ISSN Print: 2394-7500
ISSN Online: 2394-5869
Impact Factor: 5.2
IJAR 2016; 2(3): 265-271
www.allresearchjournal.com
Received: 06-01-2016
Accepted: 08-02-2016

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Liver fluke (fascioliasis)

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Abstract
Liver fluke (fascioliasis) is a parasitic disease caused by a trematode parasite of the genus *Fasciola*. The fluke occur mainly in animals (cattle, sheep, goats and occasionally equine) but human can be infected. Human fascioliasis has recently been recognised as an emerging and re-emerging zoonotic disease in several countries. Approximately 17 million people have been infected and 180 million people at risk all over the world. Fascioliasis is a major problem in the field of veterinary public health as well as a great impact on economic losses. The disease can be diagnosed by faecal examination of excreted eggs however; it is not reliable in horses. In addition, molecular techniques, ELISA and Western Blot tests can be applied to confirm the diagnosis. Triclabendazole, Rafoxanide and Closantel are used for the treatment of fascioliasis but resistance has been recorded in some countries. No commercial vaccine is yet available in spite of many trials. In this review, we aim to shed light on the life cycle, diagnosis, treatment, resistance and control of *Fasciola* in details.

Keywords: Fascioliasis, Liver Fluke, Triclabendazole, ELISA.

1. Introduction
Fascioliasis, or liver fluke is a human and an animal parasitic disease caused by endoparasitic trematodes of the genus *Fasciola* [27] which live in the bile ducts [31]. *Fasciola hepatica* and *F. gigantica* are the most common species of liver flukes that cause hepatobiliary system infection mainly in cattle and sheep that they have an impact on public health. It has been stated by (Parkinson *et al*., 2007) [27] that *F. gigantica* is responsible for the liver fluke in tropical areas, whereas *F. hepatica* is found more commonly in temperate climates [27]. *F. gigantica* is restricted to Asia and Africa, however, *F. hepatica* has been found globally [27].
Human fascioliasis is caused by *F. hepatica* as it is recently been recognised as an emerging and re-emerging zoonotic disease in several countries. Approximately, 17 million people have been infected and 180 million people at risk all over the world. Most of the infected or at risk populations are in the USA, parts of Europe, South Africa, the Middle East and Asia [14]. The reservoir hosts are mainly ruminants including cattle, sheep, goats, alpacas and deer but pigs and humans can be infected [16].
Other animals such as kangaroos, wombats and rabbits may maintain the contamination of pastures as reservoir hosts. Equines can also occasionally be infected with liver fluke [30].
It has been reported that the disease is significant in livestock-rearing areas, as it has major impacts on the economic losses in the animal industries such as death, decrease in production rates and liver condemnation [20]. In addition, meat and milk output reduction in infected animals and additional costs derived from flukicide drugs [5]. Yearly, fascioliasis result in more than US $2000 million lost and more than 600 million animals have been infected globally [19].
In addition, up to 40 million sheep and 6 million cattle are present in the areas where fascioliasis is endemic. Moreover, US $10 million is spent only for liver fluke drenching and a further $50-80 million as a lost production costs per year. Other significant economic losses in sheep are decreased production of wool quality and lambing percentages as well as poor lamb growth rate. Fascioliasis has also been identified as a major problem of veterinary public health [19].
The impacts of climate change have been described in a recent review of animal and zoonotic helminthoses [17]. It has been found that an increasing production of cercaria is associated with global warming [17]. For a better understanding of the wider complication effects of climate changes on the prevalence and incidence of fascioliasis in livestock animals, a qualitative risk assessment framework has been promoted in some countries such

as in the UK [19]. It was reported that climate change has the potential effects on the intermediate host (snail) of the fluke [19]. Additionally, it has an impact on the survival of stages of the liver fluke in the environment. In Scotland for example, the occurrence and prevalence of fascioliasis were increased and it was predominantly associated with warming of weather. It was suggested by (McCann *et al*., 2010) [19] that rising of water table is due to increasing of rainfall which allows the snails to extend its natural habitat.

The clinical signs of fascioliasis are acute, sub- acute and chronic according to the maturity of the flukes, and the diagnosis is mostly based on finding of excreted eggs of the liver flukes in faecal samples. To improve diagnosis, an Enzyme Linked Immuno-Sorbent Assay (ELISA) technique was developed [28].

Liver fluke infection is rarely found in horses and few studies carried out on the prevalence of equine fascioliasis [28]. The problem with conducting research on equine liver fluke is the difficulty of diagnosis as the most *Fasciola* worms do not reach maturity in horses therefore, faecal examination of excreted eggs is not reliable [30].

Triclabendazole (TCBZ), a benzimidazole derivative, is effective against both adult and juvenile flukes. Although many drugs are effective but they can only provide interim control of liver fluke because sheep and cattle can easily be re-infected. Furthermore, resistance to triclabendazole reported in Australia and some European countries such as Spain, UK, Ireland and Netherland [7]. Moreover, no commercial vaccine has yet been produced against liver fluke infection. Hence, anthelmintics draining and fencing of the intermediate host habitats and management of pasture are the basic principles of control [7].

2. Equine Fascioliasis

It was reported by (Owen, 1977) [25] that horses considered as a less common host along with some other exotic animals for example, beaver, coypu, elephant, and kangaroo. Although equine fascioliasis recorded all over the world, the rate of infection is relatively low in comparison with other herbivorous animals such as ruminants [25]. In the endemic places where the incidence is nearly 100% in ruminants, the liver fluke infection rate is apparently rare. It might be relevant to make a comparison of the fluke infection between horses and pigs, as generally both species are greatly resistant to this infection [23].

For the first time, (Owen, 1977) [25] suggested that the fluke infection in horses may not be as less common as previously recorded. A survey in the West of Ireland revealed that 91 percent of donkeys and 77 percent of horses were positive for faecal egg examination. However, the total number of horses and donkeys was not recorded.

The epidemiology, immunology and pathogenicity of liver fluke infection in horses have not been recorded in many countries such as Ethiopia. Furthermore, the roles that equine may play in the transmission of fascioliasis to other susceptible animals as well as humans are not known [9].

In Ethiopia, a study determined that the high prevalence of infection rates among working donkeys without significant effects of their ages, and explained the differences of infection between the different regions [9]. The environmental conditions play an important role in the development of various stages of the parasite and its intermediate host [9].

Although few studies carried out on the prevalence of equine fascioliasis, the coprological examination of donkeys showed that the prevalence of liver fluke is to be 44.4% and the post-mortem finding revealed it to be 41.9%. It was reported that 20% of donkeys slaughtered for zoo carnivores in Turkey was positive for *Fasciola hepatica*. Another study revealed that around 189,000 horses, 296,000 donkeys, and 68,000 mules were infected with liver flukes in Turkey [18].

Diagnosis of liver fluke infection in cattle is sometimes complicated as the cow might mention to be infected and excreted fluke eggs but not in the next days, this might also be a big problem in horse *Fasciola* diagnosis [25]. It is really difficult for investigators to conduct faecal examination as most flukes do not reach the mature stages in equines. This makes faecal examination for excreted eggs unreliable. A proper alternative method is the serological tests including enzyme-linked immunosorbent assay (ELISA) as well as western blot [19].

3. Parasite Characteristics

General characteristics: Liver flukes are flatworms (leaf shaped) that live in or on other organisms. They use suckers to attach to their host. The infective stage (encysted metacercariae) cannot be dissolved by the stomach acid by having a hard cover around them. Their host specificities are different according to different types of flukes. Most flukes are hermaphrodites. Some flukes get their nutrition from blood and some from bile through the pharynx. This parasite can eat blood cells, mucus and body cells [34], (Figure 1A and 1B).

Fig 1A: The adult liver fluke (*Fasciola hepatica*) taken from sheep [33].

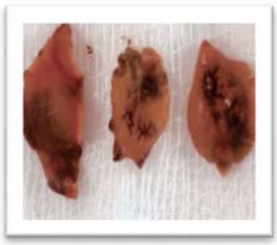

Fig 1B: Gross appearance of the adult flukes of *Fasciola hepatica*. The adult fluke is flat, brownish, and measures approximately 2.5 to 3 x 1 cm [8].

4. Characteristics of Fluke Eggs

In definitive hosts, fluke eggs are passed with the bile from the common bile duct of the liver into the first part of small intestine and subsequently excreted with faeces. They consist of a fertilized ovum surrounded by many yolk granules. The eggs are yellowish brown in colour, oval in shape, around 130-145 µm in length and 70-90 µm in width and they have an operculum *(Figure 2).* Undeveloped eggs are passed out with faeces onto the pasture and undergo embryonation outside the hosts. Several physical and chemical factors influence the development of eggs such as temperature, humidity and oxygen tension [6].

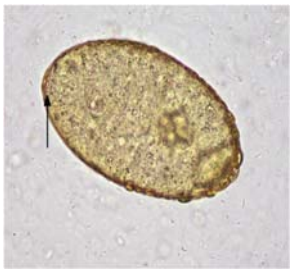

Fig 2A: Saline preparation of *Fasciola hepatica* egg in the stool (40x magnification). The arrow indicates the operculum of the egg [8].

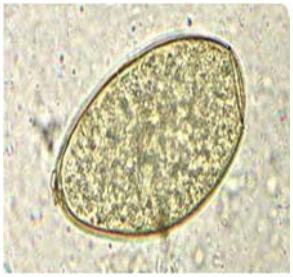

Fig 2B: *Fasciola hepatica* eggs in an unstained wet mount wet (400x magnification) [32].

5. Liver fluke life cycle

The life cycle of both liver flukes (*F. hepatica* and *F. gigantica*) are similar. The prepatent period of *F. hepatica* is about 10-12 weeks and its whole life cycle may complete within 17-18 weeks whereas, the life cycle of *F. gigantica* takes longer and the prepatent period is about 13-16 weeks. *F. hepatica* may stay for years in untreated sheep but it can survive in cattle for less than one year. Both liver flukes are hermaphrodites and one adult fluke can produce around 20 000 eggs daily [24].

Fluke eggs are excreted with the faeces of the infected mammalian animals (definitive hosts). After about 9-15 days [24], eggs will hatch and release motile and ciliated miracidia [8].

The optimal temperature required for hatching of eggs is 15 °C to 24 °C [22], because of the short life span of the miracidia, they have to find an intermediate host (freshwater *Lymnaea* snail) within three hours otherwise die. Inside the infected snails miracidia develop to sporocysts, rediae stages and cercariae consequently. Subsequently, motile cercariae pass from infected snails. Then, cercariae attach to plants and vegetables where they encyst as infective metacercaria [8]. Under unsuitable circumstances this may take several months.

Definitive hosts including human get infection through ingestion of food contaminated by metacercaria. Metacercariae encysted when reach the small intestine. Then, migrate through the gut wall, peritoneum followed by penetration of the liver capsule. The immature flukes migrate through the liver parenchyma for 6-8 weeks and enter the small bile ducts. After that, migrate to larger bile ducts or sometimes gall bladder. Finally, the mature flukes lay eggs and reach the small intestine with bile and ultimately with faeces to the environment, where another life cycle begins [1]. (Figure 3).

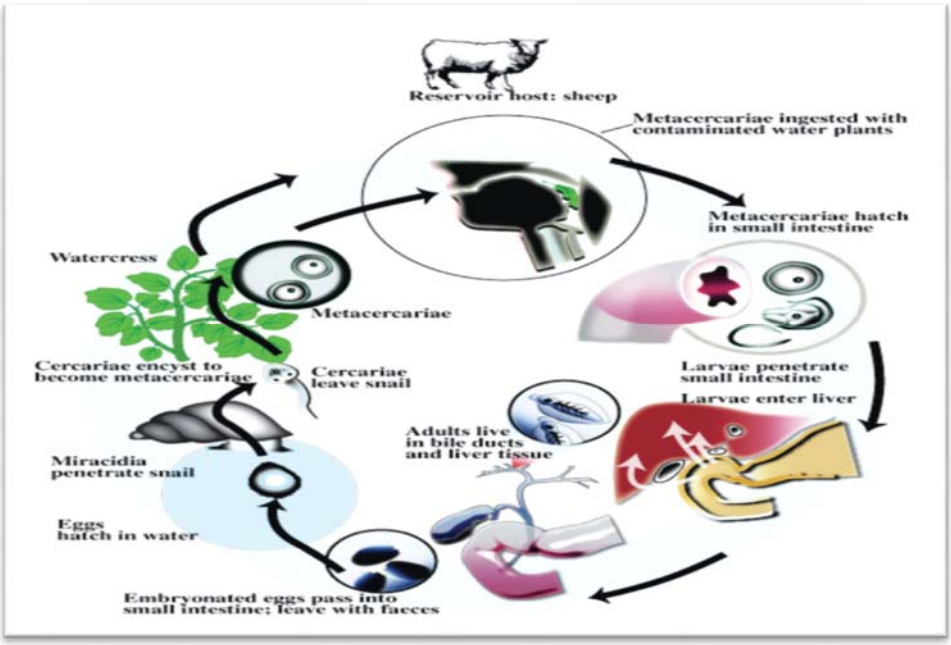

Fig 3: Shows the life cycle of *Fasciola* spp. (reprinted from the Institute of Tropical Medicine (ITM), Antwerp, Belgium)

6. Epidemiology and risk factors

Generally, fascioliasis is caused by *Fasciola hepatica,* (common liver fluke in cattle and sheep) and has been found in many different countries in the world. Although liver fluke is more common in animals than in humans, the number of infected people is more than 2 million people globally [8]. It has been diagnosed in more than 51 countries and in all continents. The disease has been found in Latin America, the Caribbean, Europe, the Middle East, Africa, and Oceania. Human fascioliasis has been recorded in the tropical areas including Africa, Asia as well as South Africa. Human fascioliasis is sporadic in some countries, while it is endemic or hyperendemic in others such as Peru (Marcos *et al*., 2006). Furthermore, Bolivia, Egypt, Iran and Puerto Rico are known to have the highest rate of human fascioliasis [8].

Approximately, 5% of the cattle in the United States are infected and the infection in beef cattle in California and Florida is about 53% and 68% respectively (Graham *et al*., 2001). An investigation revealed that 495 cattle livers have been investigated from an abattoir in Van in Turkey, 53.7% were infected with *F. hepatica*, and 1.8% with *F. gigantica* [30]. Another study has found that *Fasciola* was detected in 15.43% of cattle based on faecal examination. *F. hepatica* was diagnosed in 20% of donkeys slaughtered for zoo carnivores in Turkey. According to a study, approximately 189,000 horses, 296,000 donkeys, and 68,000 mules were positive for liver fluke in Turkey [30].

Amphibious snails from the genus Lymnaea act as an intermediate host for *Fasciola* that are widely distributed all over the world. Lymnaea truncatula and L. natalensis are the most common intermediate host for *F. Hepatica* and F. gigantica respectively. Although, there are many *Lymnaea* species that play their role in the life cycle of *F. Hepatica* for example; L. tomentosa in Australia and New Zeeland. In addition, L. columella in North America, Australia and New Zealand, L. bulimoides in Southern USA and the Caribbean. Furthermore, L. humilis in North America, *L. vector* in Southern America and *L.* diaphena in South America. Other vital Lymnaea species of *F.* gigantica are L. auricularis in Europe, USA, Middle East, and Pacific islands, L. rufescens and L. acuminta in India and Pakistan and L. rubiginosa in Malaysia [30].

When a suitable environment available for the intermediate host (optimal temperature and moisture), a large number of metacercariae will be released from the infected snail. The required temperature is about 10 °C at which both the snails and the flukes will develop. Their activity will stop, if the temperature decreases to below 5 °C. When the temperature reaches 15 °C and above their activity increases significantly, with the optimum being 22-26 °C. The optimal moisture level will be achieved when rainfall exceeds transpiration [30].

7. Diagnosis

Fascioliasis can be diagnosed by many different techniques. Faecal examination (sedimentation) looking for eggs is an appropriate way for diagnosis., but this often is not revealing particularly during the first stage of the life cycle when they do not reach their maturity. The most sensitive and quantitative technique is ELISA, which detects antibody against excretory and secretory antigen product from the mature flukes [8]. Both computerised tomography (CT) and ultrasonography as radiographic techniques have also been applied for confirmation [1]. Moreover, CT scan can also be used to identify the abnormalities of the patients' abdomen caused by fascioliasis. The abnormalities can be seen as small, indiscrete, hypodense lesions 2–10 mm in diameter, and micro abscesses arranged in a tunnel-like branching pattern, with frequent sub capsular locations of the lesions [10].

In addition, magnetic resonance imaging (MRI) can be applied diagnostic to detect different changes associated with liver trauma by migration of the fluke through the hepatic parenchyma. Biopsy from liver tissue can also be taken although it is not usually indicated. Necrotic debris, track-like destruction of parenchyma, poly-morphonuclear infiltration with abundant eosinophils, fibrosis and bile duct proliferation are the classical findings from the biopsy specimens.

Western blot, haemagglutination (HA), indirect fluorescence antibody test (IFAT), immunoperoxidase (IP), counterelectrophoresis have been applied for the immunodiagnostics of *F. hepatica* in cattle by using secretary and excretory antigen [29]. Also, Invasive techniques including percutaneous cholangiography and endoscopic retrograde cholangiography can be used [1].

8. Treatment and Resistance

In animals, triclabendazole (TCBZ) is the most effective and widely used anthelmintic against immature and mature flukes [21]. It has been stated by (Boray *et al*., 1983) [3] that frequently using of TCBZ have an effect on the reduction of the liver fluke infection to a negligible level. Centre of disease control (CDC) recommended that TCBZ is the first line agent that can be used for the treatment of human fascioliasis. (Aksoy *et al*., 2005) [1]. has reported dizziness, fever and abdominal pain one week after the using of the treatment.

Many studies have been conducted on using of TCBZ showing high efficacy against *Fasciola* spp. However, it has been revealed that in a later study, the significantly low level of efficacies of TCBZ is the indication of resistance of *F. hepatica* against triclabendazole in sheep [1]. Moreover, closantel is an effective drug for liver fluke treatment as it has been proved by (Maes *et al*., 1990) [15] that no fluke eggs were found after one week of using closantel. However, a study has been carried out in the Netherland and found eggs three weeks after the treatment by closantel. This may be interpreted as a resistance of the liver flukes for closantel [15].

Although praziquantel is the drug of choice for other trematodes, it is ineffective against *Fasciola* spp. [21].

Human fascioliasis can be treated with bithionol as an alternative of TCBZ in animals. When it is used frequently some side effects appear including nausea, vomiting, abdominal colic, pruritus, urticaria, and rash [1].

9. Immunity

It has been stated by (Haroun and Hillyer 1986) [12] that sheep can develop no or little acquired immune response against liver flukes. Accordingly, sheep are very susceptible to the pathological effects of *Fasciola*, and most acutely infected cases die due to liver fluke. Challenge infection including decreased egg production, reduced migration and retarded liver fluke, as well as increase the number of metacercariae cannot provide a protective immune response against fluke burden [6].

Sheep produce predominantly IgG1 against *Fasciola* infection, which reach the highest level at about 5 to 6 weeks after a primary infection. Therefore, the lack of acquired immunity in sheep may be somehow related to insufficient cellular responses [6].

Immuno-histological studies showed that the migratory tunnels of the liver parenchyma of sheep infiltrated by eosinophils and neutrophils, as well as macrophages and T and B lymphocytes during an acute liver fluke infection. There is not an evidence of leukocyte infiltration where the actual parasites are found. Cellular immune response has been substantially increased, but was noticed only around the portal tracts and lesion sites, not around the fluke [12].

In cattle, antibody responses show a substantial predominance of the IgG1 isotype over IgG2 which is consistent with the responses of cattle to other parasitic infections [12]. The titters of IgG1 reach the peak from 8 to 10 weeks of infection then decrease slowly [11].

It has been suggested by (Brown *et al*. 1994) [4] that CD4 cells are vital in the response of immune system of chronic

liver fluke in cattle. On the other hand, recent studies have demonstrated involvement of both CD4 and CD8 cells in the peripheral blood lymphocyte (PBL) responses in acutely infected cattle [20].

In humans the most migrating flukes become entrapped in the liver parenchyma. The clinical symptoms of human fascioliasis are abdominal pain, fever and sickness and systemic eosinophilia and elevated levels of IgM, IgE and IgG have also been observed [6].

10. Pathogenesis, pathology and clinical signs

Pathogenesis of liver fluke is primarily based on two stages of *Fasciola* development; in parenchyma and bile ducts. (Joseph, B., 2007) [13] Has reported that acute and sub-acute disease are rarely seen in cattle, but often in sheep. However, chronic disease is by far the most common type of infection in both sheep and cattle. The clinical signs of acute infection is characterised by weakness, sudden death, anaemia (Figure 5) followed by eosinophilia and dyspnoea. Sub-acute and chronic disease is characterised by anaemia, hypoalbuminemia, emaciation, ascites and submandibular oedema (bottle jaw) (Figure 5 and 6) [13].

11. Acute infection

This type of infection is mostly associated with immature flukes and often seen in autumn and early winter, several weeks after ingestion of large number of metacercariae (usually more than 2000)[1]. The immature flukes migrate through the liver parenchyma and subsequent disease results from direct trauma. The parasite can cause coagulative necrosis due to release of toxic excretion (e. g. Cathepsins), the lesions may differ from mild to severe infection such as splenomegaly, haemorrhage, fibrinous exudate on the liver surface. Flukes might be seen in tunnels of migration [1, 13]. Other clinical signs such as fever, hepatomegaly, hypergammaglobulinemia and eosinophilia can also be observed [1].

12. Sub-acute infection

This type is usually seen in the late autumn and winter in the UK, about 6–10 weeks after ingestion of smaller numbers of metacercariae approximately (500-1500). At this time, some flukes may reach the bile ducts, while others may still migrate through the liver parenchyma and sub-capsular haemorrhage may present without rupture [13, 21].

13. Chronic infection

This form of fascioliasis is usually seen in late winter and early spring and mostly associated with mature flukes due to ingestion of a few hundred metacercariae. The mature flukes reach the bile ducts and cause ulceration of the epithelium as well as severe hyperplasia of the epithelial layer. Mechanical effects by the adult flukes may occur and cause biliary retention and toxic production. Consequently, the host would show anaemia and hypoalbuminemia. Each adult fluke can take more than 0.5 ml blood daily [1]3. Plasma proteins can also be lost through the bile ducts into the gut because of increase in the permeability of hyperplastic bile duct epithelium [21]. Accumulation of the parasite and bile would cause the distension of the bile ducts in sheep, swine and horse, whereas in cattle erosions and inflammatory lesions are more prominent [13, 17].

Fig 4: Sheep with pale conjunctiva, due to anaemia

Fig 5: Sheep with bottle jaw (oedema) due to chronic fascioliasis.

Fig 6: Calf with bottle jaw due to chronic fascioliasis.

14. Post mortem lesions

Liver may have an irregular outline, and appears pale and firm. The ventral lobe is most commonly affected and reduced in size. The liver in the chronic infection is characterised by hepatic fibrosis and hyperplastic cholangitis. A number of different types of fibrosis may be present and includes post-necrotic scarring (mainly in the ventral lobe and associated with healing of fluke tracts), ischaemic fibrosis (infarction as a consequence of damage and thrombosis of large blood vessels, and fibrosis (damage by flukes in the small bile ducts). In cattle calcification of bile ducts, enlargement of the gallbladder and aberrant migration of the flukes is more common. Encapsulated parasites are often seen in the lungs. If adult cows are re-infected, parasitic migration to the foetus and resultant prenatal infection might occur [13].

15. Control

Control measures should be done on a preventative rather than curative. Three effective control strategies have been used which are: using of anthelmintic to reduce the number of liver fluke in the definitive hosts and the number of fluke eggs on the pastures, reduce the number of intermediate host and reduce of exposure to infection by managing the fluke prone areas.

1. Using anthelmintics: the correct time to use anthelmintics based on weather and climate conditions. Drugs play a crucial role in the control of fascioliasis. More frequent treatments are necessary if you use drugs that are only effective against advanced mature flukes aged 12–16 weeks or older. Using triclabendazole-based flukicides, the most effective drug against both early mature and adult liver flukes. The best control measures may be achieved if this drug use three times yearly. August/September: to prevent pasture from contamination and to eliminate adult flukes came from autumn and winter. January /February: to completely remove of flukes picked up during late spring and early summer. April/ May: to remove flukes picked up during summer and early autumn [13, 24].
2. Snail control: The second available strategy for control of *Fasciola* spp. is the control of snail as it acts as an intermediate host for the parasite. This can be done by; Chemical control: although chemical control is effective, snails cannot be eradicated by chemicals because they reproduce so readily. Improved drainage: Irrigation projects can provide habitats to the snails. Cleaning of vegetation regularly may reduce the contamination of herbage [13, 2].
3. Disease control by farm management: This is the third effective strategy for control of *Fasciola*. This can be accomplished by: Fencing the snail-infested grazing areas consist only a small part of the animals' pasture. Therefore, Fencing off these contaminated areas would be the most economic and efficient method of controlling fascioliasis. Spending a little money on fencing may prevent a serious outbreak of liver fluke disease [13, 7].

It has been reported that a number of climate-based forecasting systems applied to measure the risk of liver flukes in Great Britain, Northern Ireland and the south-eastern United States of America. Using of new technologies for example, Geographic Information Systems (GIS) has development for the spatial risk models of liver fluke. Spatial risk models of fascioliasis have been used for East Africa, Ethiopia, the South American Andes region, Central Chile, Louisiana, USA, Australia and Cambodia, whereas fine spatial models have not been used in the UK [18].

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