Advances in Biological Research 11 (5): 278-285, 2017
ISSN 1992-0067

DOI: 10.5829/idosi.abr.2017.278.285

Fascioliasis: Systematic Review

Nuraddis Ibrahim

Jimma University, School of Veterinary Medicine, Jimma, Ethiopia

Abstract: Fascioliasis is among important parasitic diseases which limit productivity of ruminants in particular cattle. *Fasciola hepatica* and *Fasciola gigantic*a are the two liver flukes commonly reported to cause fascioliasis in ruminants. *Fasciola hepatica* may be acquired by man, but not directly from cattle. A person must ingest the *metacercaria* in order to become infected. The geographical distribution of trematodes species is depending on the distribution of suitable species of snails. The genus *Lymnea* in general and *Lymnea truncatula* in particular are the most common intermediate host for *Fasciola hepatica.* The interaction between moisture and temperature determines the survival and reproduction rate of the snails and the parasites. To support a diagnosis, account should be taken of grazing history and seasonality of fascioliasis in the locality. There should be fluke eggs in the faeces and characteristics hepatic lesions at necropsy. Serological tests such as the enzyme linked immune sorbent assay (ELISA) have shown promise, particularly for diagnosis of infections in cattle. In addition to rendering the liver unsuitable for human consumption, it causes loss through death, reduction in meat and milk inhibited reproduction, morbidity impaired growth reduction in carcass weight, reduction in wool growth and quality, decreased feed intake, conversion and lowered resistance. Reduction of pasture contamination with *metacercariea* will reduce future risk. This can be done by preventing the snails from becoming infected with *F.hepatica* or by diminishing the size of the snails' population. Strategic anthelmintic treatment with appropriate fluckicide and a combination of control measure includes drainage; fencing and molluscicde should be used to control and prevent the disease.

Key words: *Fasciola* · *Lymnea truncatula* · *Metacercariea* · Molluscicde · Snails · Fluckicide

INTRODUCTION

Fasciolosis is among important parasitic diseases in tropical and subtropical countries which limit productivity of ruminants in particular cattle. *Fasciola hepatica* and *F. gigantic*a are the two liver flukes commonly reported to cause fasciolosis in ruminants [1].

In general, the distribution of Fasciolosis is world wide. How ever, the distribution of *F.hapatica* is limited to temperate areas and high lands of tropical and subtropical regions [2]. The definitive host for *F. hepatica* are most mammals, among which sheep and cattle are the most important once.

The geographical distribution of trematodes species is depending on the distribution of suitable species of snails. The genus *Lymnea* in general and *L. truncatula* in particular are the most common intermediate host for *F. hepatica.* This species of snail was reported to have a worldwide distribution [3]. *F.gigantica* is found in most continents, primarily in tropical regions [4].

The economic losses due to fasciolosis are caused by mortality, morbidity and reduced growth rate, condemnation of liver, increased susceptibility to secondary infections and the expense of control measures [5].

Diagnosis is based primarily on clinical sign and seasonal occurrence in endemic areas but previous history of Fasciolosis on the farm or identification of snail habitats; post mortem examination, Haematological tests and examination of faeces for fluke eggs are useful. Coprological analysis is still commonly employed to diagnose Bovine Fasciolosis, despite the fact that eggs cannot be detected until the latent period of infections, when much of liver damage has already occurred [6]. 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 diagnosis of fasciolosis [3]. Therefore, the objective of this review paper is to highlight the overall aspects of fasciolosis.

Corresponding Author: Nuraddis Ibrahim, Jimma University, School of Veterinary Medicine, P.O. Box: 307, Jimma, Ethiopia. Tel: +251-0471116778, Mob: +251917808966.

The Parasite and Morphology: Fasciolosis is a disease of sheep, goat, cattle [7] and occasionally affects humans, thus considered as a zoonotic infection [8, 9]. According to WHO [9] and Soulsby [2], the taxonomic classification of the organisms that cause fasciolosis is presented as follows:

Phylum:	Platyhelminthes,
Class:	Trematoda,
Sub- class:	Digenea,
Super Family:	Fasciolidea,
Genus:	Fasciola,
Species:	*Fasciola hepatica* and *Fasciola gigantica*

The adult parasite *F. hepatica* has a flat leaf-like body, typical of flukes and measures 20 to 30 mm long by 8 to 15 mm wide [10]. It has an anterior elongation (a cephalic cone) on which the oral and ventral suckers, which are approximately of equal size, are located. The intestine of the adult parasite is highly branched, with numerous diverticulae extending from the anterior to the posterior of the body. The pair of testes, also highly branched, is located in the posterior half of the body. The relative compact ovary is located just above the testes and is linked to a short convoluted uterus opening to a genital pore above the ventral sucker. The vitellaria are highly diffuse and branched in the lateral and posterior region of the body. *F. gigantica* is a parasite very similar to *F. hepatica*, its length may vary 25 to 75 mm long by 15 mm wide. In addition, the cephalic cone is proportionally shorter than that of *F. hepatica* and its body even more leaf like in shape [2].

The egg of *F. hepatica* measures 150 ìm by 90 ìm in size and also very similar in shape to that of *F. gigantica* [2]. The egg of the latter is larger in size (200 ìm x 100 ìm) [10]. *Fasciola* eggs should be distinguished from the eggs of other flukes, especially from the large eggs of *Paramphistome*. *Fasciola* eggs has a yellowish brown shell with an indistinct operculum and embryonic cells whereas *Paramphistome* egg has transparent shell, distinct operculum with embryonic clear cells and possess a small knob at their posterior end [2].

Epidemiology: The epidemiology of fasciolosis is dependent on the ecology of the snail intermediate hosts. The most important intermediate hosts of Fasciola are *Lymnaea truncatula* and *Lymnaea natalensis*. In developed countries, the incidence of *F. hepatica* ranges up to 77% [11]. Evidence suggests that sheep and cattle may be considered the main reservoir host species, pigs and donkeys being secondary [12]. In tropical regions, fasciolosis is considered the single most important helminthes infection of cattle with prevalence rates of 30-90% in Africa, 25-100% in India and 25-90% in Indonesia [11]. *F.hepatica* is a temperate species and it is found in Southern America, Northern America, Europe and Australia and Africa, but found in the highlands of Ethiopia and Kenya [13]. It is the major cause of liver fluke disease in Ethiopia. Its tropical counterpart, *F. gigantica,* on the other hand is widely distributed in tropical countries, in Africa and Asia, parasitizing domestic ruminants and other herbivores in almost every continent.

The epidemiology of fasciolosis depends on the grazing habitat preference of the animal. Njau and Scholtens [14] reported that *metacercaria* can survive up to 3 months after harvesting in hay from endemic highland areas that are consumed by the ruminants in arid and lowland areas, particularly during the dry season when suitable grazing pastures are scarce; local crowding of animals along the banks of streams and ponds during the dry season. When nutritional conditions are generally compromised also provides an important dynamics for infection transmission [13].

F.hepatica has a number of survival mechanism for evading host immune responses, including changing its surface antigen during migration. Releasing proteolytic enzymes that can clave immune globulins and modulating the host immune response [11].

Factors That Affect the Distribution of the Fasciolosis: The main factor determining the timing and severity of hepatic fasciolosis are those influence the number of *metacercaria* accumulating on herbage. In particular temperature and rainfall affect both the spatial and temporal abundance of snail hosts and the rate of development of fluke eggs and larvae [15].

Availability of Suitable Snail Habitats: One of the most important factors that influence the occurrence of fasciolosis in an area is availability of suitable snail habitat [2]. Snail habitats may be permanent or temporary. The later expand and contract depending on water availability. Construction works such as road building may alter drainage patterns and disease risk. Improvement of peaty pasture by lime application may increase risk by reducing soil acidity and allowing snail colonization [11]. The availability of these intermediate hosts depends on climatic conditions for instance; *L. truncatula* prefers wet mud to free water and permanent habitats include the bank of the ditches or streams and the edges of small ponds. Hoof marks, wheel ruts or rain ponds may provide following heavy rainfall or flooding, temporary habitats.

Temperature: Temperature is an important factor affecting the rate of development of snails and of the stage of the parasite out side the final host. A mid day /night temperature 10^0C or above is necessary both for snails to breed and for the development of *F. hepatica* within the snail and all activity ceases at 5^0C. This also the minimum ranges for the development and hatching of *F. hepatica* eggs. However, it is only when temperatures rise to 15^0C and are maintained above that level, that a significant multiplication of snails and fluke larval stages ensures [3]. Temperature above 10ºC (50^0F) is necessary before the snail hosts will breed or before *F.hepatica* can develop within the snail.

The summer infection of the snail by *miracidia* hatching from egg in the spring and early summer results in the emergency of *cercaria* and the consequent contamination of herbage 5-8 weeks. For any climatic region; *cercarial* shedding is a fairly regular occurrence with minor differences in timing determined by year to year variations in weather patterns. The winter infections of the snails are a separate cycle occurring when snails are exposed to *miracidia* in the autumn. Fluke development cases in the snails during winter but resumes as temperature rise the following spring. The relative importance of this cycle depends on the mortality rate of the snails during the winter [16], which varies from region to regions and year to year. The clinical outcome of the disease (of infection) depends largely on the density of *metacercaria* on the herbage. This will be greatest when weather conditions have been favourable for snail reproduction and survival. A high intake of *metacercaria* over a short time will produce acute disease; lower numbers over a longer period lead to chronic disease. The degree to which immunity influences the course of infection differs with species. Sheep and goat do not develop a strong protective immune response to *F. hepatica* and remain vulnerable throughout their lives. Cattle eventually expel most but not their entire fluke's burden and gain partial but not complete protection against re infection [17]. No development therefore can takes place during the winter in most countries.

Moistures: The risk of hepatic fasciolosis is determined by the number of infected *lymnaied* snails in the grazing area. The disease has a predictable seasonal pattern in region where snails are active for only parts of the year. Some *lymneaid* snails have a more aquatic habitat than others but all are restricted to damp or wet environments. In general, they prefer non acidic low lying swampy areas with slowly moving water, but land with small streams, springs, blocked drainage, or spillage from, for example, water troughs may also be potentially hazardous infecting grounds. Land frequently irrigated is also highly suitable for infections to take place. Snails burrow in to the soil to survive in dry periods and release *cercaria* where free water is present [11].

The ideal moisture conditions for snail breeding and the development of *F. hepatica* within snails are provided when rainfall exceeds transpiration and field saturation is attained such conditions are also essential of the development of fluke eggs, for *miracidium* searching for snails and for the dispersal of cercaria being shed form the snails [3]. It has been shown experimentally that all stages eggs, young and adults of *L. truncatula* can with stand desiccation for considerable periods, the adult for periods of a year or more, provided that they are favourable covered with mud. Moisture is the critical factor determining the presence and extent of snail habitats, which serve as transmission foci for liver flukes. The interaction between moisture and temperature determines the survival and reproduction rate of the snails and the parasites.

Etiology: *Fasciola hepatica* is the most common and important liver flukes and has a cosmopolitan distribution. *Lymneaid* mud snails are intermediate hosts and release the infective form, the *metacercaria,* on to herbage. Hepatic fasciolosis is mainly of economic important in sheep or cattle but other species may provide a reservoir of infections. *Fasciola hepatica* may infest all domestic animals, including *equidaie* and many wild life species, but chronically infected sheep are the most important sources of pasture contamination [18]. Human cases are usually associated with the ingestion of marshy plants such as water cress.

Life Cycle: Adult *Fasciola* live in bile duct producing eggs that are excreted with the faeces. Hatching occurs in moist conditions only after the first larval stage, *miracidium,* has formed and when ambient temperature rises above 5-6ºC (41-43ºF). *Miracidia* must find and invade the tissue of suitable host snails within 24-30 h. After several cycles of asexual multiplication, the fluke leave the snails as *cercaria.* These attach to herbage and transform in to *metacercaria* by secreting a tough protective cyst wall. After ingestion by the final hosts, each *metacercaria* releases an immature flukes which crosses the intestinal wall and migrate across the peritoneal cavity to the liver. The migration is sometimes misdirected and ectopic flukes can be found in lungs, particularly in cattle. The young *F.hepatica,* migrate through the hepatic parenchyma for about 4-5 weeks,

growing from 0.1-10 mm. After entering the bile ducts, they more than double in size before egg laying starts about 10-12 weeks after infestation. Adult sheep and cattle may remain carriers for many years because of the longevity of the adult flukes [19].

Pathogenesis: Acute hepatic fasciolosis is caused by the passage of young *F.hepatica* through the liver parenchyma. Clinical sign occur 5-6 weeks after the ingestion of large number of *metacercaria.* By this time, the migrating flukes are large enough to do substantial mechanical damage to liver. Acute hepatic insufficiency and haemorrhage will result. Quiescent spores of *Clostridium novyi* may become activated by the anaerobic necrotic conditions created in the liver parenchyma by migrating *fasciola hepatica*, causing infectious hepatic necrotic hepatitis (black disease) in sheep and cattle [20]. This migration has also been thought to stimulate the development of occasional cases of bacillary haemoglobin urea in cattle.

Chronic hepatic fasciolosis develops only after the adult flukes establish in the bile ducts. They cause colongitits, biliary obstruction, fibrosis and a leakage of plasma protein across the epithelium. Although this protein can be resorbed in the intestine, there is poor utilization and retention of nitrogen leading to hypoalbuminemia. There is also a loss of whole blood due to the feeding activity of the flukes. This exacerbates the hypoalbuminemia and eventually gives rise to anaemia. It places a continuous drain on iron reserves [21]. So that the anaemia, which is initially norm chromic, became hypo chromic. These changes are more severe in sheep on a low plane of nutrition [4]. Chronic infection may limit growth rate and feed conversion in growing heifers and growth rate in beef cattle. *F.hepatica* infection has been reported to increase the susceptibility of cattle to salmonella Dublin and predispose to prolonged infection and faecal excretion [22]. Food intake is reduced and this leads to a reduction in efficiency of utilization of metabolizable energy and a reduction in calcium and protein deposition in the carcass. The fibrotic response of the liver to fluke induced damage varies with the host and may partially account for differing species susceptibilities. The severe reaction in cattle, which includes calcification of the bile ducts, appears to hinder the establishment and feeding of challenge infections. There by reinforcing immune responses both horse and pigs are generally highly resistant to infection with *F.hepatica* but differ in their mode of resistance. Horse overcome the migrating flukes at an early stage so that few reach the liver, while in the pig the resistance mechanism operates in the liver parenchyma [4].

Clinical Pathology: In acute fasciolosis there is a severe normochromic anaemia, eosinophilia and severe hypoalbuminemia. Blood concentrations of a number of serum enzymes indicating liver damage are elevated. Glutamate dehydrogenase is off particular value when the young flukes are migrating through the liver parenchyma but concentration falls after they enter in the bile ducts [15]. Increase in aspartate aminotransferase can be measure from 4 weeks and are useful as measure of immature infection egg will not be present in faeces as the flukes are still juvenile.

In sub acute and chronic disease, weight loss is associated with a severe hypo chromic macrocytic anaemia, hypoalbuminemiae and hyperglobulinemia. Sub mandibular oedema and ascities occur only occasionally in the sub acute condition but more frequently in chronic disease [6]. Serum a- glutamyl transpeptidase concentrations are raised by the activities of adult *F.hepatica* in the bile ducts. Other liver function tests are not significantly affected. A diagnosis of chronic hepatic fasciolosis can be confirmed by the detection of large number of characteristics operculated fluke eggs in the faeces. These eggs are thin walled and stained yellow brown by biliary pigments. They are dense and do not rise in all flotation solution. Zinc sulphate solution SGI-30 is recommended. Sedimentation tests are more accurate [18]. Operculated fluke eggs are also characteristics of paramphstomosis and care is needed to differentiate the two.

Necropsy Finding: Acute hepatic fasciolosis is characterized by a badly damage, swollen liver. The peritoneal cavity may contain an excess of blood stained serum. The liver capsule has many small perforations and sub scapular haemorrhages. The parenchyma shows tracts of damaged tissue and is more friable than normal. The immature flukes are often so small that they are not readily discernible. They are most easily demonstrated by slicing a piece of liver thinly and shaking in water, permeating the flukes to settle to the bottom. The size of the flukes may allow estimation of the duration of the infections and this help to determine which pasture are hazardous [23].

Chronic Hepatic Fasciolosis: Leaf like flukes, measuring some 3.5x1 cm, are present grossly enlarged and thickened bile ducts, particularly in the ventral lobe of the liver. The bile ducts may protrude above the surface of liver and cysts may present due to blockage of ducts with flukes and desquamating epithelial cells. Calcification of the bile duct walls is a common finding in cattle but not in sheep.

The hepatic parenchyma is extensively fibrosed and the hepatic lymph nodes are dark brown in colour. Anaemia, oedema and emaciation are attendant abnormalities [24].

Diagnostic Confirmation: In fluke endemic areas, fasciolosis must be considered as a possible factor in any such outbreaks. To support a diagnosis, account should be taken of grazing history and seasonality of fasciolosis in that locality. There should be fluke eggs in the faeces and characteristics hepatic lesions at necropsy. As these may be ubiquitous finding in endemic areas, a judgment is necessary to determine whether the severity of the lesions is sufficient to incriminate the fluke as the sole or major contributing etiological factor. Serological tests such as the enzyme linked immune sorbent assay (ELISA) have shown promise, particularly for diagnosis of infections in cattle. A rise in antibody can be detected by 2 weeks of infections and keeps rising until weeks of [25].

Bovine Fasciolosis in Ethiopia: Various reports indicated that, Ethiopia is one of the countries with suitable climatic condition for the existence of fasciolosis. Both *Fasciola hepatica* and *Fasciola gigantica* are found in Ethiopia and transmitted by the snail called *Lymnaea truncatula* and *Lymnaea natalensis,* respectively. Their pathogenic significance depends on the favourability of environment they live. In Ethiopia, *F. hepatica* is wide spread in areas with altitude of 1200 - 2560 meters above the sea level. Both *Fasciola* species co-exist in area with altitude ranging between 1200 to 1800 meters above the sea level [13].

Public Health Significance: Fasciolosis is emerging as an important disease in man particularly in countries such as Bolivia, Peru and Egypt [6]. *Fasciola hepatica* may be acquired by man, but not directly from cattle. A person must ingest the *metacercaria* in order to become infected [12].

Human cases of fasciolosis have been reported in South America, Europe, Africa, Australia and Far East with an estimated 2.4 million cases world wide. The number of people infected with *F.hepatica* has increase significantly since in 1980. Several geographical areas have been described as endemic for the disease in humans. Human acquire infection through ingestion of *metacercariae* that are attached to certain aquatic plant and vegetables. Infection may also be acquired by the consumption of contaminated water or the ingestion of food items washed with such water. The distribution of the disease is predominantly rural, being associated with cattle and sheep breeding. The degree of pathogenicity of *F. hepatica* to man depends on many factors, particularly the number of worm present and the organ infected. Mechanical and toxic damage are characteristics [26].

Economic Significance: Fasciolosis causes major economic losses in sheep, goat, buffaloes and cattle [27]. It is the cause of tremendous loss to the grower of these animals. In addition to rendering the liver unsuitable for human consumption, it causes loss through death, reduction in meat and milk inhibited reproduction, morbidity impaired growth reduction in carcass weight, reduction in wool growth and quality, decreased feed intake, conversion and lowered resistance [28].

Treatment: Not all compounds are equally effective against all stages of development of *F. hepatica* in the body. For treatment of acute fasciolosis, it is essential to chose and product highly effective against the juveniles that damage the liver parenchyma. For chronic disease, a compound active against the adult fluke is required product safety is an important consideration as hepatic detoxificating mechanism are already impaired. Flukicides can be used therapeutically for treating the disease or prophylactically to prevent outbreaks. Some binds to plasma protein (closantel) or erythrocyte (clorsulun) their by extending their period of protection. All flukicide either have milk withholding periods or are prohibited from use in animals providing milk for human consumption and so the best time to treat dairy cattle is at the drying of stage. Many products combine a flukicide with a nematocide, but this should only be used when there is simultaneous risk from the two types of parasites [29]. Recently a new fasciolocide was successfully tested in naturally and experimental infected cattle in Mexico. This new drug is called compound alpha and is chemically very much closed to triclabendazole.

Prevention and Control: Preventive measures are required in endemic areas of fasciolosis can causes death without warning or significant production losses. An integrated strategic approach is more cost beneficial than reliance on routine dosing and is less likely to induce anthehelmentic resistance, but requires detailed knowledge o f the local epidemiological cycle. In some countries, where, risk varies from year to year. Production of likely disease levels are issued based on analysis of metrological data and field observation. This enables control measures to be intensified when necessary computer model have been devised to assist this processes [15].

Segregation of stock from sources of infection is the ideal method of control but not always feasible in practice. Identification and mapping snail habitat may enable grazing plants to be devised that avoid areas at time of higher risk. Where habitats are restricted in size clearly defined, it may be possible to exclude stock by fencing. Stock on heavily contaminated land may be protected from acute fasciolosis by taking advantage of the interval between the ingestion of *metacercariae* and the onset of diseases. Treatment during this period with a product effective against young flukes will eliminate the migrating parasite before they damage the liver because for serious liver damage a further dose may be necessary depending on the duration of metacercaria intake and the trematode interval of the chosen product. Some *metacercaiae* will continue to be ingested after the main danger period has passed and so treatment with a product active against adult *F. hepatica* will be needed some week later to insure against possible losses from chronic fasciolosis. Additional strategic dose may be required in region where the winter infections of the snails are of significance. The precise timing of each of these doses depends on the local epidemiological pattern [30].

Reduction of pasture contamination with *metacercariea* will reduce future risk. This can be done by preventing the snails from becoming infected with *F. hepatica* or by diminishing the size of the snails' population. To achieve the first objective, adult fluke should be eliminated from the bile ducts of all grazing stock in spring and early summer. This prevents egg excretion and minimizes the number of snails-seeking miracidia at this crucial stage in the epidemiological cycle. There may however be wild life source of *F. hepatica* eggs which cannot be controlled in this way snail number can be reduced by restricting the size of their habitat. This can be done, where feasible, by draining boggy areas and by making sure that ditches, land drain, water troughs etc. are well maintained [31].

Chemical snail control was widely practiced before reliable animal treatments became available *lymnaeid* snails have enormous reproductive capacity and can quickly re colonize wet land. Application therefore has to be very though to have a significant season long effect and there must be no possibility of invasion from neighbouring land 37 [32]. Chemical can be applied in spring for maximum impact on snail population before breeding starts, or later in the season when snails are plentiful but before *cercariae* start to emerge. Efficacy is reduced if luxuriant plant growth hinders penetration to soil level. Inorganic compounds such as copper sulphate or sodium pentachlorophenate are effective but may be potentially hazardous to man stock and the environment safer and more selective low volume mollusc ides such as n-trityl morpholine have been developed [15].

Vaccines for *F. hepatica* are under development. One of these which use a combination of defined fluke derived cathepepsin l protinase and haemoglobin molecules has given 72% protection against infection in cattle in early trials [33]. Immune response to successful vaccination strategies are qualitatively different from those induced by natural infection.

CONCLUSION

Fasciolosis is a disease of sheep, goat, cattle and occasionally affects humans, thus considered as a zoonotic infection. *Fasciola hepatica* is the most common and important liver flukes and has a cosmopolitan distribution. The epidemiology of fasciolosis is dependent on the ecology of the snail intermediate hosts. The most important intermediate hosts of *Fasciola* are *Lymnaea truncatula* and *Lymnaea natalensis*. Human cases are usually associated with the ingestion of marshy plants such as water cress. Human cases of fasciolosis have been reported in South America, Europe, Africa, Australia and Far East. Fasciolosis causes major economic losses through death, reduction in carcass weight, reduction in wool growth and quality, decreased feed intake, conversion and lowered resistance. In fluke endemic areas, Fasciolosis must be considered as a possible factor in any such outbreaks. To support a diagnosis, account should be taken of grazing history and seasonality of fasciolosis in that locality. There should be fluke eggs in the faeces and characteristics hepatic lesions at necropsy. Serological tests such as the enzyme linked immune sorbent assay (ELISA) have shown promise, particularly for diagnosis of infections in cattle.

REFERENCES

1. Keyyu, J.D., J. Monrad, N.C. Kyvsgaard and A.A. Kassuku, 2005. Epidemiology of *Fasciola gigantica* and Amphistomes in cattle on traditional, small-scale dairy and large-scale dairy farms in the Southern Highlands of Tanzania. Trop An Health Prod., 37: 303-314.
2. Soulsby, E.J.L., 1982. Helminthes, Arthropods and protozoa of domesticated Animals, seventh edition, Balliere. Tindall, London, UK. pp: 40-52.

3. Urquhart, G.M., J. Duncan, L. Armour, J. Dunn and A.M. Jennings, 1996. Veterinary parasitology second edition, Black well science, UK. pp: 103-113.
4. Dalton, J.P., 1998. Fasciolosis. School of Biotechnology, Dublin city, Ireland.
5. Malone, J.B., F. Gommes, O.F. Nachet and E. Agamen, 1998. A geographic information system on the potential and Abundance of *F.hepatica* and *F. gigantica* in East Africa based on food and Agriculture organization data basis, Veterinary Parasitology, 78: 87-101.
6. Rokni, M.B., J. Massoud and E.B. Kia, 2003. Comparison, Of Adult Somatic and cysteine proteinase antigens of *Fasciola gigantica* in Enzyme linked Immunosorbent Assay for diagnosis of bovine Fasciolosis. DIE seminar on biotechnology, proceeding November 9-13, Tehran, Iran.
7. Andrews, S.J., 1999. The life cycle of *fasciola hepatica* in fasciolosis in Dalton, j.p.ed. CABI publishing walling ford, UK pp: 1-30.
8. Okewole, E., G.A.T. Aogundip, J.O. Adejinmi and A.A. Olaniyan, 2002. Clinical evaluation of chemo prophylactic regime against ovine helimentheasis in fasciola endemic farm in Ibadan, Nigeria, Israel Journal of Veterinary Medicine, 56(1): 15-28.
9. World Health organization (WHO), 1995. Control of food borne Trematode infections. Technical Report series 849, (61).
10. Dunn, A.M., 1978. veterinary helminthology 2nd ed. Butler and tanner, ltd, London, UK pp: 15-159.
11. Spithill, T.W. and J.P. Dalton, 1999. progress in development of liver fluke vaccine parasitology today, 14(6): 224-228.
12. Mas-coma, S., M.D. Barguest and J.G. Esteban, 1999. Human fasciolosis In: fasciolosis Dalton, J. P. ed. CABI publishing, walling ford, UK pp: 411-434.
13. Yilma, J.M. and J.B. Malone, 1998. A Geographic information systems fore cost model for strategic control of Fasciolosis in Ethiopia, Faculty of veterinary medicine, Addis Ababa university, Ethiopia, Veterinary Parasitology, 78: 103-123.
14. Njau, B.C. and R.G. Scholtens, 1991. The role of traditionally harvested hay in the transmission of ovine fasciolosis in the Ethiopian highlands. Veterinary Research Communication, 15(5): 369-372.
15. Roberts, J.A. and Suhardono, 1998. Approach to control of fasciolosis in ruminants. International Journal for Parasitology, 26: 971-981.
16. Ginetsinkaya, T.A., 1988. Trematodes, their life cycles, biology and evolution. Amerind publishing Co., Pvt. Ltd, New Delhi, pp: 559.
17. Boray, J.C., R. Jackson and M.B. Strong, 1985. Chemoprophylaxis of fasciolosis with triclalbendazol. New Zealand Vet. Journal, 33: 182-155.
18. Boray, J.C., 1985. Flukes of domestic animals. In Gaafar, S.M., Howard, W.E. and Marsh, R.E. (Ed) *Parasites, pests and predator*. Elsevier, Amsterdam, Oxford, New York, Tokyo, pp: 179-218.
19. Haas, W. and B. Haberl, 1997. Host recognition by trematode miracidia and cercariae.In Fried, B. and Craczyk, T.K. (Eds) Advanced in trematode biology. CRC press, Boka Laton, Florida, pp: 197-228.
20. Preveraud-sindou, M., G. Dreyfuss and D. Rodeland, 1994. Comparison of the migration of *fasciola hepatica* sporocyst in *lymnea truncatila* and other related snails families. Parasitology Research, 80: 342-345.
21. Fried, B. and J.K. Graczyk, 1997. Advances in trematodes biology. CRC Press Boka Laton, Florida, pp: 466.
22. Westcott, R.B. and W.J. Foreyt, 1986. Epidemiology and control of trematodes in small ruminant. Veterinary clinic. North America: Food Animal Practice, 2(2): 373-381.
23. Kithuka, J.M., N. Maingim, F.M. Njeruh and J.N. Ombui, 2002. The prevalence and economic impact due of bovine fasciolosis in Kenya. An Analysis of abattoir data anderstepoorl journal of veterinary research (south Africa), 69(4): 256-262.
24. Blood, D.C. and Radostitis, 1994. veterinary medicine: a text book of disease of cattle, sheep, pigs, goat and horses 8th ed. ELBS, Baillie tindall 68.
25. Briskey, D.W., 1998. Diagnosis of liver fluke infection in cattle, Veterinary Bulletin, 68: 1-4.
26. Elmer, R. noble and Grenn, A. noble, 1982. Parasitology, the biology of animal parasite 5th ed. pp: 172. Lea and Tebigen, Phiadelphia.
27. Food agriculture organization (FAO) of united nation, 1994. Disease of domestic animal caused by flukes: epidemiology, diagnosis and control of *fasciola*, paramphistome, dicrocolium, eurytrema and schistome infection in ruminants in development countries FAO/UN, vialedelle terme di Caracalla, Rome, Italy, pp: 49.
28. Olsen, O.W., 1991. animal parasites: their life cycle and ecology, 3rd ed. Royal university.park press. London. pp: 120-128.
29. Ross, E.M. and A.G. Gilman, 1985. Pharmaco dynamics. Mechanism of drug action and the relationships between drug concentration and effect. In Gilman, A.G., Goodman, L.S., Tall, T.W. and Furad, F. (Eds). *The pharmacological basis of therapeutics*. Macmillan publishing company, New York, pp: 35-48.

30. Haseeb, M.A. and B. Freid, 1997. Mode of transmission of trematodes infection and their control. In Freid, B.and Craczyk, T.K. (Eds) Advances in trematodes biology. CRC press, Boka Laton, Florida, pp: 31-50.
31. Hansen, J. and Brian Perry, 1994. The epidemiology, diagnosis and control of helminthes parasites of ruminants. A handbook Rome: Food and Agricultural Organization f the United Nations, pp: 72.
32. Boyce, W.M. and C.M. Curtney, 1990. Seasonal transmission of *fasiola hepatica* in North Central Florida (USA). International journal for parasitology 20: 695-696.
33. Hourdin, P., D. Rondelaud and J. Cabaret, 1993. The development of *fasciola hepatica* parthetnitaea in *lymnaea truncatula* by modification of *mullerius capillaries* infection. International Journal for Parasitology, 23: 235-243.