for example, Oxidative stress would be one of the consequences of the activity of inflammatory cells such as neutrophils, macrophages and eosinophils in producing oxygen-derived free radicals, nitric o\][P BNxide and their products. A useful indicator of oxidative stress is the concentration of reduced glutathione (GSH) in cells. For chronic Fasciolosis, confirmatory diagnosis could easily carried out by coproscopic examination employing sedimentation technique. *Fasciola* eggs have high specific gravity and sedimentation is preferred to floatation. When the latter is employed, floating medium such as ZnSo4 should be used. As *Fasciola* eggs may be confused with *Paramphistomum* eggs, addition of meth line blue in the feacal suspension will facilitate ease identification by providing a blue and contrasting microscopic field **(As rat *et al*., 2004)** F.gigantica infection in large ruminants is commonly diagnosed by examining feces for fluke eggs using microscopy. Immunodiagnostic methods with higher test sensitivity for F.gigantica exist and have been used in some surveys in South-East Asia **(Nguyen *et al*. 2012).** Their use for routine diagnosis of F.gigantica infection in livestock however remains limited in developing countries due to high costs, low labor transport limitation for getting samples to laboratories.

1:10: Identification of Fasciola spp:

1.10:1: Morphological identification:

The traditional identification methods are based on discrimination of morphology between species: F. hepatica and F. gigantica, described by **Dunn (1978)**. A more recent technical approach includes a computer image analysis system (CIAS) that is based on the standardized measurements of distances between organs of flukes **(Thanh, 2012)** .

1.10.2 Cytogenetic identification:

The number and morphology of metaphase chromosomes have long been used to characterize species. Phylogenetic studies have mainly used information regarding chromosome numbers and morphology rather than fine details of chromosome organization, and hence have been applicable only for high-level comparisons **(Session, 1990).**

1.10.3 Molecular identification :

PCR technology and DNA sequencing techniques facilitate identification of species, clarification of strains and genetic populations. The selected gene or sequence must be common, highly conserved within, and sufficiently divergent between taxa. Ideally, the variable regions should have adjacent conserved regions so that “universal” oligonucleotide primers may be chosen **(Thanh, 2012).**

1.11. Control and prevention of bovine Fasciolosis :

1:11:1 chemical methods:

Several control methods against ruminant Fasciolosis are available and can either be used independently and as a combination of two or more of them. These methods involve reduction in the number of intermediate snail hosts by chemical or biological means, strategic application of anthelmintics, reduction in the number of snails by drainage, fencing and other management practices and reduction in the risk of infection by planned grazing management **(As rat, 2004).** Chemotherapy has been used for years in animal populations to decrease the animal reservoir and reduce agricultural losses. Until recently, however, bithionol was the only treatment available for fascioliosis and its cost, high doses, and the extended length of treatment effectively prohibited its use for large scale campaigns. The antithelmintic triclabendazole is not yet approved in most countries, but shows great promise for its utility in single-dose chemotherapy efforts to control morbidity and transmission in endemic areas **(Sun, 1999).**

During the rainy season, mature stages of the *F. gigantica* is indicated. Because animals are often infected with a wide range of helminthes the need for broad-spectrum compounds active against Trematode, cestodes and nematodes and their larval stages is obvious. Albendazole is highly active against all stages of parasitic nematodes and, is also active against tapeworms but it shows variable activity against liver flukes **(Probert , 1994).**

Oxyclozanide either alone or with levamisole shows reasonable activity against mature *F. gigantica*58. During mid- to the end of the dry period large burdens of immature *F. gigantica* and amphistomes are expected. triclabendazole has been found to be effective against both immature and mature *F. gigantica* **(Suhardono *et al*., 1991).** Oxyclozanide or Niclosamide and triclabendazole can be administered to treat against the immature amphistomes and immature liver flukes, respectively However, in communal grazing areas it is imperative that the anthelmintics treatments mentioned above should be village-based as cattle in communal areas are grazed together and there is no benefit for only a few farmers to carry out the recommended control measures. The anthelmintics treatment should be organized and preferably done at the same time within a village.

Molluscicide The most frequently used public health intervention is the application of molluscicides to decrease the population of Lymnaea snails, the intermediate hosts of Fasciola hepatica. Molluscicides have been particularly popular because they also decrease transmission of many other Trematode of importance, such as the various *Schistosoma* and *Fasciola species* **(Malek, 1980)**.

Molluscicides have been used successfully as a short-term control method of snail intermediate hosts and can be cost effective but have gained little acceptance61. The main problems being environmental pollution and killing of non targeted aquatic organisms **(Roberts and Suhardono 1996)**. Also due to rapid recovery of the snail populations **(Brown, 1994)** during brief periods of favorable conditions, recolonisation should be expected and this may necessitate regular molluscicide application. However, as already mentioned above, snail densities and transmission are seasonal in the country and measures to control snails only need to be applied when high densities of infected snails are expected . However, molluscicides have mainly been used or recommended for use in dams because the more extensive habitats such as rivers make the cost prohibitive **(Suhardono *et al.*, 1991)**. Therefore, attempting to control the snails, using molluscicides, especially in the high veld region and communal grazing areas would prove to be difficult due to the widespread distribution of the snail habitats, the great biotic potential of the snails and the recurrent labor and equipment costs .Molluscicide application is probably practical in intensive farming husbandry systems especially in the low veld region where snail habitats are not widespread **(Pfukenyi *et a*l., 2005)**.

1.11.2. Biological control:

(Pfukenyi *et al*), Biological control agents of snails have been studied and tested against the snail host , Free-ranging ducks or geese, which feed on snails, have also been proposed as a possible means for control of *F. gigantica* (Rai *et a*l., 1996) but the degree at which control is likely to be achieved has not been measured. Effective control would require that ducks were present in sufficient numbers to feed on snails in a habitat before they shed Cercariae, and this may be achievable along limited stretches of the shore of lakes and streams where stock drink, 2005).

1.11.3. Immunological control:

In cattle, using irradiated metacercariae as the immunizing vaccine, a 98 % reduction in *F. gigantica* fluke burdens in vaccinated calves has been reported3. Using a range of immunizing regimes it has been shown that vaccination of Zebu calves with irradiated metacercariae reduced fluke burdens by 45–68 % **(Younis *et al*., 1986).** The use of Glutathione S-transferase (GST) isolated from *F. gigantica* as a vaccine alone or in combination with either aluminum hydroxide or saponin in sheep against *F. gigantica* infection has also been evaluated. The highest fluke reduction was observed in the group vaccinated with GST-saponin (32 %), but the reduction was not statistically significant in comparison with the control group **(Pfukenyi *et al.*, 2005).**

1.11.4. Epidemiological Studies:

Other study aimed to investigate the different pathological conditions reported in livers of slaughtered cattle and sheep leading to their total condemnation at abattoir Alkadroo, North Khartoum. Data were obtained of the slaughtered cattle were brought from Ethiopia and sheep from Elgadarief state. The prevalence of fascioliosis in cattle was 91%,while in sheep it was 0.19%.The incidence of Fasciolosis were significantly higher in cattle compared to sheep. In cattle, fascioliosis prevalence was mostly higher during winter compared to summer and autumn **(Mohamed, 2012).**

Other study was conducted to determine the prevalence rate of *Fasciola gigantica* infection in slaughtered cattle and sheep through collection of records during 1998-2007 in the White Nile State, Sudan. Four localities, Kosti, El Jebelein, Ed Dueim and El Geteina slaughterhouses were included in this study. Ranges of 19659 to 37327 heads of cattle and 22215 to 65466 heads of sheep were slaughtered annually during this period. The lowest prevalence rate (4.00%) for *F. gigantica* infection was reported in cattle in the year 1999 while the highest (10.41%) was recorded in 2000. The lowest prevalence rate (1.38%) in sheep was in 2007 and the highest (3.73%) was in 2002. The overall prevalence rate of *F. gigantica* infection was higher in cattle (6.05%) compared to sheep (2.37%). The prevalence rate was higher in the wet season compared to the dry and cold season, but the difference was insignificant ($p \geq 0.05$). Localities, the prevalence was found to be 9.2% in Ed Dueim, 9.1% in El Jebelein, 6.2% in Kosti and 2.9% in El Geteina for cattle. The prevalence rate in sheep, however, showed no significant difference ($p > 0.05$) with regard to season or locality (**Babiker *et al*., 2011).**

In Abyei area The study was designed to compare information on parasitic diseases occurrence in nomadic cattle herds. Collection of data from the veterinary records, Veterinary drug centre's, questionnaire and external parasites, faecal and blood samples from animals were carried out for one year. The clinical records showed that, parasitic diseases constitute a major problem, and formed 53% of the total diseases recorded in the clinical records. the fecal samples from cattle using floatation and sedimentation methods showed that: *Paramphistomum* sp.

constituted 11.25%, *Fasciola gigantica* 5.00%, *Schistosoma bovis,* 1.50%, **(Gad Alkareem *et al*., 2012).**

Other Study was conducted in Juba main slaughter house for three months as of May to July 2012. A total of 4,642 indigenous Nilotic and exotic Ankole cattle breeds were investigated. Nilotic cattle revealed high prevalence of 96.8% compared to 2.5 % in Ankole cattle attributed to *Fasciola gigantica* during post-mortem examination. Likewise, fecal examination revealed a prevalence of 89.6% in Nilotic and 2.9 % in Ankole cattle breed. Fasciolosis was found to be responsible for a condemnation of 36.4% inspected livers suggesting Fasciolosis as one of the priority diseases of economic importance in South Sudan **(Mousa *et al*., 2013)**.

A cross sectional study was carried out from October 2010 to March 2011 with the objectives of determining the prevalence, risk factor and economic importance of bovine Fasciolosis in Dessie municipal abattoir. Over all prevalence of 25.2% (126 of 500) was observed. Based on origins of animals, prevalence rates of 30.51%, 28.4%, 25.81% and 19.77% were recorded in Kutaber, Worehimmenu, Dessie Zuria and Tewulederae, respectively. 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. Prevalence's 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. The prevalence of *F*asciola species is different in different study areas and the highest prevalence of *F. hepatica* was observed in Kutaber (20.34%) followed by Dessie Zuria (17.74%) and the highest prevalence of *F.gigantica* was observed in Worehimmenu (9.88%) followed by Kutaber (2.54%). **(Belay *et al.*,2012).**

Other study was conducted in the northeastern areas of Punjab province (Pakistan) to analyses the monthly and seasonal pattern of Fasciolosis in buffaloes and its relation to some climatic factors (temperature, humidity, rainfall and pan-evaporation) was also worked out. The fecal samples of buffaloes were collected from April 2003 to March 2005 on a 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).**

A total of 208 cattle were randomly selected among slaughter houses, household and livestock farms to determine the prevalence of fascioliosis. Epidemiological studies on fascioliosis of 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 was high in comparatively low land areas (37.14%) as compared to high altitudes (30.09%). This study will provide necessary information regarding Fasciolosis in cattle of Ladakh for their effective Control and hence for a better production which will be beneficial resource to poor people where live stock rearing is one of the important sources of livelihood (**Kuchai *et al*., 2001**).

Also a cross-sectional study was carried out from November,(2011) to March, 2012 at Nekemte municipal abattoir 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 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 (**Petro's *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, which is .

The prevalence of liver condemnation due to Fasciolosis over the period(**2006-2009)** was calculated. The $\chi2$ test, simple linear regression analysis and $\chi2$ 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 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 sub acute 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. (**Lammer *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, morph anatomic, morph metric, 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 the species, and isoelectric focusing

(IEF) of fluke soluble protein confirmed the presence of both *F. gigantica* and *F. hepatica* in Egypt (**Lofty *et al.*, 2001**).

In 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. Fecal and blood samples were collected from 282 cattle from May 20 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 fecal examination. The mean number of EPG in infected cattle was 42.8%, 4.4%. 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**.** (**Yidirim *et al*., 2007).**

The aim of previous study was to establish 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 the retrospective study revealed that 8302 (6.7%) livers out of 123790 examined livers 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 determine 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 anthelmintics 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 Fasciolosis in abattoirs located within some selected Local Government Areas of Benin City, Nigeria. a total 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 day of August and 2nd of December, 2012, using standard histochemical 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 feeding and water consumption. (**Odigie,et *al.*,2013**).

Another study was conducted during the period between January 1999 and December 2000, the distribution and seasonal patterns of *Fasciola gigantica* infections in cattle in the High veld and low veld 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 low veld 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 High veld and in nine dams in the low veld communal grazing areas. Each site was sampled for relative snail density and the vegetation cover and type, physical and chemical 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 and 2 500 (15.4 %) of the samples were positive for *F. gigantica* eggs. Significantly higher prevalence's were found in the High veld compared to the low veld ($P < 0.001$), 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 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 formal ether sedimentation technique and the blood by Indirect ELISA kit (Bio-X-Diagnostic, ID VET Janelle-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 (**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 proved 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 known to be 38.1%, 28.8% and 13.6%, respectively and prevalence of Fasciolosis in medium body conditioned cattle, sheep and goats were known to be 30.0%, 20.5% and 11%, respectively. The prevalence of Fasciolosis in good body conditioned cattle, sheep and goats were proved 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 anthelmintics and awareness should be created on the prevention and control methods of Fasciolosis. (**Abdul hakim *et al(2012).***

CHAPTER TWO

2. MATERIALS AND METHODS

2.1. Study area:

Khartoum state is the capital of Sudan, which is located in north Eastern part of centre of Sudan .The state is located between 21^0, 25 - 24^0, 45^0 East and 15 ^{0}, 9-16^0 ,45 ^{0} North. The state cover 20,373 KM^2 distance .Khartoum state is divided in to three administration govern ate Khartoum, Omdurman, and Khartoum North. It surrounded 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 Gazer a State in the East. The climate is semi-desert, dry and hot in summer (maximum temperature of 47^c and minimum temperature of 22.7^c). The range of rain fall is 150mm per year.

The population in Khartoum state is 513,000 for Sheep, 19,000 of goat,6,585 camel, 240,000 for cattle. 20360 in Jubal Albia and 562 in Khartoum (Agriculture Census 2009).

2.1.2. Study design:

A cross-sectional study was conducted in cattle slaughtered in the abattoirs(Ganaua, Sahafa). This study was done to estimate the prevalence of Bovine Fasciolosis, and to investigate the risk factors which associated with the disease. The abattoirs were visited to collect samples and some data by filling out the questionnaire. Most of the cattle slaughtered were male adult Zebu, small number of cross breed bull from dairy farm and occasionally a few female.

2.1.3. Sampling methods:

Probability sampling methods were used to select the animals. At first for the multistage sampling, three localities were selected from the state (Khartoum, Bahri, Omdurman). Then from each locality two administration units were selected, the abattoirs selected from subunit. Finally, animals were selected by randomly sampled used systematic sampling method and examined though ante mortem and postmortem examination. *The prevalence was calculated using the formula described by Martin (**Martin,** **et al.** **1987**) as follow.*

Prevalence rate = $\frac{\text{Total number of diseased animals with Fasciolosis}}{\text{Total number of cattle at a particular point in time}} \times 100$

2.1.4. Sample size determination:

The sample size was determined by simple random sampling method use 95% confidence interval and calculated by using the formula given by **Thrusfield** (**1995**), with 5% absolute precision and at (91%) expected prevalence according to the a previous study by Mohamed (2012).

$$n = \frac{(1.96)^2 * P^{\wedge}_{exp} * Q^{\wedge}}{d^2}$$

n = sample size

P^ = expected prevalence

d^2 = desired absolute precision

Q^ = $(1-P^{\wedge}_{exp})$

$$n = \frac{(1.96)^2 * (0.91)*(1-0.91)}{} = 125 \text{ cattl}$$

This was Multiplied by 3 to increase the precision of the information about the disease. So the size became 375 animal

2.1.5. Questionnaire execution:

The abattoirs were visited in each sub-unit and just one animal from each herd was examined and filled out the questionnaire. The individual risk factors attributes were included, age, breed, sex, origin of the cattle of the disease, body condition, other diseases, appearance signs of disease. The farm attributes was include the size of the herd, hygiene and health practiced, the type of grazing, sours of water, vegetation, used treatments, presence of other animals and presence of snail Then divided these risk factors to categories.

2.1.6. Ante-mortem Inspection:

The ant mortem examination of animal was to observe the external abnormalities like emaciation, sumandibular edema animals were examined before slaughter and each and every animals were tagged with identification number before the slaughter commences and each animal which was examining during the ant mortem were also observed for the postmortem results**.** Attention was given to the factors such as age, body condition and origin of the animals to determine the impact of these factors on the disease picture.

2.1.7. Post mortem examination:

The slaughtered houses were visited on an average of once a week for nine (9) weeks (two months) from October and December 2014. The cattle were slaughtered between 9.00 am and 2.00 pm. Before the animals were slaughtered, the breed , sex and other risk factors of the disease were noted. The inspection of the meat was made possible through the cooperation of the veterinary staff on duty at the abattoir. For each cattle slaughtered, the abattoir workers assisted in bringing out the liver. The liver was thoroughly examined alongside the bile duct for adult of Fasciola species. The fluke recovery and count was made following the approach of **Hammond and Swell (1975):** the gall bladder was removed and washed to screen out mature flukes. The infected liver was placed on a clean board and dissected in-situ using scissors, the bile duct was opened and all the flukes found were removed and placed in a Petri dish. The total number of flukes removed from each liver was recorded. Collected flukes from each animal were examined and classified on the basis of size and shape **(Soulsby, 1982.**

Categorization of the affected livers was carried out based on the approach of Ogunrinade and Adegoke (1982) as follows: severely affected, Moderately affected, , Lightly affected, almost the entire organ is involved, the liver is cirrhotic and triangular in outline as the right lobe is often atrophied. The intensity of the fluke infection (mean fluke burden) was also correlated with the pathological lesions.:

2.1.7. Carpological examination:

Carpological examination was conducted on fecal samples collected directly from the rectum of the animals into a universal bottle containing 10% formalin and transported to Veterinary Laboratory for examination. Sedimentation technique was used to detect the presence or absence of fluke eggs in the fecal sample collected, as described by **Antonia *et al*. (2002**). 3g of feces were mixed with 1 liter of water (The sieve and glass were washed at each stage). The filtrates were placed in the centrifuge in a conical tube for 10 minutes 5000rpm. The supernatant was discarded and the sediment was left at the bottom of the centrifuge tube, a small quantity of the sediment was scooped with the scapular and placed on a clean slide. The prepared slide was examined under the light microscope using X 10 objective of the microscope. One drop of 1% methylene blue was added to the preparation to make the eggs more visible. The yellow Fasciola eggs showed distinctly against the blue background **(Monica, 1987).** large and oval in shape. It has an indistinct operculum (lid). It contains an unsegmented ovum surrounded by many yolk cells **(Monica,1987)** To differentiate between eggs of *Paramphistomum* species and *Fasciola* species, a drop of methylene blue solution was added to the sediment where eggs of *Fasciola* species showed yellowish colour while eggs of *Paramphistomum* species stain by methylene blue (**Hansen and Perry, 1994**).During sampling information on sex, breed, and approximate age of individual animals were recorded. Age was classified as young (<4 years) and adult (≥4years) (Cringoli et al., 2002). Samples that were not processed within 24 h were stored in a refrigerator at 4°C.

2.7. Data management and statistical analysis:

The data was entered into Microsoft excel sheet. Data was summarized and analyzed using statistical package for social sciences (SPSS) version 16 computer program. The Pearson's chi-square (X^2) test at a significance level of 5 and 95% CI was used to determine the differences in the prevalence of Fasciolosis infection among cattle, between ages , body conditions of cattle and other factors. Multivariable logistic regression model we describe the risk factor , number of positive cases , odds ratio, confidence intervals and p-value .The difference on the risk factors was consider as statistically significant if the p.value was less than0.05

CHAPTER THREE

RESULT

The study was conducted to estimate the prevalence and risk factors of Fasciolosis in cattle slaughtered at Khartoum State ,Sudan . A total of 375 cattle were investigated for Fasciolosis, 32 cattle were found to be infected with Fasciolosis giving a prevalence rate of 8.5% (32/375) through meat inspection and fecal examination .

Table 1: Prevalence of Fasciolosis in 375 cattle examined in Khartoum State:

Result	Frequency	Percent	Cumulative Percent
+ve	32	8.5	100.0
-ve	343	91.5	91.5
Total	375	100.0	100.0

Distribution of 375 cattle examined for Fasciolosis by risk factors:

The result of the study showed the distribution of 375 cattle examined for Fasciolosis by age. 96 (25.6%) cattle examined were young, 279 (74.4%) were adult. The result of study showed the distribution of cattle's examined for Fasciolosis by sex, 363 (96.8%) animals were found male and 12(3.2%) female. During study found that the distribution of 375 cattle examined for Fasciolosis by breed, the frequency table showed that 355 (94.7%) of examined cattle were local, and 20(5.3%) were cross. The result showed distribution of body condition. 363 (96.8%) cattle examined were good body condition and 12 (3.2%) were poor body condition. According to result the distribution of 375 cattle examined by source of animal showed that 344(91.7%) cattle from Darfur, 14 (3.7%) from white Nile and 17 (4.5%) cattle from Khartoum. The result of the study showed that the distribution of 375 cattle examined by grazing system , and frequency table showed that 16(4.3%) of examined cattle were indoor and 359 (95.7%) cattle were found to be out door. The result of the study showed that the distribution of cattle examined by vegetation, 361 (96.3%) of examined cattle were in vegetation and 14 (3.7%)were in no vegetation. Distribution of cattle examined by using drug the result showed that 284 (75.7%) of examined cattle were animals used drug and 91(24.3%) were animals not using drug. The result of the study showed that the distribution of cattle examined by present of snail, the frequency table showed that 320 (85.3%) of examined cattle were found in present snail and 55 (14.7%) were in no present snail. According to the result distribution of cattle in frequency table by manure disposal 49 (13.1%) of cattle found in disposal manure and 326(86.9%) not disposal. The result of the study showed that the distribution of 375 cattle examined by water body. The table of frequency showed that 350 (93.3%) of examined cattle were found in water body and 25 (6.7%) were in no water body. The knowledge of disease during study was observed and distribution of cattle showed that 373 cattle(Table3.4) were found in knowledge of disease and 2cattle in without knowledge of disease.

Table 2:Frequency distribution of 375 cattle examined for bovine Fasciolosis in Khartoum State according to potential risk factors investigated:.

Risk factors	**Frequency**	**Percent (%)**	**Cumulative percent(%)**
1- Age			
Young (<4 years)	96	25.6	25.6
Old (>5years)	279	74.4	100.0
2- Sex of animals			
Male	363	96.8	100.0
Female	12	3.2	3.2

3- Breed			
Cross	20	5.3	5.3
Local	355	94.7	100.0
4- Body condition			
Good	363	96.8	96.8
Poor	12	3.2	100.0
5- source of animals			
Khartoum	17	4.5	4.5
Whit Nile	14	3.7	8.3
Darfur	344	91.7	100.0
6- grazing system			
In door	16	4.3	4.3
Outdoor	359	95.7	100.0
7- vegetation			
Yes	361	96.3	100.0
No	14	3.7	3.7
8- using drug			
Yes	284	75.7	75.7
No	91	24.3	100.0
9- present of snail			
Yes	320	85.3	14.7
No	55	14.7	100.0
10- Manure disposal			
Yes	49	13.1	13.1
No	326	86.9	100.0
11- water body			
Tab	25	6.7	6.7
Dam	350	93.3	100.0
12- knowledge			
Yes	373	99.5	100.0
No	2	5	5

Cross tabulation of bovine Fasciolosis with potential risk factors in 375 cattle slaughtered at Khartoum State is shows in last:

(3:1). Age specific prevalence. Total number of old animals was 279 and young animals was 96 ,the prevalence of bovine Fasciolosis, was higher in age group >5 years (11.1%) than that of age groups <5 year (1.0%).

(3:2) Sex specific prevalence: Total number of male was 363 animals, 30 animals were found infected, when the number of female 12 and infected animals was 2, The prevalence of bovine Fasciolosis was higher in female (16.7%). than that of male (8.3%).

(3:3) specific prevalence: Breed The prevalence of Fasciolosis were found also higher in cross cattle (10.0%) from total of 20 animals. than that of local breed cattle (8.5%) from total of 355 local cattle.

(3:4) Body condition specific prevalence: Total number of good body condition was 363 animals . Among these, 27 animals were found infected, the prevalence was 7.4%. Total number of poor body condition was 12 animals .Among these 5 animals was found infected .the prevalence was 41.7%.

(3:5):Source of animals:17 animals were from Khartoum state, 14 cattle from whit Nile state and 344 animals from Darfur . the prevalence of disease were found (11.8%, 7.1%, 8.4%) respectively in above source.

(3:6): Grazing system specific prevalence: Total number of indoor was 16 animals. Among these, two animals was found infected, the prevalence of Fasciolosis was 12.5%.Total number of outdoor was 359 animals. Among these, 30 animals was infected, the prevalence was 8.4%.

(3:7) Vegetation specific prevalence: Total number of cattle found in vegetation was 361 animals. Among these, 29 animals was found infected, the prevalence of

Fasciolosis was 8.0%.Total number of cattle out of vegetation was 14 animals. Among these, 3 animals was infected, the prevalence was 21.4%.

(3:8) Drug use specific prevalence: Total number of animals used drug was 284 animals. Among these, 17 animals were found infected, prevalence was 6.0%.Total number of animals not used drug was 91 animals. Among these, 15 animals was found infected, prevalence of Fasciolosis was 16.5%.

(3:9) Present snail specific prevalence: Total number of animals present snail was 320 animals. Among these, 31 animals were found infected, prevalence was 9.7%.Total number of animals not present snail was 55 animals. Among these, one animal was found infected, prevalence of Fasciolosis was 1.8%.

(3:10) Manure disposable specific prevalence: Total number of animals in heard which manure disposable was 49 animals. Among these, one animal are found infected, prevalence was 2.0%.Total number of animals in not disposable manure was 326 animals. Among these, 31 animals was found infected, prevalence of Fasciolosis was 9.5%.

(3:10) Source of water specific prevalence: Total number of animals in tab water was 25 animals .Among these, 3 animals was infected, prevalence of Fasciolosis was 9.4%.Total number of animals in dam water was 350 animals. Among these 29 animals was infected, the prevalence of Fasciolosis was 90.6%.

(3:12) Knowledge of disease specific prevalence: Total number of animals in knowledge was 373 animals. Among these 32 animals was infected, prevalence of Fasciolosis was 8.6%.Total number of animals no knowledge was two animals Among these, the infected was zero, the prevalence of Fasciolosis was 0% Table (3.5).

Table 3:Cross tabulation of bovine Fasciolosis diagnosed through post-mortem and fecal examination with potential risk factors in 375 cattle's slaughtered at Khartoum State:

Risk factors	No. of animals examined	Number of positive %
1- Age		
Young (<4 years)	96	1(1.0%)
Old (>5years)	279	31(11.1%)
2- Sex of animals		
Male	363	30(8.3%)
Female	12	2(16.7%)
3- Breed		
Cross	20	2(10.0%)
Local	355	30(8.5%)
4- Body condition		
Good	363	27(7.4%)
Poor	12	5 (41.7%)
5- source of animals		
Khartoum	17	2(11.8%)
Whit Nile	14	1(7.1%)
Darfur	344	29(8.4%)
6- grazing system		
In door	16	2(12.5%)
Outdoor	359	30(8.4%)
7- vegetation		
Yes	361	29(8.0%)
No	14	3(21.4%)
8- using drug		
Yes	284	17(6.0%)
No	91	15(16.5%)
9- present of snail		
Yes	320	31(9.7%)
No	55	1(1.8%)
10- Manure disposal		
Yes	49	1(2.0%)
No	326	31(9.5%)
11- water body		
Tab	25	3(12%)
Dam	350	29(8.3%)
12- knowledge		
Yes	373	32(8.6%)
No	2	0(.0%)

Univariate analysis for the association between Fasciolosis and potential risk factors using the Chi-square test:

In the Chi-square test the results showed that there was significant association between (Age, Body condition, Vegetation ,Using drugs, Present of snail and Manure disposable) and Fasciolosis the p-value (0.002 , 0.000, 0.078, 0.002,0.054 and 0.081) respectively. Table (3.4).

In the Chi-square test the results showed that there was no significant association between risk factors and Fasciolosis such as (sex of animals, breed of animals, sours of animals, grazing system, source of water, knowledge 0.665) p-value 0,305,0.809,0.875,0.562,0.739 respectively. Table (3.4).

Table 4: Univariate analysis for the association between Fasciolosis and potential risk factors in 375 cattle examined in Khartoum state. using the Chi-square test:

Risk factors	No. of animals examined	Number of positive (%)	d.f	X^2.value	p-value
1- Age			1	9.278	0.002
Young (<4 years)	96	1(1.0%)			
Old (>5years)	279	31(11.1%)			
2- Sex of animals			1	1.051	0.305
Male	363	30(8.3%)			
Female	12	2(16.7%)			
3- Breed			1	.058	0.809
Cross	20	2(10.0%)			
Local	355	30(8.5%)			
4- Body condition			1	17.436	0.000
Good	363	27(7.4%)			
Poor	12	5 (41.7%)			
5- source of animals			2	.267	0.875
Khartoum	17	2(11.8%)			
Whit Nile	14	1(7.1%)			
Darfur	344	29(8.4%)			
6- grazing system			1	.337	0.562
In door	16	2(12.5%)			
Outdoor	359	30(8.4%)			
7- vegetation			1	3.098	0.078
Yes	361	29(8.0%)			
No	14	3(21.4%)			
8- using drug			1	9.730	.002
Yes	284	17(6.0%)			
No	91	15(16.5%)			
9- present of snail				3.724	0.054
Yes	320	3(1.9.7%)			
No	55	1(1.8%)			

10- Manure disposal Yes No	49 326	1(2.0%) 31(9.5%)	1	3.044	0.081
11- water body			1	.605	0.739
Tab Dam	25 350	3(12%) 29 (8.3%)			
12- knowledge Yes No	373 2	32(8.6%) 0 (.0%)	1	.188	.665

Multivariate analysis for the association between Fasciolosis diagnosed through post-mortem, fecal examination and potential risk factors in 375 cattle examined in Khartoum state using logistic regression. the result showed string of association by odds ratio between disease and age of the animals ,body condition and vegetation.

Table 5: Multivariate analysis for the association between Fasciolosis examination and potential risk factors in 375 cattle examined in Khartoum state using logistic regression:

Risk factors	No. of animals examined	Number of positive (%)	Exp (B) odds ratio	p-value	95% CI	
					lower	Upper
1- Age						
Young (<4 years)	96	1(1.0%)	Ref	-	-	-
Old (>5years)	279	31(11.1%)	24.88	0.o46	1.066	580.835
2- Body condition						
Good	363	27(7.4%)	Ref	-	-	-
Poor	12	5 (41.7%)	10.555	.004	2.106	52.907
3- vegetation						
Yes	361	29(8.0%)	Ref	-	-	-
No	14	3(21.4%)	0.033	0.029	0.002	0.710
4- using drug						
Yes	284	17(6.0%)	Ref	-	-	-
No	91	15(16.5%)	1.899	0.127	0.832	4.331
5- present of snail						
Yes	320	31(9.7%)	6.696	0.160	.472	94.988
No	55	1(1.8%)	Ref	-	-	-
6-Manure disposal						
Yes	49	1(2.0%)	Ref	-	-	-
No	326	31(9.5%)	14.297	.0.185	.280	729.430

CHAPTER FOUR

4.1.Discution:

Fasciolosis is widespread ruminant health problems and causes significant economic losses to the livestock industry, which is caused by Trematode of the genus Fasciola that migrate in the hepatic parenchyma, and establish and develop in the bile ducts (**Troncy, 1989) .**Apart from its veterinary and economic importance throughout the world, Fasciolosis has recently been shown to be a re-emerging and widespread zoonosis affecting many people **(Esteban *et a*l., 2003).**

The overall abattoir and fecal examination prevalence of Fasciolosis in the present study was 8.5% which is higher than many other studies from different abattoirs in Sudan by **(Salam *et al*., 2009)** in Agar gel precipitation test (AGP) he found that prevalence was 8%. The prevalence of this study is lower than that of the study conducted by **Abdul (1992)** and **Adem (1994)** with the rates of 47% and 56.6% at Sodo and Ziway municipality abattoir. More over the current study is lower than that of the study conducted by **Gebretsadik *et al*. (2009)** with the prevalence of 24.32%. In Sudan previous study the prevalence was 91% in abattoir Alkadroo, North Khartoum **(Mohamed, 2012).** in the White Nile State Kosti, El Jebelein, Ed Dueim and El Geteina slaughterhouses The lowest prevalence rate was (4.00%) for *F. gigantica* in cattle, while the highest (10.41%). The overall prevalence rate of *F. gigantica* infection was higher in cattle (6.05%) Localities, the prevalence was found to be 9.2% in Ed Dueim, 9.1% in El Jebelein, 6.2% in Kosti and 2.9% in El Geteina for cattle.(**Babiker *et al*., 2011).** In Abyei area the prevalence of *F.gigantica* was 5% **(Gad Alkareem *et a*l., 2012).** in Juba prevalence of 89.6% in Nilotic and 2.9 % in Ankole cattle breed, South Sudan **(Mousa *et al*., 2013).**The variation in prevalence may be due to climate ecological conditions such as altitude, rainfall, temperature, livestock management system and suitability of the environment for survival and distribution of the parasite as well as the intermediate host might have played their own role in such differences.

In our study the total number of old animals was 279 and young animals was 96 ,the prevalence of bovine Fasciolosis, was higher in age group >5 years (11.1%)

than that of age groups <5 year (1.0%). And chi-squire test showed significant association between age and Fasciolosis this result agree with **Abdul hakim and Addis, (2012),** and disagree with **Abele *et al.* (2011)**who reported that the prevalence of higher in young than adult animals , Such variation of the prevalence was exist it is not statistically significant (P>0.05). This could be best explained by the fact that young animals are usually kept in door or around the home and are not allowed to go far with adult animals for grazing so that they have reduced chance of exposure to infective parasitic stages when compared to adults.

According to result Body condition was observed and chi-squire test showed significant association with the disease, Higher infection with fascioliosis was observed in poor body condition 41.7% compared with good body condition 7.4%, the prevalence agrees with **Abdul hakim and Addis (2012)**. This finding confirms the importance of fascioliosis in causing weight loss and emaciation as a characteristic sign of the disease. Besides, the high prevalence of fascioliosis infection in poor conditioned animals could be justified by the fact given by **Devendra and Marca (1983)** who indicated animals of poor body condition are vulnerable to parasitic diseases. The significant variation in the prevalence of fascioliosis in relation to body condition could be further justified by the fact that as the body condition improves, infecting with fascioliosis decreases because *Fasciola* worms are known to suck blood and tissue fluid and even damage the parenchyma of the liver due to the migrating immature worms . Moreover, Chollengitis and liver cirrhosis induced in chronic Fasciolosis could reduce bile flow to the duodenum and hence reduced lipid emulsification,, digestion and absorption of fatty acid and lipid soluble vitamins (Gargili et al., 1999).

In this study vegetation was found with significant association with fascioliosis by chi-squire, total number of cattle found in vegetation was 361 animals, 29 animals was found infected (8.0%), and the number of cattle out of vegetation was 14 , 3 animals were infected (21.4%).This result could be attributed to that animals grazing in vegetation area may be treated against the disease, in dry period scarcity of vegetation may in force the animals graze in floating grasses . in the fact vegetation play major role to protect metacercariae **Suhardono *et al*. (2006)** reported that exposure to direct sun killed metacercariae within eight hours.

The chi-squire test showed significant association between Drug use and bovine Fasciolosis (p-value 0.002).Total number of animals using drug were 284 animals. 17 animals were found infected(6.0%)., while the total number of animals not using drug was 91 animals, 15 animals was found infected16.5%. this result attributed to effect if drug use. **Joseph, (2007)** reported that the Drugs play an important role in the control of Fasciolosis. An efficient strategic control program relying on a minimum number of treatments per year and aimed at long–term elimination of pasture contamination requires drugs that are effective against both mature and early immature flukes. More frequent treatments are necessary if you use drugs that are only effective against advanced mature fluke aged 12–16 weeks or older . According to the present result t snail were found with significant association with the disease. 320 animals in the presence snail ,31 animals were found infected (9.7%).and 55 animals are found in not present snail, Among these, one animal was found infected (1.8%). This agrees with **Tesfay *et al*., (2012).**

Biologically availability of moisture, optimal temperature and suitable snail habitat are among factors influence the occurrence of Fasciolosis in a certain area **(Urquhart *et al*., 1996)**. An optimal temperature of 10 ºC and 16ºC are necessary for snail vectors of *F. hepatica* and *F .gigantica* for development of the Fasciola in the intermediate snail hosts. Moreover, such conditions are required for completion of the life cycle such as development of fluke eggs, miracidia searching for snails and dispersal of cercaria **(Urquhart *et al*., 1996). Tesfay *et al* (2012)** reported that variation of these environmental and ecological factors on different agro ecological zones leads to variation of the prevalence of Fasciolosis from one study area to other localities In this study the Chi-squire test showed significant association of manure disposable with bovine Fasciolosis. Total number of animals in heard with manure disposable was 49 animals. Among these, one animal was found infected (2.0%) .Total number of animals with no disposable manure was 326 animals. Among these, 31 animals were found infected (9.5%). This may be attributed to sufficient temperatures for killing the eggs is only produced in the depth of storage of large bodies of manure, but in small bodies of manure and also in the surface portion of stored manure or in drier parts of manure heaps, the temperature produced by biological process is not sufficient to inactivate the eggs **(Nicholson *et al*., 2005**), therefore, as a final conclusion, it could be recommended to heat these kinds of manure before using them as fertilizer in grazing areas.

4:2:Conclusion:

Fasciolosis is a serious health problem of cattle which causes liver condemnation in the abattoir, reduction in the production of the animal This study indicated the prevalence of bovine Fasciolosis in the study area during the study period 8.5% Among the assessed risk factors;, age, body condition and vegetation were found to be the associated potential risk factors with the occurrence of disease. There was paucity of information on control and prevention options of the disease.

4:3:Recommendations:

Animal diseases continue to constrain, in a variety of different ways, livestock productivity, agricultural development, human well-being and poverty alleviation in many regions of the developing world. The most important and read measurable direct effects of disease are often losses in productivity. These include the effects due to death, illness leading to condemnation, poor weight gain and poor feed conversion. Disease of livestock have many additional direct and indirect impacts on human nutrition, community development and socio-cultural and also reduction in farm income, contributing to food insecurity and poor nutrition, therefore the control of the parasites based on the current finding the following points were Recommended;

(1) Standard regulations and functional meat inspection policies should be formulated for organs and carcass approval/ rejection .

(2) Awareness creation for farmers should be advocated

(3) Improving of the veterinary service and infrastructure in prevalence area with provision of modern antihelemntics and treatment is giving based of the weight of the animal.

(4)Control of intermediate host snails through drainage Practicing zero grazing is important in the control of the disease

(5) Regular deworming of animals before and after the rainy season is important

6- Application of molluscide drugs are important in the control of the intermediate hot factors and its indirect economic loss

(7)Drainage of swampy area is also important in the reduction of the intermediate host

(8)Further epidemiological investigation should be encourage

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Appendix 1

Frequencies table of 375 cattle diagnosed from Khartoum state according

To risk factors:

Age

		Frequency	Percent	Valid Percent	Cumulative Percent
‘Valid	young	96	25.6	25.6	25.6
	Old	279	74.4	74.4	100.0
	Total	375	100.0	100.0	

Sex

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Female	12	3.2	3.2	3.2
	Male	363	96.8	96.8	100.0
	Total	375	100.0	100.0	

Breed

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	cross	20	5.3	5.3	5.3
	local	355	94.7	94.7	100.0
	Total	375	100.0	100.0	

Body condition

		Frequency	Percent	Valid Percent	Cumulative Percent
	good	363	96.8	96.8	96.8
	poor	12	3.2	3.2	100.0
	Total	375	100.0	100.0	

Sours:

		Frequenc-y	Percent	Valid Percent	Cumulative Percent
Valid	Kh	17	4.5	4.5	4.5
	Whit	14	3.7	3.7	8.3
	Darfur	344	91.7	91.7	100.0
	Total	375	100.0	100.0	

Grazing:

		Frequenc-y	Percent	Valid Percent	Cumulative Percent
Valid	Indoor	16	4.3	4.3	4.3
	Outdoo-r	359	95.7	95.7	100.0
	Total	375	100.0	100.0	

Vegetation:

		Frequenc-y	Percent	Valid Percent	Cumulative Percent
Valid	no	14	3.7	3.7	3.7
	yes	361	96.3	96.3	100.0
	Total	375	100.0	100.0	

Use drug

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	yes	284	75.7	75.7	75.7
	no	91	24.3	24.3	100.0
	Total	375	100.0	100.0	

Present of snail:

		Frequenc -y	Percent	Valid Percent	Cumulative Percent
Valid	no	55	14.7	14.7	14.7
	yes	320	85.3	85.3	100.0
	Total	375	100.0	100.0	

man. Disposal:

		Frequenc -y	Percent	Valid Percent	Cumulative Percent
Valid	yes	49	13.1	13.1	13.1
	no	326	86.9	86.9	100.0
	Total	375	100.0	100.0	

s. water:

		Frequenc-y	Percent	Valid Percent	Cumulative Percent
Valid	0	1	.3	.3	.3
	tab	24	6.4	6.4	6.7
	dam	350	93.3	93.3	100.0
	Total	375	100.0	100.0	

Knowledge:

		Frequenc-y	Percent	Valid Percent	Cumulative Percent
Valid	0	2	.5	.5	.5
	yes	373	99.5	99.5	100.0
	Total	375	100.0	100.0	

Appendix 2:

Cross tabulation table of 375 cattle diagnosed from Khartoum state according To risk factors:

Appendix 2.1 **age:**

Result	Age		Total
	Young	old	
+ve	1(1.o4%)	32(11.5%)	33(8.8%)
-ve	95(98.95%)	247(88.5%)	342(91.2%)
total	96	279	375

Appendix 2.2: sex:

Reslut	Sex		Total
+ve	2(16.7%)	30(9.o1%)	32(8.5%)
-ve	10(83.3%)	333(91.7%)	343(91.5%)
total	12	363	375

Appendix 2.3: breed:

Result	breed	Total

	Local	cross	
+ve -ve	3(8.5%) 325(91.5%)	2(10%) 18(90%)	32(9.3%) 343(91.7%)
total	355	20	375

Appendix 2.4: body condition:

Result +ave	Body condition		Total
	Good	poor	
-Ve	27(7.4%) 336(92.6%)	5(41.7%) 7(58.3%)	32(8.5%) 343(91.5%)
total	363	12	375

Appendix 2.5 source of anima :

Result	Source of animal			Total
	Khartoum	White Nile	Darfur	
+ve -ve	2(11.8%) 15(88.2%)	1(7.1%) 13(92.9%)	29(8.4%) 315(91.%)	32(8.5%)343(91.5%)
total	17	14	344	375

Appendix 2.6 grazing:

Result	grazing		Total
	indoor	Out door	
+ve _ve	21(2.5%) 148(7.5%)	30(8.4%) 329(91.6%)	32(8.5%) 343(91.5%)
total	16	359	375

Appendix 2.7: vegetation

Result	vegetation		Total
	yes	no	
+ve -ve	29(8.o3%) 232(91.97%)	3(21.4%) 11(78.6%)	32(8.5%) 343(91.5%)
total	361	14	375

Appendix 2.8:using drug:

Result	Using drug		total
	yes	no	
+ ve	17(6%) 267(94%)	15(16.5%) 76(83.5%)	32(8.5%) 343(91.5%)
total	284	91	375

Appendix 2.9: present of snail :

Result	Present of snail	total

	yes	no	
+v ve	31(9.7%) 289(90.3%)	1(1.8%) 54(98.2%)	32(8.5%) 343(91.5%)
total	320	55	375

Appendix 2.10: manure disposal:

Result	Manure disposal		total
	yes	no	
+ve -ve	1(2.o4%) 48(97.96%)	31(9.5%) 295(90.5%0	32(8.5%) 343(91.5%)
total	49	326	375

Appendix 2.11: water body:

Result	Water body		total
	tap	dam	

+v -ve	3912%) 22(88%)	29(8.3%) 321(91.7%)	32(8.5%) 343(91.5%)
total	25	350	375

Appendix 2.12: knowledge :

Result	knowledge		Total
	yes	no	
+v -ve	32(8.5%) 341(91.5%)	0(0%) 2(100%)	32(8.5%) 343(91.5%)
total	373	2	375

Association of different potential risk factor with Fasciolosis diagnosis by fecal examination Using chi-square test(x)2

Appendix 3.1: Age.

	X^2	df	p-value

Pearson Chi-Square	9.278	1	0.002
Likelihood Ratio	12.938	1	.000
N of Valid Cases	375		

Appendix 3.2:sex

	Value	df	p-value
Pearson Chi-Square	1.051	1 1 1 1 1	.617 .357 .306 .617 .357
Continuity Correction	.250		
Likelihood Ratio	.849		
Fisher's Exact Test	1.048		
N of Valid Cases	375		

Appendix 3.3: breed

Appendix 3.3: breed	X^2	df	p-value
Pearson Chi-Square	**.058[a]**	1	.809
Continuity Correction	.000	1	1.000
Likelihood Ratio	**.056**	1	.814
Fisher's Exact Test N of Valid Cases	**375**		

Appendix 3.4: source of animal:

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	.267[a]	2	.875
Likelihood Ratio	.247	2	.884
Linear-by-Linear Association	.138	1	.711
N of Valid Cases	375		

Appendix 3:5:body condition:

	Value	df	p-value
Pearson Chi-Square	17.436[a]	1	.000
Continuity Correction	13.327	1	.000
Likelihood Ratio	10.141	1	.001
Fisher's Exact Test			
Linear-by-Linear Association	17.390	1	.000
N of Valid Cases	375		

Appendix 3:6:grazing

	X^2	**df**	p-value
Pearson Chi-Square	.337[a]	1	.562
Continuity Correction	.015	1	.902
Likelihood Ratio	.300	1	.584
Fisher's Exact Test			
Linear-by-Linear Association	.336	1	.562
N of Valid Cases	375		

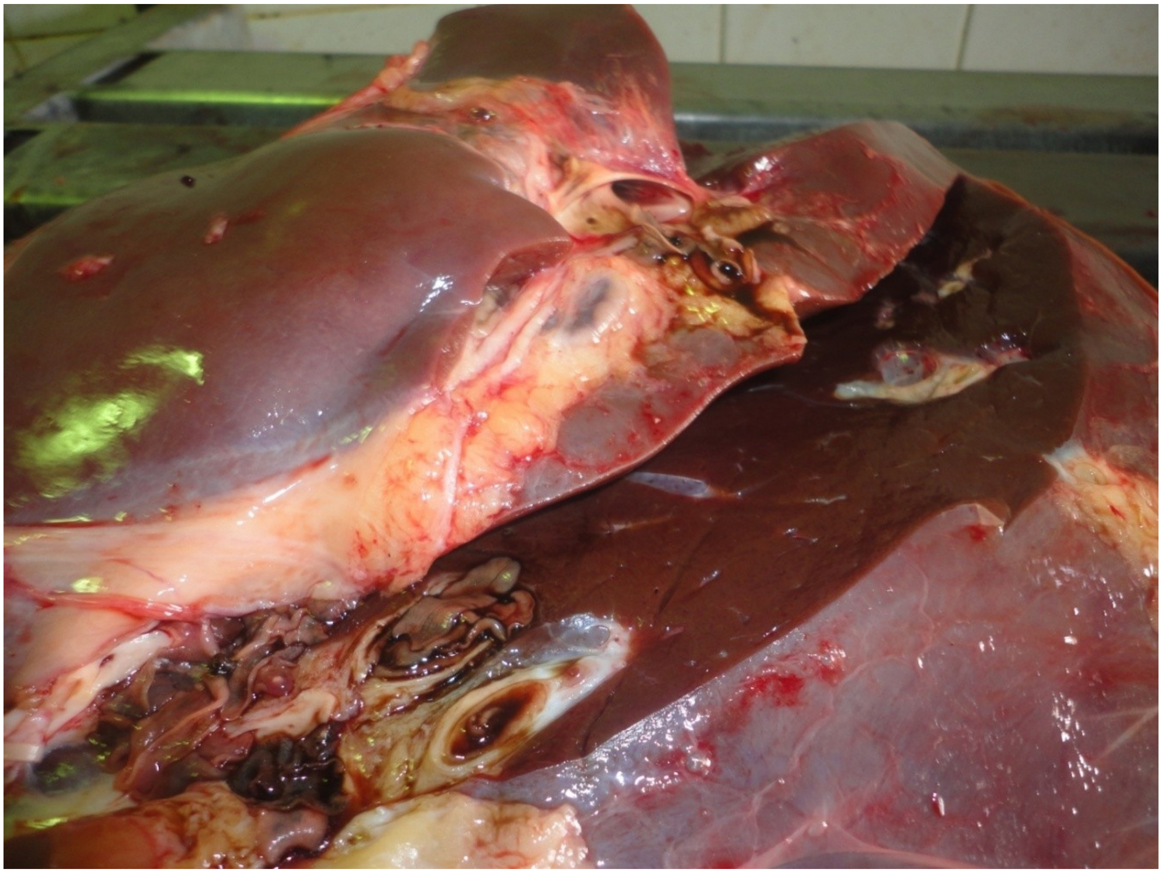
Appendix 3: 7:using drug

	X^2	**df**	**p-value**
Pearson Chi-Square	3.098[a]	1	.078
Continuity Correction	1.620	1	.203
Likelihood Ratio	2.299	1	.129
Fisher's Exact Test			
Linear-by-Linear Association	3.090	1	.079
N of Valid Cases	375		

Appendix 3:8:vegetation:

	X^2	df	p-value
Pearson Chi-Square	9.730[a]	1	.002
Continuity Correction	8.432	1	.004
Likelihood Ratio sher's Exact Test	8.544	1	.003
Linear-by-Linear Association	9.704	1	.002
N of Valid Cases	375		

Appendix 4.1


Figer1: the adult parasite in the liver bile duct

Appendix 4.2:

Figer 3:feacal examination at Suba

Appendix 5

Questionnaire

To obtain information about fasciolosis in cattle and information regarding geographical localization and risk factors influencing the presence of the disease.

General information:

1.Name: ……………………………………………………………………………..

2. Gender: ……………………………………………………………………….

3. Level of education:

…………………………………………………………………

Information about the fasciolosis risk factors.

1. Age:

Young ( )

Old ( )

2. Sex:

Male ( )

Female ( )

3. Breed:

Local ( )

Cross ( )

4. Body condition:

Good ( )

Poor ( )

5. Source of animal

Darfur ( )

White Nile ( )

Khartuom ( )

6. Grazing.

Indoor ( )

Outdoor ( )

7. Using drug.

Yes ( )

No ( )

8. vegetation

Yes ( )

No ( )

9. sourse of water

dam ( )

tab ( )

present of snail

Yes ( )

No ( )

11.manuar disposable

Yes ( )

NO ( )

Knowledge of disease

Yes ( )

No ( )