



Forbes, A. (2017) Liver fluke infections in cattle and sheep. *Livestock*, 22(5), pp. 250-256. (doi:[10.12968/live.2017.22.5.250](https://doi.org/10.12968/live.2017.22.5.250))

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Deposited on: 09 November 2017

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Liver fluke infections in cattle and sheep.

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Introduction

The liver fluke (*Fasciola hepatica*) is an extraordinarily successful parasite that is found in most temperate regions of the world; in the sub-tropics and tropics it is joined or replaced by *Fasciola gigantica*. The molluscan intermediate hosts of *F. hepatica* are amphibious snails of the genus *Lymnaea* of which *Lymnaea truncatula*, now called *Galba truncatula*, appears to be the most common and widespread. In regions where *G. truncatula* is absent, other species of *Lymnaea* are competent vectors (Graczyk and Fried, 1999). The liver fluke life cycle is complex and involves several motile, developmental and infective stages. There is a wonderful description of the steps towards the discovery and piecing-together of all the free-living life cycle stages in a paper published in 1937 (Taylor, 1937). Although the free-living stages of the liver fluke life cycle are affected by topography, temperature and rainfall, the basic biology is similar, irrespective of location, however, the parasitic stages in the final hosts show greater variability.

Liver fluke infections have been recorded in a broad range of mammals from several different classes. The focus of this article is on fasciolosis in cattle and sheep, but many other species can act as final hosts for *F. hepatica*, including domestic ruminants (Reddington et al., 1986), deer (Alexander and Buxton, 1994), camelids (Hamir and Smith, 2002), rodents (Poitou et al., 1993), lagomorphs - which can act as a wildlife reservoir (Walker et al., 2011), horses (Raftery et al., 2017; Williams and Hodgkinson, 2017), swine (Mezo et al., 2013; Ross et al., 1967a) and man (Mas-Coma et al., 2005). In addition, there is a report of liver fluke infection in a bird, the emu (Vaughan et al., 1997). Though there may be some small genetic differences between *F. hepatica* isolated from sheep and cattle (Vilas et al., 2012; Walker et al., 2007), it is the same species that infects both. It is important that clinicians know of the differences in the patterns, pathology and pathophysiology of liver fluke infection between sheep and cattle in order that they can understand the likely course of disease, its treatment and control.

The initial stages of liver fluke infection

Following ingestion by the grazing animal, the metacercariae pass into the rumen, where the concentrations of carbon dioxide and temperatures of ~39°C (Dixon, 1966) initiate the dissolution of the outer cyst wall. This continues as they pass into the abomasum, where the enzyme pepsin begins to digest the cyst wall and in the duodenum where the cyst wall is further broken down by the enzyme trypsin. The final trigger for the emergence of the juvenile fluke from the cyst is a chemical present in the bile, glycocholic acid, which stimulates the fluke to make vigorous movements and escape through the partially digested cyst wall (Sukhdeo and Mettrick, 1986).

Although the studies that were conducted to elucidate the route of migration of juvenile *F. hepatica* from the small intestine to the liver were conducted in sheep (or laboratory animals) (Sukhdeo et al., 1987), it seems highly probable that this process is similar in all ruminants. The newly emerged juvenile fluke pass through the gut wall and enter the abdominal cavity where they encounter the abdominal wall and travel along its inner surface (Sukhdeo and Sukhdeo, 2002), which eventually

leads to the diaphragm, against which lies the ventral lobe of the liver, where the juvenile fluke penetrate the diaphragmatic surface of the liver capsule (Ross et al., 1966). Evidence that this is the most common route taken by juvenile flukes can be found in the pathology associated with the initial migration through the parenchyma, which are commonly found on parietal aspect of the liver lobes adjacent to the diaphragm.

Migration through the liver parenchyma

After penetrating the liver capsule, the young fluke tunnel through the parenchyma, feeding as they go, over a period of around 8 weeks until they reach the bile ducts. During this time their migrations they create haemorrhagic tracts in the liver tissue, resulting in local damage. Histological cross sections of the liver containing juvenile fluke show large numbers of red blood cells at the head end.

Acute fasciolosis in sheep

Acute fasciolosis is a specific terms used to describe a disease arising from the damaging effects of high numbers of juvenile fluke migrating through the liver parenchyma. It is thus observed 6-8 weeks post-infection. Under experimental conditions, single challenge infections of >4000 metacercariae lead to acute fasciolosis (Boray, 1967). Challenges of this magnitude result in juvenile fluke burdens in the liver parenchyma in excess of 1000 accompanied by very high mortality in sheep in the absence of treatment (Boray, 1967; Ross et al., 1967b).

There is another expression of liver fluke infection in sheep, known as sub-acute fasciolosis; in this condition, which can also be fatal, the infection has progressed to a stage where there are large numbers of juvenile flukes in the liver parenchyma, but also many adult fluke in the bile ducts. It should also be remembered that, as there is no demonstrable acquired immunity to *F. hepatica* in sheep, acute fasciolosis can be super-imposed on an existing chronic fluke infection. For example if sheep have experienced several years of low-grade infections and are then suddenly exposed to a large metacercarial challenge in a high risk year.

Parenchymal migration of juvenile *F. hepatica* in cattle

The specific syndrome of acute fasciolosis appears to be extremely rare in cattle in the field and under experimental infections 2500-25,000 metacercariae did not result in disease or mortality in calves over the risk period of 8 weeks post-infection (Dow et al., 1967). The best researched explanation for this is that in cattle with high (>1300) metacercarial challenges, the juvenile fluke get blocked in the parenchyma, fail to develop, become walled-off and eventually die and disintegrate in situ (Ross, 1965, 1967). This is not an acquired response as the cattle in the experiment were fluke-naïve. Whilst the migration of juvenile flukes through the bovine liver clearly results in some local pathology, limited or no significant adverse effects on health, appetite and growth are observed during this phase of the liver fluke life cycle in infected cattle, even under high challenge, experimental infections (Cawdery et al., 1977; Reid, 1973; Ross et al., 1966).

Residence in the bile ducts

Once the juvenile flukes have traversed the liver parenchyma, they take up residence in the bile ducts typically from about 8 weeks post infection. There the flukes feeding habits change to a diet comprising solely blood, which they ingest via their oral suckers, which attach to the lining of the bile

ducts. The flukes continue to grow and reach sexual maturity 9-11 weeks post infection, after which egg-laying commences. *F. hepatica* is highly fecund and in infected sheep can produce on average 20-25,000 eggs per day (Happich and Boray, 1969), resulting in a potential daily output of over a million fluke eggs per day in a moderately infected sheep. There is no evidence that liver fluke in the bovine host are less fecund, because adult burdens tend to be lower in cattle compared to sheep, then net output of fluke eggs per animal may be less (Dixon, 1964).

Pathology

From around 25 weeks post infection, bovine livers showed extensive pathology, typical of chronic fasciolosis in the bovine, including extensive fibrosis of the parenchyma, particularly in the ventral lobe of the liver, and enlargement of the bile ducts with associated fibrosis and calcification (Dow et al., 1967). The damage to the bile ducts is thought to result from irritation caused by the spines that are present on the fluke cuticle; it is thought that, along with the oral and ventral suckers, these spines help stabilise the fluke in the bile ducts (Behm and Sangster, 1999). In the sheep the pathology in the bile ducts is less marked and the enlargement seen is mainly due to hyperplastic cholangitis.

Longevity

In the absence of treatment, liver fluke live shorter lives in cattle compared to sheep, probably related to the bile duct pathology, described above, which makes feeding very difficult once the duct walls have thickened, fibrosed and calcified (Dow et al., 1967). Whilst fluke mortality in cattle increases over a period of 6-18 months post-infection, during which 75% of the initial burdens are lost, live fluke have been found after ≥ 26 months (Ross, 1968). On the other hand it appears that liver fluke in sheep can live and remain reproductively viable as long as the sheep lives; up to 11 years post-infection has been recorded (Durbin, 1952).

Feeding

Adult Liver fluke feed almost exclusively on blood, which is ingested from capillaries within the walls of the bile ducts (Todd and Ross, 1966). It has been estimated that each adult *F. hepatica* is responsible for a loss of 0.2 to 0.5 ml blood per day (Holmes et al., 1968). The resultant anaemia is essentially haemorrhagic and in chronic fasciolosis, packed cell volumes can be reduced to 12-15% (normal 35%) in both sheep and cattle. This can be readily detected on clinical examination and has potential to be assessed semi-quantitatively using the Famacha© method (Olah et al., 2015).

Impact of Fasciolosis

Sheep

The clinical manifestations in sheep range from sudden death in acute fasciolosis to chronic ill-thrift. In non-fatal infections; adverse effects are associated with anaemia, hypoalbuminaemia and diverse changes related to liver dysfunction (Dargie and Berry, 1979; Rojo-Vazquez et al., 2012; Sykes et al., 1980). Manifestations at farm level include inappetence, low growth rates in young animals; poor wool growth and sub-optimal reproductive performance in breeding stock (Crossland et al., 1977; Dargie et al., 1979; Ferre et al., 1994; Hawkins, 1984). In addition, fasciolosis is a risk factor for

infectious necrotic hepatitis, caused by *Clostridium novyi* type B infection, which can lead to Black Disease (Osborne, 1962).

Cattle

The mechanisms through which liver fluke affect cattle are essentially the same as those in sheep, though, in terms of expression, acute disease is extremely rare in cattle, in which chronic infections are the norm. In both species, the lack of a functionally effective acquired immunity means that the impact of fasciolosis is seen as much in adult animals as in youngstock (Cawdery et al., 1977; Charlier et al., 2014). Several studies have highlighted the potential impact of liver fluke infection on reproductive performance and milk yield (Charlier et al., 2007; Howell et al., 2015; Lopez-Diaz et al., 1998; Oakley et al., 1979).

In cattle, fasciolosis is a risk factor for *Salmonella dublin* infection on dairy farms (Vaessen et al., 1998), though the results of co-infection experiments are equivocal (Aitken et al., 1978; Hall et al., 1981). Another important effect of *F. hepatica*, which has been explored much more in cattle than in sheep, is its role in immunomodulation (Naranjo Lucena et al., 2017). Of particular interest are the interactions between *F. hepatica* and *Mycobacterium* spp. in cattle, both in terms of susceptibility to infection (Garza-Cuartero et al., 2016), but also in relation to the effects on the responses to the intra-dermal skin test (Claridge et al., 2012; Flynn et al., 2007), which is the standard screening test for bovine tuberculosis in the United Kingdom (UK) and elsewhere.

Implications for treatment and control

Treatment

Of the flukicidal products currently licensed for use in cattle and sheep in the UK, only one – triclabendazole administered orally – has claims against juvenile fluke from 2 weeks of age onwards (Boray et al., 1983). Therefore it becomes not only the drug of choice for acute fasciolosis in sheep, but essentially it is the sole option. Unfortunately triclabendazole resistance has been reported from many parts of the world and appears to be relatively widespread in the UK, though precise estimates of incidence are not available to date. When resistance does appear, triclabendazole appears to be ineffective against all stages of *F. hepatica*, including adult parasites (Brennan et al., 2007).

Three other flukicides – closantel, nitroxylnil and rafoxanide - have efficacy claims against late stage juvenile flukes 6-8 weeks of age (Fairweather and Boray, 1999) and these are the only options for treating acute fasciolosis where triclabendazole is no longer effective. In terms of pharmacokinetics, these three drugs are highly bound to plasma proteins (Fairweather and Boray, 1999) and it seems likely that their activity is triggered when the juvenile fluke enter the bile ducts and feed exclusively on blood. This would explain the variation between efficacy studies in terms of the time of onset of action, which may depend on the entry of fluke into the biliary system; this in turn means that efficacy against even late parenchymal stages may be quite limited.

One solution to treatment, which has not yet been fully exploited is the synergy exhibited by various flukicidal combinations (Fairweather and Boray, 1999); these have been shown to provide enhanced activity against juvenile fluke and also isolates that are resistant to triclabendazole. In Australia, one such product has been licensed; it is a three-way combination of ivermectin (200 mcg/kg), nitroxylnil (10 mg/kg) and clorsulon (2 mg/kg) in an injectable formulation. The efficacy claims include activity

against all ages of fluke from 2-week old juveniles upwards and against TCBZ-resistant isolates (Hutchinson et al., 2009), however, at present it is only licensed for use in cattle.

The treatment of chronic infections comprising adult fluke in both sheep and cattle is relatively uncomplicated insofar as all licensed flukicides have efficacy against these stages; in addition, all are effective against triclabendazole-resistant liver fluke (Coles et al., 2000; Coles and Stafford, 2001; Hanna et al., 2015). One theory as to why resistance appears to be extremely uncommon in flukicides other than triclabendazole is that the juvenile fluke stages may be considered in refugia, that is to say they are not exposed to discriminatory doses of drug and therefore selection pressure for resistance is low. Of course there are also refugia in snails and on pasture.

Because chronic fasciolosis is the most common presentation of liver fluke infection in cattle, the options are diverse and include several formulations and combinations, as farmers may have preferences for certain types of administration, particularly in heavy animals (Webster et al., 2008). Treatment of cattle at housing with flukicides of different profiles can provide good clinical efficacy and allow good growth performance, irrespective of spectrum of activity (Forbes et al., 2015).

Control

Strategic treatments for the control of fasciolosis in both sheep and cattle are greatly facilitated by having flukicides that kill virtually all stages present in the animal at the time of treatment as this will extend the period until egg reappearance to 9-12 weeks and therefore reduce the frequency of administration required to minimise pasture contamination (Parr and Gray, 2000). Unfortunately, it may be this pattern of use that has led to the emergence of triclabendazole resistance as treatments at or close to the pre-patent period can be highly selective for anthelmintic resistance (Falzon et al., 2014). There is a plausible argument that triclabendazole should not be used strategically and be used only in sheep with acute fasciolosis in an attempt to preserve its efficacy. Because of the irregular spatial and temporal patterns of acute fasciolosis, therapeutic use is unlikely to select strongly for resistance.

Because of the relative resilience to the migrations of juvenile fluke in the liver shown by cattle, they may be used to graze fields in the late summer/early autumn when high numbers of metacercariae may be present after a warm, wet summer, when it would be dangerous to graze sheep (Ross and Todd, 1968). Cattle grazed in this way could be treated with a flukicide from 8 weeks onwards after grazing the high-risk ground to remove the potentially more threatening bile-duct-dwelling late juvenile stages and adult fluke (Forbes, 2017).

Conclusion

Whilst there are a number of common features of liver fluke infection in sheep and cattle, there are a number of differences and clinicians can benefit from an understanding of this, particularly in relation to susceptibility, clinical manifestations, treatment and control.

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