The Epidemiology And Control Of Liver Flukes In Cattle And Sheep

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Key Points

- *Fasciola hepatica*, *F. gigantica* and *Fascioloides magna* are liver flukes causing disease of economic and welfare importance in cattle and sheep worldwide
- Their life cycle involves a snail intermediate host and thus requires suitable moisture and temperature conditions for at least three months of the year
- Drug treatment is the mainstay of control and needs to be applied with an understanding of the life cycle and epidemiology of the parasites concerned.

Synopsis

Fasciola hepatica, *F. gigantica* and *Fascioloides magna* are liver flukes causing disease of economic and welfare importance in cattle and sheep. *F. hepatica* is the most widespread parasite, occurring on all continents except Antarctica. *F. gigantica* is restricted to tropical regions, whilst *F. magna* is found in areas of North America and central Europe. Damage to the liver due to *F. hepatica* and *F. gigantica* results in clinical disease and/or production losses, particularly reduced milk yield and slower growth rates. *F. magna* appears to have little effect in cattle but causes high mortality rates in sheep. The fluke life cycle involves an aquatic or amphibious snail intermediate host and thus requires suitable moisture and temperature conditions for at least three months of the year. *F. magna* also requires the presence of deer. Drug treatment is the mainstay of control and needs to be applied with an understanding of the life cycle and epidemiology of the parasite.

The Epidemiology and Control of Liver Flukes in Cattle and Sheep

Introduction

The liver flukes are digenean trematode parasites that cause economically important disease of domestic livestock. This chapter discusses three of the most important species of liver fluke: *Fasciola hepatica* (the common liver fluke or cattle fluke), *F. gigantica* (the tropical fluke), and *Fascioloides magna* (the giant liver fluke or deer fluke). The two *Fasciola* species are best documented as

infecting domesticated ruminants, although wild herbivores and most mammals can also be infected. In terms of zoonotic importance, 17 million people are estimated to be infected with these parasites in more than 70 countries worldwide. [1]

Conversely, *F. magna* is primarily a parasite of wild ungulates, but can infect sheep and cattle as dead end or aberrant hosts.

F. hepatica is the most widespread species, occurring in 70 countries worldwide in temperate climates, including parts of Latin America, the Caribbean, Europe, the Middle East, Africa, Asia and Oceania. In the USA, *F. hepatica* is limited to areas of high rainfall and poorly drained pasture within Texas, the Gulf coast, Great Lakes, and northwestern states.

F. gigantica is present in tropical regions of Africa and Asia. The two *Fasciola* species co-exist in areas of North Africa, the highlands of east Africa, and Asia, and there is evidence of hybridisation in some regions. [2,3]

F. magna originated in North America, and is currently endemic in five parts of the US and southern Canada: the north Pacific Coast, Rocky Mountain trench, Great Lakes region, northern Quebec and Labrador, and the area comprising the Gulf Coast, lower Mississippi and southern Atlantic seaboard.[4] It has been introduced into parts of Central Europe through imported game animals and is now present with one population in northern Italy, and a genetically distinct population that originated in Czech Republic and southwestern Poland, and has since spread to forests in Austria, Slovakia, Hungary, Croatia, Serbia and Germany. [5]

Recent increases in cattle movements and climate change have led to liver flukes expanding their range:[6] in the US, *F. hepatica* and/or *F. magna* are now found in 26 states and in 24% of slaughtered cattle. Similarly in Europe, *F. hepatica* is now found in areas that were once considered free of fluke.[7,89]

The liver flukes exert a considerable economic burden on livestock farming, with subclinical losses contributing a large proportion of the cost. Reduced milk yield and fertility, slower growth rates and reduced feed conversion are seen even with low burdens. [10,11] Emerging resistance to flukicide drugs is a challenge to control in Europe and Australia. [12,13]

Life cycle

The life cycle of all three fluke species involves a definitive (mammalian) host and an intermediate (snail) host. The life cycle is dependent on suitable habitat, moisture and temperature to sustain the intermediate host. The life cycle of *F. hepatica* is described below, with species variations described afterwards.

Fluke eggs are passed into the environment with faeces, *via* the gall bladder. If suitably mild and moist conditions exist, embryonation occurs. Moisture is essential for egg survival and embryonation, with eggs quickly dessicating in dry conditions. Embryonation takes 6 months at 10 °C, decreasing to 8 days at 30 °C. [14] At higher temperatures, viability periods are decreased. [15] At temperatures between 0° and 10° C, eggs remain viable for at least 2 years, but they are killed if exposed to temperatures below -5° C for longer than 2 weeks. [16] Aerobic conditions and a pH of between 4.2 and 9 are also required. [15]

Eggs must be liberated from faeces to create the correct conditions for hatching to occur, a process that is aided by water or mechanical disturbance. An active miracidium hatches from the egg and swims energetically for up to 24 hours to find an intermediate snail host. [17] The miracidium penetrates the body of the snail and becomes a sporocyst, from which, following parthenogenic multiplication, up to 200 rediae burst. [18] Each redia then gives rise to around 20 cercariae. This final larval stage then migrates out of the snail, around 4-7 weeks after infection. Following a short active phase of up to 2 hours, the cercariae encyst on nearby plant matter or on the surface of water as metacaercariae, the infective stage. A proportion of metacercariae may survive on pasture for up to

a year, although the infective load will decrease during this time. Survival relies on suitable moisture and temperature levels, with the heat and drought of a typical Australian or US summer, or temperatures below -10 °C causing mortality. [16,19,20] Metacercariae may survive in damp hay for a short time, however will not survive in silage under anaerobic conditions (B. John, unpublished results).

Once inside the host, excystation occurs and the newly excysted juvenile fluke migrate through the walls of the small intestines, and into the abdominal cavity within a few hours. Penetration of the liver capsule takes up to a week, and juvenile flukes then burrow through the liver parenchyma for up to 6 weeks before reaching the bile ducts where they remain. Fluke can survive for several years in sheep. Fluke are hermaphrodite although they reproduce mainly by cross-fertilisation, [21] with eggs being produced from 10 to 12 weeks post- infection. Therefore, the complete life cycle takes at least 16 weeks, although it may take much longer.

For the tropical liver fluke, *F. gigantica*, the life cycle is similar except that the temperatures required for the host snail species and parasite development are higher, and the time scales are longer. The prepatent period is 12-16 weeks, and the time for the full life cycle is at least 20 weeks.

F. magna also has a similar life cycle, with required temperature and moisture levels being similar to *F. hepatica,* but with considerably longer time required. The pre-patent period is at least 30 weeks and the full life cycle takes around 7 months to complete. Fluke migrate through the liver until they encounter another fluke, whereupon, in deer, the immune system of the host leads to the formation of a fibrous capsule where the hermaphrodite flukes mature and remain for up to 5 years.[22,23] In deer, eggs are able to pass out of the pseudocyst and reach the environment. [24] However in cattle, eggs remain trapped within the pseudocyst and therefore cattle do not contribute to the completion of the life cycle. In sheep, the formation of the pseudocyst is not effective and eggs can be excreted, if the sheep survives for long enough. [25,26]

Intermediate hosts

Lymnaeid snails are the intermediate host species for all the liver flukes. *Galba truncatula* (Figure 1) is the preferred host of *F. hepatica* in most parts of the world, and has been found in parts of Africa, North and South America, and Asia. *G. cubensis* and *G. bulimoides* are the main host species in North America.[27,28] These snails live in semi-aquatic habitats on the banks of streams or ponds, wet flushes and drainage ditches, or anywhere where exposed wet mud allows algae to grow. Damage to pasture caused by trampling by livestock or tractor tyres, combined with wet conditions, can cause snail habitats to expand. The snails are small, measuring 1-10 mm in length, and can survive periods of drought by aestivation. Where *F. hepatica* and *F. magna* co-exist, they share intermediate snail species.

Members of *Lymnaea auriculara sensu lato* are the preferred host of *F. gigantica.* [29] In Africa this is predominantly *L. a. natalensis*, whereas in the Indian subcontinent *L. a. rufescens* is the main host species. [30] *L. auriculara* are less able to aestivate and therefore live in permanent water bodies, being found deep in rivers and lakes.

Figure 1. *Galba truncatula* seen under the dissecting microscope

Effect on the host

Clinical signs

For the two *Fasciola* species, two main forms of disease are seen: acute fasciolosis, caused by migration of juvenile flukes through the liver parenchyma, and chronic fasciolosis, caused by adult flukes in the bile ducts. Acute fasciolosis occurs 6 to 8 weeks following ingestion of large numbers of infective metacercariae [31]. Liver damage and blood loss caused by migrating flukes leads to anaemia, proteinaemia, weight loss, and frequently in sheep, death. Sheep are more susceptible to acute fasciolosis, although sudden death can occasionally be seen in cattle.

Chronic fasciolosis is seen 4-5 months after ingestion of smaller numbers of metacercariae [31] and is associated with adult fluke in the bile ducts. Typical signs include loss of condition, anaemia, submandibular oedema, ascites, decreased milk yield, and fibrosis and, in cattle, calcification of the bile ducts may be seen at post-mortem examination. Additionally, sub-clinical infections in cattle are common, and may result in considerable reduction in growth rates and milk production. For *F. hepatica*, reductions in milk production of 3-15%, reduced growth rates in cattle of 6-9 % and negative effects on reproduction have been reported [32–37].

F. magna in cattle are contained within a pseudocyst [25,26] and usually do not cause clinical signs .[22,38] In sheep, the pseudocyst does not form effectively and the mature fluke migrates throughout the liver and other tissues such as lungs, causing haemorrhage and death in most cases. [22]

Liver damage caused by fluke can allow *Clostridium novyi* bacteria to enter and result in sudden death from infectious necrotic hepatitis (Black disease).

Immunology

Most immunological research on flukes has been on *F. hepatica.* Cattle and sheep can become infected at any age, and do not develop protective immunity. [39,40] The predominant immune response in naturally infected animals is Th2/regulatory, [41] which is likely to be a host adaptation to chronic infections, to avoid excessive tissue damage resulting from inflammatory Th1 cytokines, and is also induced by fluke antigens. [42,43] Antibodies are detectable from 2-3 weeks after infection and levels remain high throughout the period of infection. [44,45] However, these Th2/regulatory responses do not give protective immunity against liver fluke. *F. hepatica* has the ability to modulate the immune system to promote its own survival, and this has been shown to have bystander effects on co-infecting pathogens such as *Mycobacterium bovis*, although the practical implications are still unclear. [46–50] Conversely, some sheep breeds and rats have an innate immunity to *F. gigantica* and others can acquire it. This may be due to differences in antigen expression between the two parasites, or because *F. hepatica* is able to suppress the protective response.[51]

Diagnosis

Diagnostic methods include faecal egg count, antibody detection in milk or serum and antigen detection in faeces (Table 1). Pre-mortem diagnosis of *F. magna* in cattle and sheep is difficult as eggs are not usually produced. For all methods, sensitivity tends to be worse in animals harbouring only a light infection, where a missed diagnosis is likely to be of least importance. [52]

Table 1. A summary of the performance of some of the tests commonly used to diagnose liver fluke infection in cattle

Test	Sensitivity	Specificity	Comments	Suitable for	References
Faecal egg count (traditional sedimentation or Flukefinder®)	43-65%	90-100%	Pre-patent period is 8-12 weeks following infection (*F. hepatica*) or 13-16 weeks (*F. gigantica*). Sensitivity depends on egg count and weight of faeces used. Most methods are based on sedimentation	*F. hepatica* and *F. gigantica,* individuals or pooled samples for groups	[11]
Serology	79-95%	80-93%	Can detect infection 2-4 weeks post infection Remains positive for several weeks after cure.	Validated for *F. hepatica*. Likely to cross react with other species	[11,44]
Milk antibody detection	92% 96%	88% 80%	Individual Herd	Validated for *F. hepatica*. Individual or herds. Likely to cross react	[44,53]

				with other species	
Copro-antigen ELISA	40-98%	92-94%	Detects infection 6-8 weeks post infection, Returns to negative 1-2 weeks post treatment	Validated for *F. hepatica*. Likely to cross react with other species individuals or pooled samples for groups	[11,54–57]
Post-mortem diagnosis	63-93%	100%	Sensitivity varies: Lower at meat inspection, higher if liver is sliced up and soaked	All 3 species	[58]

For *F. hepatica* and *F. gigantica*, faecal egg detection is easy and cheap, although it has the disadvantage that only patent infections can be diagnosed. Using traditional sedimentation methods, 10-50 g of faeces per animal can be tested and eggs identified with a dissecting microscope (Table 2, Figure 2). Taking 5 g from 10 sheep in a group to make 50 g is a convenient way of testing a pooled sample.[59] For cattle, taking 10 x 10 g samples, mixing well and testing 10 g sub sample is equally sensitive.[60] For individuals, Flukefinder® (Richard Dixon, ID, US) is a convenient way of rapidly testing 2-3 g per animal. Flukefinder® is a unit made up of two sieves and uses the same principle as sedimentation. In spite of the smaller volume of faeces, the sensitivity is comparable to traditional sedimentation [61] and offers a considerable time saving.

Table 2. Morphology of *F. hepatica, F. gigantica* and *F. magna* and their eggs

	Egg		Adult parasite	
	Appearance	Length	Appearance	Length
F. hepatica	Oval, operculated, orange. Eggs cannot be reliably distinguished.	120-164 µm[62]	Leaf shaped, dorso-ventrally flattened, *F. gigantica* more elongated	10-30 mm[63]
F. gigantica		129-204 µm[64]		30-55 mm[63]
F. magna		109-175 µm[24]	Similar but lacking anterior cone	30-80 mm[22]

Figure 2A. *F. gigantica* adult. B. *F. hepatica* adult. C *F. hepatica* egg seen under dissecting microscope. It is morphologically indistinguishable from the eggs of *F. gigantica* and *F. magna*.

(A and B used by permission of E.J. LaCourse. C used by permission of J. Graham-Brown)

Commercial ELISAs are currently only validated and marketed for *F. hepatica,* but it is likely that these cross-react with the other two species, which could limit their use in areas where more than one species co-exist. [65,66] Antibodies can be detected from 2-4 weeks post infection. [44,67] Antibody tests are more sensitive than egg detection in the early stage of infection, and can remain high for several weeks after treatment. Antibody levels do not directly correlate with parasite burden, but do give an indication.[11] On dairy farms, bulk milk antibody detection is a convenient way of screening the whole herd for *F. hepatica.* [44,55,68] A positive result indicates that approximately 25% of the herd is sero-positive.

Copro-antigen detection can detect infections slightly earlier than faecal egg counting, but performance has been variable, with a different cut-off from that recommended by the manufacturer needed to increase the sensitivity to acceptable levels. [11,69]

All three parasites can be diagnosed post-mortem by identification of parasites in the liver. In sheep and cattle with *F. hepatica* or *F. gigantica* infection (Figure 2), thickened bile ducts, liver fibrosis and scarring may be seen with either current or previous infection. In *F. magna* infection, black pigmentation in the hepatic parenchyma, lymph nodes and other tissues, necrosis and haemorrhage due to migration, and, in cattle, white fibrous capsules are seen. [23]

Epidemiology and control

Effects of climate and environment

Liver fluke only occurs in regions where conditions support the intermediate snail host, and suitable moisture and temperature levels are needed for at least three months for completion of parasite development within the snail. As a result, in many areas, only one complete life cycle takes place each year and fasciolosis is a seasonal disease. Due to the time taken for the parasite to mature within the snail, the peak infectious period begins when high numbers of metacercariae reach the pasture, which, assuming animals are present and excreting eggs onto pasture, occurs around 10-12 weeks after the snails become active. In the USA, *F. hepatica* is found in the Gulf Coast and western states, where high rainfall, poorly drained pastures and soil types that can support the intermediate host snail are found.[70–72] The weather conditions mean that snails are most active during the relatively warm winters, and hence the peak infectious period is spring, before snail numbers decline due to hot dry summer conditions.[73,74] Counterintuitively however, drought conditions can lead to higher infection levels as livestock congregate around the few remaining drinking and grazing areas. In Northern European climates where cold winter weather is the limiting factor, snails are most active during the warm summer months and infectious metacercariae on pasture peak in late summer to early autumn.

Snail numbers and hence infectious levels on pasture also vary largely between years, with wetter, milder conditions leading to more severe fasciolosis outbreaks. Changes to farming systems such as increases in pasture irrigation can introduce liver flukes to new areas.[72]

In tropical regions where *F. gigantica* is present, conditions are generally limited by moisture. In areas where the main snail habitat is rivers and lakes, peak numbers of infected snails found at the end of the rainy season. As habitats dry out and water levels drop, oxygen concentrations can become too low to support snails, but infection levels rise as animals congregate in these areas.

Man-made water supplies such as irrigation canals and reservoirs can be an ideal snail habitat, for example in the Andean highlands, Pakistan and Cambodia. [75–77] Irrigation alters the seasonality of the liver fluke life cycle by enabling a longer period of snail activity than would otherwise have occurred, or enabling two periods per year. In other cases where rainfall is very low, irrigation is the only source of water and leads to new areas of snail activity. [29]

Aspects to consider in *F. hepatica* control

1. Localised risk factors

Grazing management can reduce exposure of animals to liver fluke risk pastures. [78] On some farms, it may be possible to fence off or drain high risk areas, although this is often challenging as snail habitats can be localised, temporary and difficult to identify, or too widespread throughout the available pasture. As an alternative, avoiding grazing the high risk pastures during the most risky times of year may be possible.[79] Snail control using molluscicides is currently banned in most countries due to adverse environmental effects.

The local climate and/or timing of crop irrigation determines when the peak transmission periods are likely to be and thus when the optimal time for treatment is. In some countries, forecasting systems are available to help farmers decide when to treat. Wet weather leading to standing water

during the time of year when temperatures are above 10° C but below 30° C is the key feature of high risk years. [70]

2. The species and production type of animals present on farm.

Sheep are at risk of acute fasciolosis, therefore a drug active against immature stages may be needed at around 8 weeks after the peak snail season (However, in the USA none of the available anthelmintics have activity against the immature stages, therefore this would not be possible). An anthelmintic treatment in spring using a product effective against mature stages may help to reduce pasture contamination from mature flukes that have built up in the animals overwinter. The lack of anthelmintics that target the immature stages makes raising sheep a challenge in the areas of the USA where *F. hepatica* is highly prevalent.

Cattle are unlikely to suffer from acute fasciolosis, therefore treatment should be aimed at killing mature parasites to control chronic disease that may affect production, and to reduce pasture contamination. In warmer climates, such as in the southern USA, the optimal time for treatment is late summer or autumn.[70,71] This is the earliest time at which the parasites that infected the animals during the peak transmission time of winter and spring are likely to have reached maturity. In northern Europe, where infection occurs mainly during the summer, the most efficient time to treat is during the late autumn and winter. If animals are housed for winter, a treatment several weeks post housing can be given. A single annual treatment may be enough if timed correctly, because after the intermediate host snails become inactive, infection pressure decreases until the following season. Restrictions of treatment in dairy animals mean that treatment may only be possible during the dry period, which may not fall at an ideal time of year. Therefore, where there are several animal types present on one farm, it may be preferable to graze dairy cattle and sheep on drier land at high risk times, and allow beef suckler cattle to graze the wetter areas, as they can more easily be treated and are at less risk of acute fascioloisis.

3. Animals coming onto the farm

All incoming animals including sheep, cattle, bulls, rams and seasonal sheep should be included in the control programme, to avoid bringing in animals harbouring heavy burdens or drug resistant fluke. These animals should be quarantined, tested and treated to reduce the risk.

4. Effective use of drugs

Abattoir returns or diagnostic testing should be used to inform the need for and frequency of treatment. Several drugs are available to treat liver flukes, and these vary both in terms of the life stages of the parasite killed and availability in different countries (Table 3). Accurate dosing is important and over-use of a single product should be avoided to delay the development of resistance.

Table 3. Flukicide drugs and their availability[80–82]

Drug name	**Fluke life stage treated**	**Availability in North America**
Albendazole	10 weeks onwards	USA and Canada
Clorsulon	10 weeks onwards (Can be effective from 8 weeks but higher dose required)	USA
Closantel	7-8 weeks onwards	Canada
Nitroxynil	8 weeks onwards	No
Oxyclosanide	10 weeks onwards	No
Rafoxanide	4 weeks onwards	No
Triclabendazole	2 weeks onwards (cattle), 2 days	No

	onwards (sheep)	

5. Drug resistance

Triclabendazole resistance is now widespread in much of Europe and there also are reports of closantel resistance.[13,83–85] This is a great problem on sheep units because of the risk of acute fasciolosis. In the event of suspected treatment failure, a faecal egg count reduction test (FECRT) should be performed. This has only been validated in sheep and for triclabendazole[86], but the same principle applies for cattle and for other drugs. Though at present there is no internationally recognized standard protocol for diagnosing resistance in flukes, the following protocol has worked well in Europe. Twenty x 5 g samples are taken from a penned group of sheep, before treating them with triclabendazole. The samples are tested as two pools of 50 g each using the sedimentation method. The same group is then resampled 3 weeks later. A reduction in egg count of less than 90% between the first and second testing indicates resistance is present. An alternative is to use the copro-antigen test to check drug efficacy.[87]

F. gigantica control

Although the same drugs are effective, in many countries where *F. gigantica* is endemic they are unavailable or prohibitively expensive. Little evidence exists for the beneficial effect of these drugs on productivity. In terms of timing of treatment, in areas where snail habitats are water bodies in pastoral areas, the same principles apply as for *F. hepatica,* in terms of treating 8-10 weeks after the end of the rainy season when peak snail activity occurs. In areas where irrigated rice fields exist, treatment in advance of planting has been suggested to ensure that cattle dung used as fertiliser is free of eggs and therefore does not cause infection of snails. Treatment of cattle after they have grazed rice stubble may be most effective time to prevent chronic fasciolosis. As for *F. hepatica,* the timing of this post exposure treatment depends on whether the product is effective against immature flukes or only adults. [29]

F. magna control

In cattle, *F. magna* is not usually associated with any clinical signs, and eggs are not shed as they are unable to escape the fibrous capsule within which the parasite is contained.[38] Losses are usually confined to condemnation of the liver at slaughter. Therefore, treatment is not needed.

In sheep, albendazole (7.5 mg/kg), triclabendazole (20 mg/kg), clorsulon (21 mg/kg) and closantel (15 mg/kg) are reported to be at least partially effective against mature and late stage immature (from 8-10 weeks) *F. magna*. [88–92] Of these, albendazole is approved for this purpose in the USA. Treatment should be given 8-10 weeks after peak snail activity to kill the maximum number of flukes at the earliest possible stage. As a single *F. magna* can be fatal in sheep, and none of these drugs are completely effective, the mortality rate can be high even when treatment is given in a timely fashion. Drug treatment of wild deer has proved ineffective at preventing infection in livestock, and preventing access of deer to pasture is likely to be impractical.[74,93]

Summary

The three liver fluke species present a considerable burden to cattle and sheep farming worldwide. Effective control depends on a good understanding of their life cycles and local epidemiology.

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