Liver fluke – an overview for practitioners

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ABSTRACT

Fasciola hepatica, the common liver fluke, is an important cause of production loss in cattle. Prevalence has increased in recent years and there is a growing awareness of its importance, particularly in dairy cattle. This review considers recent developments in diagnosis, the economic implications of infection, our current understanding of triclabendazole resistance and prospects for vaccine development.

KEYWORDS: liver fluke; *Fasciola hepatica*; cattle; production loss

INTRODUCTION

Fasciola hepatica, the common liver fluke, is a ubiquitous parasite affecting the health and welfare of cattle. Fluke infection costs the UK agriculture somewhere in the region of £300 million per year due to production losses; liver condemnations alone cost over £3 million per year (http://www.eblex.org.uk/). Evidence from various sources suggests that the prevalence of infection has increased considerably in recent years for a variety of reasons including changing climate, changing farming practices and increased animal movements. There are growing concerns about triclabendazole (TCBZ) resistance in sheep and the limited availability of products that can be used to treat dairy cattle; moreover fluke have the capacity to modulate the host's immune system, affecting susceptibility to and diagnosis of other pathogens including bovine tuberculosis.

This review will briefly describe the biology and life cycle of the parasite before summarising latest developments in diagnosis, anthelmintic resistance and vaccine trials. More information can be obtained from http://www.cattleparasites.org.uk/

LIVER FLUKE BIOLOGY

Mature *F. hepatica* are large, leaf-shaped trematodes; about 3 to 5cm in length and 1cm in width. They are hermaphrodite; each individual fluke can self fertilize and cross fertilize. Both the juvenile and the adult stages of the parasite feed by secreting enzymes, most notably cysteine proteases, which break down blood and the liver tissue. The parasites are covered in microscopic spines, which irritate the walls of the bile ducts causing hyperplasia of the bile duct epithelium.

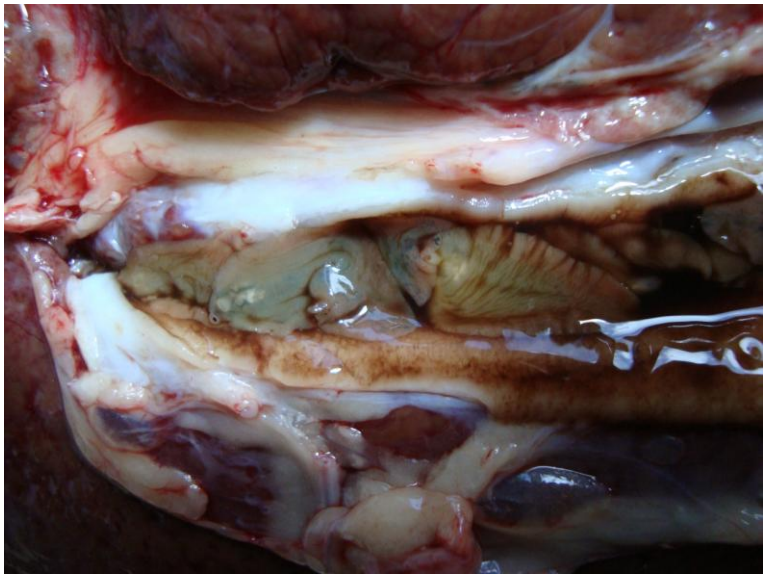

Figure 1. Adult fluke in the bile ducts of a cow.

F. hepatica are able to parasitize a range of animals. Sheep and cattle are the main hosts in UK, but deer and hares can also be infected and the University of Liverpool's diagnostic services have received an increasing number of cases of *F. hepatica* infection in horses over recent months (www.testapet.ac.uk; http://www.liv.ac.uk/diagnosteq/). In other parts of the world, *F. hepatica* causes disease in llamas, alpacas, donkeys, buffalo and humans. The World Health Organization considers *F. hepatica* to be an important threat to human health in some developing countries (http://www.who.int/neglected_diseases/diseases/en/). As far as we know, whilst fluke are genetically very heterogeneous, they can be transmitted between all these different hosts, meaning that infection can spread from sheep to cattle and vice versa.

LIVER FLUKE LIFE CYCLE

Liver fluke have an indirect life cycle involving a snail intermediate host. In the UK the principle species is *Galba truncatula,* the dwarf pond snail.

Undifferentiated fluke eggs are passed out in the faeces of infected animals and once washed out of the faeces, the eggs start to develop. When a fully developed

egg is given stimuli of increased light and temperature, the short-lived miracidium is released. It requires water to swim through and once it finds a snail, it burrows through the foot and into the body cavity. The fluke multiplies and, after about 6 weeks, cercariae are released. A snail infected with a single miracidium can produce several thousand cercariae, which are released over a period of time, probably several days. The cercariae encyst on the vegetation to form infective metacercariae. When a grazing animal eats contaminated herbage, the metacercariae hatch, the newly excysted juveniles burrow through the gut wall and migrate into the liver. In cattle it takes about 10-12 weeks for flukes to reach the bile ducts, mature and start producing eggs that can then be detected in faeces.

EPIDEMIOLOGY

For the fluke life cycle to occur, normally *G. truncatula* must be present. Other species of snail such as *Radix* spp can support parasite development, and may replace *G. truncatula* in upland, peaty environments (Relf et al 2009). Snails feed on algae on the surface of mud; hence they prefer slow moving bodies of water and a neutral pH. They also need calcium and other minerals for good shell growth. Snails are normally found on mud around the edges of ponds, streams, hoof prints and tractor ruts in muddy fields.

During the winter months, snails go into hibernation and the development of any stages of the parasite that are still in the snail at the start of winter will be arrested. In spring as the weather warms up, snails come out of hibernation and the parasite life cycle resumes. When winters are mild, fewer snails will perish and more will be present in the spring, ready to become infected as eggs develop and hatch. The rate of egg development is dependent on temperature; above 10^{o}C, development takes two to four weeks and the warmer the weather, the more rapid the development. Little development occurs in the winter when temperatures fall below 10^{o}C, but undifferentiated eggs can survive on pasture for several months, even if temperatures drop below freezing (Smith et al unpublished results).

Fasciolosis is a seasonal disease in the UK; most of the development of the free living and intramolluscan stages occurs between May & October and if the weather conditions are ideal over the summer months, large numbers of metacercariae are released from snails onto the pasture in August, September and October. Acute fasciolosis is not normally reported in cattle, but cattle that acquire infection with large numbers of metacercariae in the autumn, can develop chronic disease associated with the adult flukes in the bile ducts. This is traditionally seen in late winter and early spring and can occur in housed cattle if they have not been treated.

If weather conditions are less favourable, for example if the summer is hot and dry, then development of both snail and parasite is slowed; the result is fewer metacercariae on the pasture in the autumn and their release from snails is more gradual. Cattle become infected with smaller numbers of metacercariae; they may not develop overt clinical disease but effects on production become evident.

Whilst there is a peak in the number of infective cysts on pasture from the late summer into autumn, low numbers of metacercariae can be present on pasture all year round as they can survive on pasture for months given the right conditions. Also snails that have carried the infection over the winter can release metacercariae onto the pasture when they come out of hibernation in the spring. Cows grazing at risk pasture, even early in the season, can pick up infection. This rarely leads to clinical disease but these animals pass eggs that will develop and infect snails, perpetuating the infection on the pasture. There is little evidence that cattle develop immunity or resistance to fluke infection. Infection can be picked up at any time and animals can be repeatedly infected.

Metacercariae of *F. hepatica* remain viable on the pasture for several months, particularly in cool, damp conditions. Laboratory studies suggest that metacercariae survive for up to a year at temperatures between 0^{o}C and 20^{o}C but at temperatures above 20^{o}C they lose infectivity rapidly. Similarly metacercariae survived freezing between 0^{o}C and -20^{o}C and survived diurnal freezing and thawing (Boray and Enigk, 1965).

DIAGNOSIS

Diagnosis of infection is important to establish if fluke is the cause of disease or production loss. Abattoir reports are useful in establishing if fluke is present on the farm. Other herd tests should be regarded as a first step in investigating the presence of fluke on a farm and are useful monitoring tools when a program of control is implemented.

For individual cows, two diagnostic tests are used widely: faecal egg counts (FEC) and antibody detection ELISAs that can be used with milk or serum. FEC lack sensitivity, particularly in cattle and can only detect patent infection. Diagnostic sensitivity ranges from 30-70% depending on the amount of faeces used; increasing the quantity of faeces analysed to over 30g per animal can increase sensitivity to 90% (Rapsch et al 2006). AHVLA offers sedimentation tests on individual animals using 40g of faeces (http://www.defra.gov.uk/ahvla-en/tests-and-services).

An ELISA that detects antibody in serum and milk samples can detect early, pre-patent infection, from 2-4 weeks after infection, but serum antibodies are known to

persist for 4-10 weeks after treatment, so a positive result does not prove that an infection is actually present, just that the cow has been exposed to the parasite (Salimi-Bejestani et al 2005a). The sensitivity of most antibody-detection ELISAs is high, ranging from 86-100%, with specificities ranging from 83-96% (reviewed by Charlier et al 2014).

More recently a copro-antigen detection ELISA has become available based on a monoclonal antibody to fluke cathepsins (Mezo et al 2004). This test forms the basis of the copro-antigen tests marketed by Bio X Diagnostics, Belgium and also Biobest Diagnostics, Edinburgh. In sheep this test is useful for detecting infection two to four weeks before eggs are detected in faeces and as a means of detecting triclabendazole resistance but it has been less well evaluated and appears to be less sensitive in cattle (Gordon et al 2012a; Philip Skuce, personal communication).

In individual animals, liver enzymes, gamma-glutamyl transferase (γ-GT) and glutamate dehydrogenase (GLDH) may be raised but this is not pathognomic.

For dairy herds, bulk tank ELISAs are used routinely to establish the presence of infection within the herd, they indicate high, moderate, low or no infection and can be done three or four times a year to monitor levels of infection and efficacy of control programmes (Salimi-Bejestani et al 2005b). Several milk testing labs have the capacity to test routinely for fluke.

For beef herds, composite faecal egg counts can be informative. Normally at least 5g of faeces are required from each of 10 animals, and a single sedimentation assay carried out. This reduces the cost of diagnosis but can only provide information about whether the parasite is present within the herd and prompt further investigation. All the caveats about individual egg counts apply to composite egg counts. The copro-antigen ELISA appears unsuitable for composite faecal samples in either sheep or cattle (Skuce, personal communication).

Other factors should be taken into account when diagnosing infection including herd history, time of year and the weather conditions over the summer months. NADIS provide useful monthly updates on fluke risk for regions of GB but it is important to bear in mind that risk will vary between individual farms within an area depending on the farm environment and management practices.

PREVALENCE OF INFECTION IN CATTLE

The most recent data on prevalence of *F. hepatica* infection in cattle stems from abattoir returns, VIDA data and bulk milk tank analysis for dairy herds.

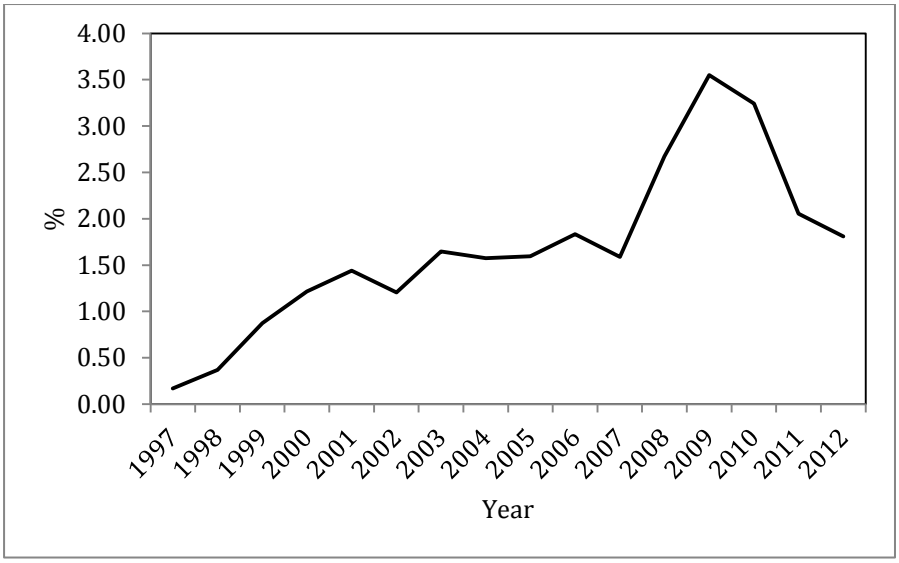

Figure 2: Diagnoses of fasciolosis in cattle as a % of total number of submissions for which a diagnosis was reached.

In a study of over 3000 dairy herds conducted in 2006/7, 72% of herds in England and 84% of herds in Wales showed evidence of exposure to *F. hepatica* (McCann et al 2010). In a more recent study (autumn of 2012) of 606 high yielding dairy herds supplying one of the UK's major supermarkets, 79.7% of herds had positive bulk tank ELISA results (Howell, unpublished results). The most recent Food Standards Agency data show that 27% of cattle livers were condemned due to *F. hepatica* at slaughter in the UK (Table 1) and an average of 2.7% of total bovine submissions to the AHVLA over the last four years (2009-2012) were diagnosed with fluke (Figure 2: http://www.defra.gov.uk/ahvla-en/category/publications/disease-surv/vida/).

Table 1: Summary of data on liver condemnation in cattle from abattoirs in Britain for 2013 (http://www.eblex.org.uk/)

	Total cattle throughput	*Fasciola* infected cattle	
		Number	Percentage
England	1,493,149	318,211	21.3
Scotland	474,499	157,740	33.2
Wales	142,933	39,215	27.4

ECONOMIC IMPACT OF FLUKE

When cattle have high worm burdens, damage to the liver is more severe and cattle may develop chronic fasciolosis and classically show loss of weight, condition and develop anaemia. A lower fluke burden may result in sub-clinical infection, which affects growth rates, carcass composition, fertility, and in dairy cattle reduced milk yields and changes in milk quality. When considering control of fluke in cattle, it is

often difficult to persuade farmers that it is worthwhile investing in control since the effects of infection, although persistent, may be subtle.

The true economic burden associated with fluke infection is not well described. Charlier et al (2008) suggested that a fluke burden of more than 10 flukes was associated with raised γ-GT and detectable liver damage. A recent study of 606 high yielding dairy herds in Britain, showed a significant ($p<0.001$) negative association between *F. hepatica* exposure and milk yield at the herd level; herds with the highest levels of exposure had a 15% lower yield compared to herds with low exposure (Howell et al unpublished results). A study in Belgium showed that closantel treatment at drying off increased milk production by 303kg over a 305 day lactation (Charlier et al 2012). The effect of fluke infection on milk quality and fertility is less well defined, several studies show differing effects, confounded by differences in farming systems (reviewed by Charlier et al 2014).

Impact of fluke infection is poorly quantified in modern beef farming systems and much more research is required to fully evaluate the cost of fluke to the British beef industry. Some recent data from ADAS suggests that infection costs between £30-£200 per animal and cattle take an extra 80 days to reach market weight (http://www.eblex.org.uk/)

Cattle infected with *F. hepatica* are thought to be more susceptible to other infections including *Salmonella Dublin* and *Clostridium* spp. (Vaessen et al 1998). There is also recent evidence to suggest that the diagnostic SICCT test for bovine tuberculosis may be compromised in fluke infected cattle (Claridge et al 2012).

CONTROL

Control of fasciolosis in cattle depends on the type of enterprise, the history of the herd and the level of challenge, which will vary from year to year depending on the prevailing weather conditions. Each control program should be tailored to a particular farm and consider the whole farm, including adult cows but also sheep including tack sheep and young stock, bearing in mind that wild life can also act as reservoirs of infection. Control usually depends on a combination of drug treatment to reduce sub-clinical economic losses and disease, treatment to reduce contamination of pasture with eggs and grazing strategies that avoid heavily contaminated pasture, particularly in the autumn. The aim should be to reduce the level of infection on a farm rather than complete elimination, which is probably unrealistic. For most farmers implementing a rational control program is a balance between the cost and the economic benefits obtained. This will differ between enterprises – control of even modest infections may have a big impact in high

yielding dairy herds but less intensively managed herds may see fewer benefits, particularly if fluke burdens are low.

There is a range of products that can be used to treat cattle for fluke. When recommending treatment plans it is important to use drugs that target the stage most likely to be present within the animal at the time, to help reduce selection pressure. Triclabendazole is the only product that is effective against the very early stages, from two weeks after infection to adults. Closantel is partially effective against 3-8 week old fluke and fully effective against adult fluke; nitroxynil is effective against fluke from 8 weeks post infection and clorsulon, oxyclozanide and albendazole are effective against adult flukes. There are a restricted number of products licensed for use in dairy cattle. Albendazole has a 60 hour milk with-hold and oxyclozanide has a 72 hour with-hold. Fasinex 240 can be used at the start of the dry period but milk for human consumption can only be taken from 50 days after treatment (http://www.noah.co.uk/ ; http://www.cattleparasites.org.uk/).

Quarantine dosing should be considered, especially if animals are bought from known fluke endemic areas, such as South Wales, North West England and West Scotland. Treatment with TCBZ should be avoided if possible but it is important to recognize that other products will not kill all the immature stages so a follow up treatment 4-6 weeks later will be required.

ANTHELMINTIC RESISTANCE

Resistance to triclabendazole was first described in the UK in the late 1990's and has now been reported on numerous occasions in fluke populations affecting sheep (reviewed by Fairweather, 2005). Exactly how common TCBZ resistance is in different regions of the UK not known, but anecdotally it appears to be highly prevalent in fluke populations in sheep rearing areas. One of the problems in assessing the prevalence of TCBZ resistance is the lack of validated tests that can be used in the field. In collaboration with AHVLA, we recently described the validation of a faecal egg count reduction test (FECRT) using composite samples from sheep and showed that of 25 farms investigated, seven showed evidence of drug failure (Daniel et al 2012). The majority of those farms (6/7) were from South Wales. Subsequently (autumn 2013), working with a farmer's co-operative in North West England, we demonstrated evidence of drug failure on all 13 farms participating in the study and on eight of those farms, treatment had no effect at all on the egg count (Kamaludeen et al unpublished results).

One question that is often asked when interpreting FECRT data is whether failure is due to the inability of a fluke-damaged liver to metabolize the drug into its active forms rather than true anthelmintic resistance (Fairweather 2011). In our

experience, when we have purchased sheep, isolated parasites that survived drug treatment, passaged them through snails and infected new sheep that were then treated with TCBZ, the fluke survived treatment demonstrating true resistance (Hodgkinson et al 2013). Others agree and suggest that the FECRT is a useful test for demonstrating true drug failure in the field (Jones et al 2014; Gordon et al 2012b)

In the Netherlands, TCBZ resistance was described in both sheep and cattle on 14 farms from the same province in 2005. A follow up study on one of those farms, after no TCBZ had been used in the intervening three years, showed that there had been no reversion to susceptibility (Borgsteede et al 2005).

In Britain, there are fewer reports of resistance to TCBZ in fluke populations in cattle, which may reflect the less intensive use of TCBZ in cattle. However resistance was described in 2010 in Scottish beef calves and is becoming more evident as awareness increases (Sargison et al 2010). It is important that farmers are warned of the risk of buying in animals carrying resistant fluke populations and take appropriate advice about quarantining animals particularly if coming from fluke endemic parts of the country.

As far as we are aware there are no published reports of resistance to closantel, clorsulon or nitroxynil in the UK although albendazole resistance has been reported in Spain and Sweden and clorsulon resistance implicated in a study in Spain (Alvarez-Sanchez et al 2006; Novobilsky et al 2012; Martinez-Valladares et al 2014).

PROSPECTS FOR A VACCINE

In view of the difficulties in controlling *F. hepatica* effectively and sustainably, a vaccine would be a major advance. There have been several vaccine trials in recent years, evaluating a range of fluke molecules. Experimental trials suggest that Cathepsin L1, L2, fatty acid binding proteins, glutathione transferase, peroxiredoxin and a *Schistosoma mansoni* derived antigen, Sm14, all have potential as vaccine candidates and elicited between 50-80% protection both in reducing fluke burdens and egg output (Dalton et al 2013). However there have been few trials where significant levels of protection have been achieved against natural challenge. Calves vaccinated with recombinant Cathepsin L1 and exposed to natural challenge in Ireland, showed a 48% reduction in worm burden after 13 weeks exposure but total worm burdens were low suggesting modest challenge (Golden et al 2010). Other trials are underway by a large consortium of groups, funded by the European Union (PARAVAC; http://paravac.eu). An effective vaccine is probably some years away; progress is hampered by a lack of suitable adjuvants and the need to test multi-component vaccines to try and increase the levels of protection achieved. Moreover we have yet to fully understand the impact of the parasite's ability to profoundly

polarize the host immune response, preventing protective immune responses developing to *F. hepatica* and possibly to other pathogens as well (reviewed by Dalton et al 2013).

CONCLUSIONS

Liver fluke is a common parasite that affects the productivity and welfare of cattle and sheep. Control is confounded by the lack of cheap, accurate, animal-side diagnostics, issues surrounding use of drugs in milking cattle and anthelmintic resistance. It is likely that changes to the UK's climate will significantly alter the epidemiology and transmission of the parasite over the coming decades. Research is urgently needed into vaccine development, better understanding of the mechanisms of TCBZ resistance and identification of new drug targets. The availability of an annotated, whole genome map of *F. hepatica* (Hodgkinson et al 2013) will accelerate developments in these areas, but better advice on control of the parasite at the farm level is also imperative.

Additional information on the sustainable control of *F. hepatica* is available at http://www.cattleparasites.org.uk

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