Department of Primary Industries
primefact

Liver fluke - a review

May 2017, Primefact 813 third edition
Stephen Love, Veterinarian / Research Officer - Parasitology, Sheep Unit, Armidale

This Primefact gives an overview of liver fluke, including a review of recently published information. Also see NSW DPI Primefact 446, '*Liver fluke disease in sheep and cattle'*, by eminent fluke expert Dr JC Boray, for more information and many helpful images.

Summary

Liver fluke (*Fasciola hepatica*) is a parasite of the liver. It affects a range of animals including livestock (and occasionally humans).

Adult fluke live in the bile ducts where they produce eggs which are passed out in the faeces. Under warm, moist conditions, the eggs hatch and the resulting larvae infect intermediate host snails.

Figure 1. *Austropeplea (Lymnaea) tomentosa* - the most common intermediate host in Australia and New Zealand

Image credit / source: Dr JC Boray

After developing and multiplying in snails, larvae are released and attach to herbage in wet areas. Animals get infected while grazing this herbage. The ingested larvae leave the intestines of the host and migrate to the liver, through which they wander for several weeks until they reach the bile ducts and become adults.

Liver fluke cause liver damage, anaemia and even death in severe cases. The risk of black disease is also increased (preventable by vaccination).

Control is by grazing management (restricting access of animals to wet, 'flukey' areas) and strategic treatments. One to three treatments with fluke drenches may be required, with the most important being in early winter.

There are some cases of fluke resistant to flukicides ('fluke drenches').

Tests available include a liver fluke egg count, a blood test (ELISA) for antibodies, and a test for fluke antigens in the faeces (coproantigen ELISA). These tests can be used to see if fluke are present in livestock, and to check if a treatment for fluke was effective.

Introduction

Liver fluke is a parasite affecting a range of livestock and other species. *F. hepatica* is the only *Fasciola* species in Australia. *F. gigantica*, which is common in nearby south-east Asian countries, does not occur in Australia.

Figure 2. Liver fluke (*Fasciola hepatica*) from sheep

Image credit / source: Dr JC Boray

Final hosts in which liver fluke can develop to sexual maturity include livestock such as sheep, cattle, horses, pigs, goats, alpacas and deer. Other species include kangaroos, wallabies,

rabbits, and humans. Animals other than sheep and cattle are not considered to be important hosts of *F. hepatica* unless, perhaps, eradication is attempted (Barger and others, 1978).

The wide range of mammalian hosts favours the maintenance and dispersal of the parasite (Barger and others, 1978).

People can be infected, for example by eating water cress from creeks in fluke-infested country. The World Health Organisation considers *F. hepatica* to be an important threat to human health in some developing countries.

Millions of sheep and cattle in Australia graze pastures where liver fluke is endemic, mainly in south eastern Australia (Boray, 2017).

Economics

Liver fluke disease (fasciolosis) costs millions of dollars each year. A recent estimate of the annual cost of fluke to the Australian sheep industry was about 25 million dollars (Lane and others, 2015).

Globally, the parasite is estimated to cost €2.5 billion per year (Mazeri and others, 2016). Costs arise from lost production, stock deaths, and treatment and prevention. Liver fluke contributes to internal parasitism in general being ranked as one of the most economically important diseases of grazing livestock.

As with many worms, most of the economic cost of fasciolosis is from production losses due to more or less inapparent infections. Significant losses can occur before eggs are detectable in the faeces, which happens 2-3 months after initial infection.

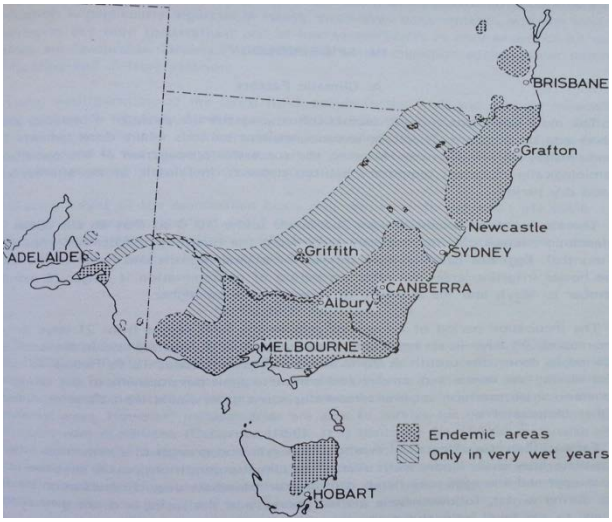
Liver fluke can reduce milk production in dairy cattle by approximately 4-30%, and also lower herd fertility, depending on intensity of infection and the nutritional status of animals (Elliott and others, 2015).The thresholds for economic losses in dairy cattle are reported to be >25% of animals in a herd being infected, or approximately 30-40 flukes per animal, possibly as low as ten flukes in dairy cattle (Kelley and others, 2016).

Fanke and others (2017), in a study of German dairy cattle farms, found reduced milk yield in multiparous cows were the major reason for annual production losses due to gastrointestinal nematodes (GIN)(€13.33 per cow) and *F. hepatica* infections (€7.95 per cow). Added to this were annual costs for anthelmintic treatment against GIN infections in adult cows (€10.00 per cow), and *F.hepatica* infection associated annual costs due to repeated artificial insemination (€10.13 per cow) and prolonged calving intervals (€9.40 per cow).

Incidence

The incidence of fasciolosis in the UK has reportedly increased in the last decade, with the distribution also changing to include previously drier areas. Reasons given for the changing epidemiology include climate change, increasing animal movements and resistance to the flukicide, triclabendazole (Mazeri and others, 2016. Also see Haydock and others, 2016).

Figure 3. Distribution of fasciolosis in Australia



Source: Barger and others, 1978; adapted from Boray, 1969

Distribution of liver fluke in Australia

Liver fluke occurs in regions, and areas on individual farms, where the environment favours fluke eggs, the intermediate host snails and larval fluke.

Liver fluke is mostly limited to the higher rainfall (>600 mm annually) areas of

- NSW (typically the tablelands in the eastern part of the state, and nearby coastal areas to the east and slopes to the west)
- Victoria and Tasmania
- small areas in Queensland and South Australia.

Liver fluke may also be found in irrigation areas.

Life cycle and biology

Adult liver fluke, which live in the bile ducts of the livers of affected final hosts, are large and leaf shaped, 30-50 mm long and 10 mm wide. The bile ducts thicken (epithelial hyperplasia) in

response to microscopic cuticular backwardly-projecting spines covering the fluke.

Liver fluke are hermaphrodites (they have testes and ovaries) and are able to self- and cross-fertilize. Daily egg production has been estimated at 20,000-50,000, over a long period in sheep. Egg production declines in cattle as resistance develops after infection (Boray, 2017; Barger and others, 1978). The eggs are passed through the bile ducts, gallbladder, then into the intestines, finally being shed in the faeces. These undeveloped (undifferentiated) eggs can survive on pasture up to several months, even under freezing conditions.

The eggs develop (differentiate) and hatch in wet areas on pasture when mean daily temperatures are over about 10 degrees Celsius. The incubation period ranges from about 1.5-3 weeks in summer, up to 13 weeks in spring and autumn (Boray, 2017; Barger and others, 1978) but time intervals quoted by other sources vary. According to Taylor and others (2016), the optimal temperature range is 22-26 degrees C.

The microscopic 'motile ciliated' larvae (miracidia) only live for a few hours after emerging from the egg. They swim through water and burrow through the foot of intermediate host snails and into their body cavity. These are 'lymnaeid' snails, most commonly *Austropeplea (Lymnaea) tomentosa* in Australia and New Zealand and, in the UK and Europe, most commonly the 'mud snail', *Galba (*syn. *Lymnaea) trunculata*, an amphibious snail with a wide distribution throughout the world (Taylor and others, 2016). *A. tomentosa* is a very efficient intermediate host, and can spread rapidly whenever physical and climatic factors are favourable (Barger and others, 1978).

(According to Ponder and others (2016), the *Austropeplea* genus needs to be revised. Further name changes for these lymnaeid snails are possible. Also see Lloyd and others, 'Identifying liver fluke snails' (Primefact 476, revised 2017) for more information).

Multiplication rates of snails, and the fluke larvae within, varies with temperature. Under most Australian conditions, the snails produce eggs, which then hatch, throughout most of the year, with reproduction rates lowest in winter, but increasingly markedly in spring through to autumn. Snails produce up to 3000 eggs a month and one generation of snails from egg to egg takes only about one month under optimal conditions. *Austropeplea tomentosa* survives in dry mud for at least one year, and tolerates low temperatures. The snail can move with and against the water current for long distances. (Boray, 2017; Barger and others, 1978). During winter months in colder areas, the snails hibernate, and development of fluke present in the snail is arrested until warmer conditions resume in spring.

Figure 4. Cercariae leave an infected snail-*Austropeplea tomentosa*

Image credit: Dr JC Boray, 2017

Once inside the snail, the fluke develop and multiply asexually through stages (sporocysts, rediae, daughter rediae, and cercariae). Larval development within the snail takes from one month (25 degrees) to 3 months (15 degrees). Hundreds up to several thousand cercariae can result from a single miracidium (Boray, 2017; Barger and others, 1978). Cercariae are released from the snail over a short period of time, probably several days (Williams and others, 2014).

The tadpole-like cercariae leave the snails, swimming until they attach to vegetation, on which they 'encyst' (i.e., form a tough protective cyst wall), becoming metacercariae, the infective stage ('infective cyst') of liver fluke.

Some research shows metacercariae (infective cysts) on herbage can remain infective for several months (up to 9-10 months) if conditions are cool and damp. Laboratory studies indicated they can withstand freezing (-20 to 0 degrees C), and diurnal freezing and thawing (Boray and Enigk, 1965), but lose infectivity rapidly as temperatures rise above 20-25 degrees. Their survival depends on moisture and moderate temperatures. Metacercariae will not survive for more than six weeks at 25 degrees, but can survive for eight weeks at temperatures of -2 degrees. It has been estimated, for example, that 50% of metacercariae will survive a normal UK winter. Heat and drought will kill metacercariae (COWS, 2016).

Regarding survival in hay or silage, research findings are variable, with some stating

metacercariae survive for 'shorter periods' in low-moisture, well-cured hay, and die after 5-8 weeks of being ensiled in silage (Kelley and others, 2016). Others say it is unlikely that metacercariae will survive in silage, and that the survival of metacercariae in hay is equivocal, it not being clear if metacercariae will survive in hay produced under normal farm conditions. However, freshly cut grass should be regarded as a potential source of infection if harvested from fluke-contaminated pasture (COWS, 2016).

When grazing animals ingest metacercariae, immature flukes 'excyst' or 'hatch' in the duodenum then penetrate the wall of the small intestine, and make their way to the liver. Upon entering the liver, the young fluke are 1-2 mm long and lancet-like (Taylor and others, 2016).

The migration to the liver is sometimes misdirected, and ectopic flukes can be found in the lungs, especially in cattle (Radostits and others, 2007).

The immature fluke migrate through the 'fleshy' part (parenchyma) of the liver for about 6-8 weeks, destroying tissue and growing from 1.0 to 10 mm (Radostits and others, 2007). The time for transit to the liver (possibly several days), then through the liver on the way to the bile ducts, varies.

Both juveniles and adults feed by secreting enzymes (proteases) which break down blood and liver tissues (Williams and others, 2014). Production losses can be significant if not clinically apparent during the migration phase of juvenile flukes, hence the importance of triclabendazole, given its efficacy against 'all stages' (2 weeks and older) of susceptible liver fluke.

Fluke then enter the small bile ducts, and migrate to the larger ducts and, occasionally, the gall bladder. In the bile ducts, they mature (doubling in size (Radostits and others, 2017) and begin to produce eggs. The prepatent period, the time from initial infection until eggs are produced, is usually 2-3 months (10-12 weeks in cattle), depending on the fluke burden. The minimum period for the completion of one entire life cycle is about 17 weeks. More typically it takes 18-30 weeks (Mazeri and others, 2016).

Adult flukes may live in the bile ducts of sheep for years. According to Merck (2017), most are shed from cattle within 5–6 months. However, Radostits and others (2007) imply cattle can be carriers for longer periods.

Immunomodulation

Liver fluke can modulate the host animal's immune system, possibly increasing susceptibility to other infections, for example *Salmonella* and *Clostridia* spp. Also recent evidence suggests diagnosis of bovine tuberculosis may be compromised in fluke-infected animals (Williams and others, 2014). Thankfully Australia is free of bovine TB.

Host factors

Horses and pigs are relatively resistant to infection by liver fluke. Horses overcome migrating fluke early, so that few reach the liver. Pigs resist migrating fluke once they have entered the liver (Radostits and others, 2007).

In sheep, there is no evidence of any acquired resistance to *Fasciola hepatica*. Acute and chronic fasciolosis can occur at any age (Boray, 2017); however adult sheep that are reinfected may suffer less liver damage (Barger and others, 1978).

Cattle have a natural resistance and, under normal conditions, clinical disease is only likely in young cattle. However, this resistance, which allows chronically infected cattle to spontaneously recover, and previously infected animals to partially resist reinfection, is due to a more intensive tissue reaction than in sheep, for example. This response results in a fibrous mechanical barrier (fibrosis) against re-infection, by impeding the usually preferred migration of young flukes into the ventral lobe of the liver. The subsequent enlargement (hypertrophy) of the right lobe helps the host survive by leaving sufficient undamaged liver tissue. In chronic cases, mineralisation (calcification) of, and fibrosis around, bile ducts also causes the elimination of liver flukes. Calcification tends to be minimal or absent in sheep (Boray, 2005; Boray, 2017).

Despite cattle being more resistant to liver fluke disease (fasciolosis) than sheep, it is only possible because of chronic fibrotic changes in the liver, as already mentioned. One source (http://cattleparasites.org.uk) states there is little evidence that cattle develop immunity as such to fluke infection. Infection can be picked up at any time and animals can be repeatedly infected. However, even with a small number of fluke present, there may be production losses (Boray, 2017) if not overt disease.

Grazing behaviour is another important factor when considering fluke disease. Cattle willingly graze wet areas including fluke habitats at any time, while sheep and goats tend to graze them

only when other feed is scarce. A 'classic' time for clinical fasciolosis to appear in sheep in NSW, Australia is a dry autumn following a good spring and summer. Prior to autumn, numbers of metacercariae (infective cysts) on pasture have been increasing, while a dry autumn results in sheep grazing fluke habitats in search of green pasture.

Liver fluke disease (fasciolosis)

Fasciolosis should be considered when there are deaths, anaemia or ill thrift in sheep or cattle that are or have been grazing on fluke-prone country (Boray, 2017).

Another common cause of deaths and anaemia, especially in goats, sheep and alpacas, is haemonchosis, caused by barber's pole worm (*Haemonchus* sp). Anaemia and ill thrift due to 'eperythrozoonosis' (mycoplasmosis), caused by the blood parasite *Mycoplasma (Eperythrozoon) ovis*, is another possibility in young sheep.

Liver fluke disease can be acute, sub-acute or chronic, depending on the size or intensity of the infection and how quickly it is acquired. Disease is due to haemorrhage and tissue damage from migrating immature fluke, and from damage to bile ducts and blood loss due to adult fluke. In cattle, less so in sheep, the duct walls become greatly thickened and often calcified (Merck).

Acute fasciolosis: death may occur, with or without abdominal pain, jaundice and anaemia. This is due to large numbers of immature, wandering flukes destroying liver tissue and causing haemorrhage. Acute disease is seldom seen in cattle (Barger and others, 1978).

Sub-acute fasciolosis: jaundice, ill thrift, anaemia, and possibly death after several weeks. Liver damage and haemorrhage is exacerbated by the reparative tissue reaction which prolongs fluke migration and causes further mechanical damage (Barger and others, 1978).

Chronic fasciolosis: the most common form in sheep, goats and cattle, and particularly in more resistant hosts, such as horses and pigs (Boray, 2017). The slowly developing clinical signs include anaemia, loss of appetite, and 'bottle jaw' (submandibular oedema, resulting from low blood protein), largely due to fluke in the bile ducts. Bile ducts thicken and the liver becomes cirrhotic (scarred). Chronic fasciolosis can occur at any time but is often most evident in autumn and winter.

In sheep, fluke burdens of more than 100 fluke are potentially lethal, the sheep dying from chronic fasciolosis. One thousand fluke generally cause death from acute or sub-acute disease (Barger and others, 1978).

Black disease (infectious necrotic hepatitis): This is an acute and fatal liver disease of ruminants such as sheep, especially, but also cattle. It is caused by a toxin produced by the bacterium *Clostridium novyi*. Tissue destruction by wandering flukes may create a microenvironment favouring activation of clostridial spores (Merck, 2016). Black disease is preventable by vaccination.

Figure 5. Cross section of fibrotic sheep liver with heavy chronic fluke infection

Image credit: Boray, 2017

Treatment and prevention

Control is by means of grazing management and strategic treatment with flukicides.

Flukicides are anthelmintics that are effective against liver fluke. See Table 1.

Extra 'tactical' treatments may be required, as indicated by diagnostic tests, along with clinical signs and loss of productivity.

In the future, with further research and improved diagnostic tests, targeted selective treatments (TST) (see below) may be an additional option.

Grazing management

As 'flukey' areas are confined to certain parts of a farm, grazing of these areas can be managed or even precluded. For example, grazing by the most vulnerable stock, sheep, goats, alpacas and young cattle, can be minimised.

Boray (2017) discusses a rotational grazing program once recommended in Australia to eliminate infection. However, the system was never widely adopted.

In this system, an effective drench is used about two weeks before moving stock to potentially contaminated areas. The second step is to alternate the grazing between fluke-infected areas and fluke-free areas.

Grazing in infected areas is restricted to a period of six weeks, which is less than the time for fluke

entering the liver to reach maturity and produce eggs.

In fluke-free areas, animals are grazed for longer periods. Here, any fluke picked up on the fluke-infested paddocks reach the adult stage (certainly by 12+ weeks after infection) but are removed by drenching about two weeks before stock are moved back to contaminated pastures.

The main obstacle with this strategy was the difficulty in organising pasture rotation and the problems of moving fences or erecting new fences.

However, Boray (2017) argued the system could be easily applied to properties where only a small number of paddocks had suitable snail habitats. In mixed grazing properties the more resistant cattle could be grazed on the known fluke-prone areas, on the basis that these animals are less likely to be affected and thus requiring less treatment.

Strategic treatments

Strategic treatments can help reduce liver fluke populations. One to three treatments may be needed per year, depending on the severity of the problem.

Early winter treatment

April-May is the most important strategic fluke drench in southeast Australia. At this time, the most effective fluke drench is the treatment of choice, partly because burdens could be heavy, and made up of a mix of adult and immature fluke. This means a triclabendazole (TCBZ)-based drench (optimally an oral formulation and optimally triclabendazole plus oxfendazole).

In cattle two other similarly effective flukicide products are available in Australia. These are the injectable products containing the unrelated flukicides, clorsulon and nitroxynil: Nitrofluke®, and also Nitromec®, the latter having a third ingredient, the broad-spectrum active, ivermectin.

As always, read and follow instructions on product labels.

Early spring treatment

The next most important strategic drench, if required, is in spring, around August-September. Adult flukes are likely to dominate infection at this time so a drench other than triclabendazole should suffice, resistance excepted. Using a non-TCBZ drench at this time means TCBZ is used less frequently, hopefully resulting in less selection for resistance to this active. Also, given that most non-TCBZ flukicides are only effective against adults +/- late immature fluke, a proportion of the fluke (juveniles < 6-8 weeks old) in the animal *possibly* will not be selected for resistance.

In beef cattle, if TCBZ was used in early winter then a nitroxynil plus clorsulon combination could be used in early spring (or summer), and vice versa.

Summer treatment

Properties badly affected by fluke could well need a third strategic drench, in summer.

Dairy cattle

Boray (2017) says this about treatment of dairy cattle:

“Treat young heifers and dry cows with a suitable anthelmintic effective against immature fluke, i.e., triclabendazole, and follow the above plan for beef cattle.

Products registered for use in lactating cows (oxyclozanide plus levamisole, clorsulon plus ivermectin, and clorsulon) are only effective against adult fluke aged 12-14 weeks or older. (The registered product that contains clorsulon alone is not currently available).

If paddocks on your property are heavily contaminated and are being grazed, you may need to treat lactating cows monthly during summer and autumn. Products to use are oxyclozanide plus levamisole, or ivermectin plus clorsulon, both these being registered for use in lactating dairy cattle. Either of these drenches also controls susceptible gastrointestinal nematodes, as well as lungworm infections on the occasions they are a problem.

On heavily contaminated pastures, good control of fasciolosis may require a triclabendazole treatment immediately after drying off, as well as a month before calving. The two treatments may especially be warranted if a less effective flukicide is used. If the pre-calving treatment is considered necessary (check with your advisor), consider the possibility that the estimate of calving date may be incorrect, or the cow may calve early, either of which could result in the treatment being too close to calving, with consequent residue issues.

Note also that some products containing triclabendazole can be used in dairy cattle, but with restrictions, whereas some (but not all) triclabendazole-based products which also contain a broad-spectrum active may be precluded from use in animals that are producing or may produce milk products for human consumption. Assume nothing; check the label”.

Targeted selective treatment (TST)

The current approach to managing liver fluke, as described in this Primefact, consists of grazing management, strategic treatments, and added 'tactical' treatments as necessary.

Future hopes, in no particular order, might include vaccines (see below), improved diagnostic tests, TST and novel anthelmintics.

In recent decades, there has been increased interest in using targeted selective treatment (TST) to manage internal parasites of grazing livestock, given that most of the parasites in a group of animals tend to be carried by a minority of animals. The 'trick' is accurately and economically identifying animals that need treatment.

The principle is that good parasite control can be achieved by treating the minority that need it, with an added potential benefit of reduced selection for anthelmintic resistance.

With a minority rather than a whole group of animals being treated, a greater proportion of the parasite population is 'in refugium' (plural, 'refugia'), i.e., not exposed to the anthelmintic, and therefore not selected for resistance to that anthelmintic.

A well-known example of this is use of the FAMACHA© system (van Wyk and Barth, 2002), a non-invasive method of detecting anaemia, in the control of the haematophagous ('blood-eating') nematode *Haemonchus contortus* (barber's pole worm) in small ruminants.

Liver fluke (*Fasciola hepatica*), of course, is also a 'blood-eater', and anaemia is commonly, if not invariably, a feature of fasciolosis (liver fluke disease).

With this in mind, Olah and others (2015) investigated the potential use of FAMACHA as a tool to enable selective treatment of chronic fasciolosis in sheep. The research was done on 288 sheep in the UK in winter, when fluke burdens are mostly mature. Measurements included FAMACHA score (eye colour), fluke counts, nematode egg counts, and packed cell volume (PCV) (haematocrit), which is a simple lab test to detect anaemia. The authors found a strong a correlation between FAMACHA score and fluke burden in their study, but these variables were only moderately correlated with PCV.

Olah and others (2015) concluded that their study furnished proof of principle for the use of FAMACHA as a decision support tool to guide TST in chronic fasciolosis in sheep. However, they did discuss various issues, including the fact that there are other causes of anaemia in small (and large) ruminants. Another issue is the risk, in untreated sheep, of acute or sub-acute fasciolosis, in which clinically evident anaemia may not always be a feature.

The hope remains however, that future research will produce improvements in sustainably managing fasciolosis, possibly including TST.

Monitoring and diagnosis using tests

The clinical and economic importance of fasciolosis or liver fluke disease (first reported in 1379) has been recognised for centuries, but diagnostic tests are far from perfect (Mazeri and others, 2016. See Table 2).

Regular monitoring for liver fluke infections should be done. For monitoring, and for diagnosis in the face of clinical disease, testing options include:

Liver fluke egg counts

This requires the collection of faecal samples and obviously only detects the presence of adult flukes. The oval, 'operculated' (having a lid or cap), golden brown eggs (130–150 × 65–90 microns) must be distinguished in the lab from those of paramphistomes (stomach flukes), which are larger and clear (Merck). The sensitivity (ability to detect true positives) is not high and ranges from 30-70% (Woodgate and others, 2016; Williams and others 2014), depending on the amount of faeces used. Specificity (ability to detect true negatives) is close to 100%, if technicians are skilled at distinguishing fluke eggs from other, similar eggs. Fluke egg counts do not give much information on the size of the fluke burden in the animal.

Composite (bulk) faecal egg counts can be informative, in beef herds for example. Dung samples are collected from 10 animals and a number of composite egg counts are performed at the lab. This indicates whether the parasite is present in the herd and allows further investigation. However, fluke egg shedding can be intermittent and the absence of eggs does not necessarily mean that animals are fluke-free, as they may be harbouring immature liver fluke (COWS, 2016).

The egg counting method for liver fluke is different from that used for gastrointestinal nematodes (roundworms). The latter have less dense eggs, which float in solutions such as saturated salt (NaCl solution (specific gravity

(SG) of ~1.2), commonly used in laboratories as a flotation solution. Fluke eggs will float in some saturated solutions (e.g., zinc sulphate) that have a higher SG, but labs more commonly use a sedimentation technique for counting fluke eggs (Hutchinson, 2009; RVC (year unknown)).

Antibody-detection ELISA

Blood samples are used for this test and, in the case of dairy cattle, milk samples. The sensitivity and specificity of most antibody-detection ELISAs (enzyme-linked immunosorbent assays) is high, ranging from 86-100% and 83-96% respectively (Williams and others, 2014), and the test has the advantage of detecting infection early, from about 4 weeks post-infection. However, antibodies can remain detectable for up to 12 weeks or more after successful treatment for fluke (Hutchinson, 2003; Brockwell et al., 2013, cited by Woodgate and others, 2016).

Bulk tank milk ELISAs are routinely used in dairy herds around the world to establish if a herd has been exposed to fluke. They indicate high, moderate and low levels of exposure and can be done routinely to monitor infection and efficacy of control programs (COWS, 2016).

Coproantigen ELISA (faecal fluke antigen ELISA or cELISA)

This relatively new test is based on a monoclonal antibody to enzymes (proteases known as cathepsins) produced by liver fluke. The test, which employs a European diagnostic kit (BIO-X Diagnostics, Belgium), is available from NSW DPI's veterinary laboratory at Menangle, and the Charles Sturt University veterinary laboratory at Wagga Wagga, NSW. It has been validated in Australia for use in cattle at least, tests showing a good correlation between coproantigen ELISA results and liver fluke burden, i.e., the test gives a good indication of intensity of infection.

Testing of bulk (combined or composite) samples instead of individual samples may be investigated to reduce cost of testing. Brockwell and others (2013), found in their studies on cattle that the coproantigen ELISA still detected a low infection in one animal when its faecal sample was mixed with up to 4 negative samples.

The test has shown high sensitivity (few false negatives) and specificity (few false positives) (Elliott and others, 2015).

According to Williams and others (2014), the test detects infections 2-4 weeks before eggs appear in the faeces.

Or, to put it another way, the test does not detect fluke until 5 or more weeks after infection (Brockwell and others, 2013). Generally it detects fluke from 6-9 weeks (cattle and sheep) post-infection, i.e. about the time fluke are in the bile ducts.

Samples can be frozen and tested later, thus allowing batching of samples. Various researchers have found that the coproantigen test is not adversely affected when done on samples from sheep and cattle that were previously frozen (Brockwell and others, 2014)

Regardless of whether fresh or previously frozen samples are used, coproantigen levels in samples collected at different times from individual animals can vary (2-6 fold) from day to day (Brockwell and others, 2013). A similar day-to-day variation occurs when doing fluke egg counts.

Other

Liver enzymes (GGT (increases with bile duct damage) and GLDH) in the blood may also be raised, but these are not specific to fluke-related liver disease.

Other tests for liver fluke, for example the 'LAMP' assay, may be options in the future. (LAMP (loop-mediated isothermal amplification) is a very specific, efficient, and rapid gene amplification procedure in which the reaction can run at a constant temperature, and avoids some of the drawbacks of the standard PCR (polymerase chain reaction) (Martínez-Vallada and Rojo-Vázquez, 2016).

Other blood tests can detect anaemia, jaundice and hypoproteinaemia (low blood protein), but clinical examination of affected animals often detects these signs as well. And then of course there are post mortem findings: liver pathology consistent with fluke disease, with or without the presence of the fluke themselves.

Parasites such as *Haemonchus* (barber's pole worm), the blood parasite *Mycoplasma (Eperythrozoon) ovis*, mineral deficiencies (copper, cobalt) and other conditions need to be considered when investigating possible fluke disease.

Measuring productivity, e.g. weight gains, milk yield, is useful for many reasons, one being that it can be an indicator that parasites are possibly having an impact.

Genetics aside, the two most important causes of ill thrift in grazing livestock are parasites and poor nutrition.

Whatever lab test is used, good times to test are April, August, and January, or thereabouts, or

when disease or production losses from liver fluke are suspected.

More information on lab tests

http://www.dpi.nsw.gov.au/about-us/services/laboratory-services/veterinary/liver-fluke

Flukicides – efficacy and resistance

Resistance to flukicides has been reported in various countries, including Australia. See Table 1.

Triclabendazole, which has been used since the 1980s, is the only flukicide active available for sheep and cattle that is effective against early immature, immature and adult fluke (>98% effective against adults), at least when oral formulations are used. In dairy cattle, it is only used at drying off time due to its long milk withholding period.

Other single-active flukicides are only effective against stages in the bile ducts, i.e., adults (>10-12 weeks old) or adults as well as late immature stages (>8 weeks or > 6-8 weeks old, depending on the product). The lowered efficacy of a number of the salicylanilides (e.g. closantel) and substituted phenols (e.g. nitroxynil) against juvenile flukes – compared, say, to triclabendazole - may be due to the high protein binding of these drugs in the blood (Merck Veterinary Manual).

The **nitroxynil/clorsulon combination**, available only for cattle in Australia, is also effective against early immature fluke (see table 1), unlike either of the actives (nitroxynil or clorsulon, at usual dose rates) acting on their own.

Boray and De Bono (1989) published **the first reports of *Fasciola hepatica* resistance** to flukicides under field and laboratory conditions. They surveyed sheep farms in Australia and, on 60% of the farms, found resistance (markedly reduced efficacy compared to susceptible strains) of 6 week old fluke to the salicylanilide **rafoxanide**, with side-resistance to **closantel**, which is also a salicylanilide. These fluke strains retained their resistant status in cattle. There was also cross-resistance to **nitroxynil**, which is a phenol; however resistance was manifested in immature fluke, rarely in adults. There was no side resistance to **oxyclozanide**, even though it, like rafoxanide and closantel, is a salicylanilide (Fairweather and Boray, 1999). According to the UK's Veterinary Medicine Directorate, there have been no reports (as of September 2016) of resistance to oxyclozanide.

Kelley and others (2016; see Table 1), cite Novobilský and Höglund (2015) as the only confirmed case of **closantel** resistant fluke. This case was in cattle in Sweden, and related to a topical (pour on) formulation containing closantel and ivermectin. However, Boray and De Bono (1989) reported closantel resistant liver fluke almost 30 years earlier (sheep and cattle, Australia).

The first confirmed case of **TCBZ** resistance in the world was in Victoria, Australia, reported by Overend and Bowen (1995).

In 1999, Fairweather and Boray said resistance was not a major issue, but proposed various ways of managing it, including grazing management, use of combinations (of unrelated flukicides) in particular, and other strategies.

Now, however, according to Kelley and others (2016), the situation worldwide is serious, largely due to TCBZ resistance becoming common in many countries.

Monitor flukicide efficacy

If using an oral triclabendazole (TCBZ)-based formulation in a susceptible liver fluke population, and since TCBZ kills most stages of fluke in host animals, a significant reduction (>95%) in egg count or coproantigen (faecal fluke antigen) ELISA should occur when testing post-treatment (Kelley and others, 2016).

(Note that a flukicide under current Australian standards is classed as 'effective' when the efficacy is >90% (>95% in the case of gastrointestinal nematodes)).

The other products in Australia that kill early immature, immature and adult flukes are the two injectable formulations for cattle that contain the nitroxynil/clorsulon flukicide combination (+/- ivermectin).

Apart from that, other flukicides on the market are only effective against adult fluke, or late immature as well as adult fluke.

Tests useful for checking flukicide efficacy:

- Fluke worm egg count. (Faecal samples required)
- Liver fluke antigen ELISA (coproantigen test). (Faecal samples required).

The antibody ELISA (which detects serum antibodies to fluke antigen), although very useful for the early detection of infection, is of no value in field resistance testing because the antibodies

persist long after treatment (Brockwell and others, 2013).

Following are some ways of checking flukicide efficacy:

Method 1

Do an egg count on day zero (the day animals are treated with a flukicide), then again 21 days later. (Some advisors prefer day 28. Wood and others (1995) suggest day 21).

Method 2

This is a variation on the first method.

If testing an adulticide (a chemical that only kills the adult stage), move livestock to a fluke-free paddock and leave there for at least 12 weeks. This ensures that all of the liver fluke in the animals become adults prior to treating and testing (Elliott and others, 2015). Along with the first method, this approach is also suitable for testing triclabendazole (TCBZ). Even though it is effective against all stages of fluke (early immature, immature, adult), when resistance does occur to this active, all stages are affected (Boray, 2005b).

Method 3: more intensive testing

An alternative is to do more intensive sampling, as done by Elliott and others (2015) in their research work. Their plan was to sample on-farm at days 0, 14, 35, 57 and 77. (Sampling was meant to happen at day 14, but weather conditions pushed this out to day 17).

The sampling 14 days post-treatment was to check for efficacy against adults. If using egg counts, a potential disadvantage of sampling at this time is that there could still be eggs remaining in the gall bladder, even if all adults had been killed by the treatment. Regarding the coproantigen test, various authors (see below) report that this test usually becomes negative by 14 days after successful removal of fluke, but some still suggest testing at day 21 is preferable.

After the sampling at 2 weeks post-treatment by Elliott and others (2105), the subsequent sampling times (approximately 5, 8 and 11 weeks post-treatment) were to check efficacy against immature stages. While this or similar protocols may be used in a research setting, it could be too onerous to do on-farm when initially testing effectiveness of flukicides.

Further comments on testing for resistance

Unfortunately, testing flukicide efficacy is less straightforward then checking for resistance of roundworms to broad-spectrum drenches.

Novobilský and Höglund (2015) discuss various issues relating to testing for flukicide resistance. A problem with fluke egg counts is the irregular release of *F. hepatica* eggs. Also they state that the commercial coproantigen ELISA can give both false-negative (Gordon et al., 2012; Novobilský and others, 2012; Brockwell and others, 2013) and false-positive results (Flanagan and others, 2011), making interpretation of flukicide efficacy complicated, especially under field conditions. Finally, they say there is an urgent need for standardised protocols for evaluation of flukicide efficacy, especially for thresholds applied for egg counts and the coproantigen ELISA.

Brockwell and others (2014; page 52) discuss advantages of testing at 21 days post-treatment, It allows time for:

- removal of adult fluke post-treatment and
- removal of eggs in the gall bladder, and
- for immature fluke surviving treatment to mature to antigen production. (If use of the coproantigen test is contemplated).

A disadvantage is the long wait.

Brockwell and colleagues then discuss the ‘pros and cons’ of testing earlier than 21 days, e.g. 7 days or 14 days post-treatment, the tests being faecal fluke egg counts, and/or the coproantigen ELISA test. Brockwell and others (2013) found both egg counts and coproantigen levels fell to zero or negative values within 7 days of successful TCBZ treatment of *artificially* infected cattle. Flanagan et al (2011) reportedly used a 14 day interval for egg counts and the coproantigen test in sheep and found antigen levels generally fell to zero by 14 days post-treatment. For those interested in testing albendazole’s efficacy against liver fluke, Novobilský and others (2012) state a 7 day interval may not be appropriate when testing albendazole (possibly because it takes some time for the fluke to die?).

Alas, misdiagnosing resistance may even happen on occasions when testing at 21 days, but for different reasons. For example, when adulticides (drugs that only kill adult fluke) are tested, and the re-test is done 21 days after treatment, the egg counts and fluke antigen may not always fall to zero, even if the treatment killed all adults. This is because the adulticide is not effective against young fluke, and some of these may mature sufficiently over the 21 days to produce eggs and antigens at the time of retesting (Kelley and others, 2016). Method 2 above, which allows all fluke to mature to adulthood, gets around this problem.

Novobilský and Höglund (2015), citing various authors, again remind us that 'lack of flukicide efficacy' does not necessarily mean resistance (Fairweather, 2011b).Under-dosing, inadequate storage of the anthelmintic, metabolic changes, improperly applied anthelmintic and co-infection with rumen flukes may also explain observed treatment failure (Fairweather, 2011b; Skuce and Zadoks, 2013; Hanna et al., 2015)". They further state that lack of standardised guidelines, including treatment thresholds, for resistance in *F. hepatica* at times makes identification of 'true resistance' and 'treatment failure' uncertain.

Managing resistance

To manage resistance and avoid undue reliance on drenches, consider the following:

Monitor fluke burdens and productivity

Check for liver fluke infections and their effects by testing for fluke and monitoring productivity of animals in order to avoid unnecessary drenching.

Use flukicides well; consider combinations

Use products correctly, according to label, and at the right dose rates.

Use effective flukicides, which necessarily means there will be regular testing to monitor flukicide efficacy.

As discussed above, under 'Treatment', rotate between unrelated flukicides or, probably better, use combinations of unrelated flukicides when possible, as advocated by Fairweather and Boray (1999). Admittedly, in the absence of good data, we are extrapolating from evidence relating to broad-spectrum drenches in sheep. This evidence suggests combinations of effective and unrelated actives are more successful at delaying resistance than using single-active drenches sequentially (the same drench each time stock are treated, possibly until the drench fails) or in some sort of rotation (for example, drench A on this occasion, with an unrelated drench (drench B) being used on the next occasion).

But we have limited options when it comes to available combination flukicides, so we have to settle for best rather than perfect choices.

Most importantly, good decisions about rotations or combinations cannot be made until you determine, by testing, what flukicides are effective on your property.

Grazing management

Use grazing management to reduce unnecessary exposure of vulnerable animals (small ruminants, alpacas, young cattle) to liver fluke, and so reduce the number of treatments needed.

Quarantine

A quarantine strategy helps avoid importing drench-resistant fluke through animals brought on to your farm. This is important if your farm has liver fluke, or if you farm is fluke-free but is able to support the parasite because the 'right' snails are present. Even if liver fluke cannot establish on your property, it is good, for reasons of health and productivity, to 'clean out' fluke-infected animals which have been brought in. (In sheep at least, liver fluke can live for years).

Bear in mind other biosecurity issues relating to imported animals, including drench-resistant roundworms, and other pests and diseases.

Ideally use a combination flukicide that is effective against all stages of fluke. The ones available in Australia are triclabendazole plus oxfendazole, with the additional option in cattle of flukicides containing nitroxynil plus clorsulon. Be aware, however, that resistance has now been reported to many of the available flukicides.

If a flukicide only effective against adult +/- late immature fluke is used, a follow-up treatment 6 – 10 weeks later (depending on the drench that was used) will be required to remove juvenile fluke surviving the first treatment.

Or, hold the cattle for 12 weeks on the quarantine paddock, assuming it is fluke-free. This allows all fluke to mature. Then treat once with the drench, followed by a test 3 weeks later to make sure the treatment was effective.

It is best to keep imported cattle in a 'non-flukey' quarantine paddock for long enough to complete treatments and to allow testing after the quarantine treatment. This will also aid in the management of other pests and diseases potentially imported with the livestock.

With triclabendazole resistance, all stages of fluke are affected, so a fluke egg count to check efficacy could be done as soon as 3 weeks after the quarantine treatment, as well as on the day of treatment.

The faecal antigen test is an alternative and may well be the test of choice in this situation as it is more sensitive – better at detecting true positives – than the fluke egg count. This test is also done on the day of treatment and 21 days later.

Figure 6. Calf with bottle jaw (submandibular oedema) due to liver fluke

Image credit / source: Boray JC, 2017

Vaccines?

Kelley and others (2016) state that there is no commercially available liver fluke vaccine. However, there are several experimental vaccines for livestock being developed. No vaccine has consistently shown efficacy at a level (>60%) in cattle to warrant commercial production, although one vaccine in sheep (the leucine aminopeptidase (LAP) vaccine) has shown high efficacy, up to 89%.

However, the authors state that a vaccine with only partial efficacy (50–60%) may still provide economic benefits to producers experiencing infection with TCBZ-resistant fluke. The benefits will depend on the intensity of fluke infection in a herd. But remember that economic losses in dairy cattle can occur with just 30–40 flukes, possibly as few as ten.

Following pages: appendix, including tables and notes.

Appendix – tables and extra notes

This section contains further information, which may be of interest to professional advisers and producers wishing to delve deeper.

Table 1. Flukicides available for sheep and/or cattle worldwide, including notes on efficacy and resistance

Family	Active ingredient	Route of admin.	Safety index[B,F]	Age of fluke killed[B]	Age of fluke killed[K]	Reports of resistance on-farm worldwide[K]
Benzimidazole derivative	**Triclabendazole** (TCBZ) and combinations	Oral; pour-on	20 (TCBZ)	≥ 2 weeks	≥ early immature	30 cases (TCBZ)
Benzimidazole	**Albendazole** (higher does rate for liver fluke than nematodes)	Oral; intra-ruminal	6.0	≥ 12 weeks	Adult	3 cases
Salicylanilide	**Closantel**	Oral; injectable; pour-on	5.3	≥ 8 weeks	≥ late immature	1 case
Salicylanilide	**Oxyclozanide** (only available in AU combined with levamisole)	Oral	4.0	≥ 12 weeks	≥ adult	NR
Substituted phenol	**Nitroxynil** (only available in AU combined with clorsulon +/- ivermectin)	Injectable	4.0	≥ 10 weeks	≥ adult	1 case
Sulphonamide	**Clorsulon** (only available in AU as a combination: +/- nitroxynil +/- ivermectin)	Oral; injectable	5.0	≥ 12 weeks (Inject.)	Oral, ≥ late immature; Injectable, ≥ adult	3 cases
(Combination)	**Nitroxynil** + **clorsulon**				≥ early immature	NR
(Combination)	**Closantel** + **oxfendazole** (no longer available in AU)		5.3	≥ 6 weeks		

Notes on table and additional notes:

Table adapted from Boray and others (2007, updated 2017), Fairweather and Boray, 1999, and Kelley and others, 2016. (See references).

Sources: B=Boray and others, 2007. F= Fairweather and Boray, 1999, K= Kelley and others, 2016. **Label claims** for efficacy can vary as a result of differences in formulation and regulatory requirements in different countries.

AU = Australia. **Boray's notes** on efficacy (age of fluke killed) relate to the Australian standard which defines >90% as 'effective' in relation to drugs acting on *Fasciola hepatica* (c.f. 95% for roundworms).

'Fluke killed': This means >90% killed, although efficacy may be higher. For example triclabendazole given orally kills >95% of susceptible adult *F. hepatica*.

'**Reports of resistance':** these are peer-reviewed published reports. Added to this there are also 'anecdotal' reports, with varying degrees of confirmatory evidence. **Resistance misdiagnosed:** Sometimes resistance is misdiagnosed. If faecal egg counts are used, this could be due to fluke eggs still remaining in the gall bladder at the time of the post-treatment test. Another possible cause of

misdiagnosis, in the case of adulticides, is flukes that were immature (not laying eggs) at the time of testing, became mature enough to produce eggs at the time of the post-treatment test.

NR = not reported.

Albendazole (ABZ): the recommended dose rate for roundworms in sheep in Australia (AU) is 3.8mg/kg live weight; for liver fluke it is 25% higher (4.75 mg/kg). To kill liver fluke, Bowman (2003) suggests 4.75 -7.5 mg/kg (sheep) and 10 mg/kg (cattle), both orally, but using this dose may constitute an 'off-label' use in AU. If using ABZ (not registered in AU for use in alpacas) in crias (young alpacas), especially at higher than label-recommended does rates, consider that albendazole toxicity in crias has been reported.

Combination here means a combination of two or more unrelated flukicides. The currently available combinations in Australia are TCBZ + oxfendazole (OFZ), and nitroxynil + clorsulon (+/- ivermectin). (OFZ-based drenches generally have no claim for liver fluke, but the literature (Boray) indicates OFZ is effective against 16 week old fluke). Previously closantel + OFZ (Closicomb®, Rotafluke®) was available in AU, but no longer. This was more effective than closantel alone, or closantel + albendazole (Fairweather and Boray, 1999; Boray and others, 2007). See notes below on 'synergism'.

All the anthelmintics (apart from Closicomb®) **in the table** are available in Australia, but not all formulations, e.g. an oral formulation may be available and not the injectable, or vice versa. Some actives are only available as part of a combination. Some products or actives are registered, e.g. clorsulon alone; nitroxynil alone; but are not commercially available).

See: https://portal.apvma.gov.au/pubcris.

Resistance: Boray and De Bono (1989) surveyed farms and found resistance of 6 week old fluke to the salicylanilides **rafoxanide** and **closantel**, but these fluke were susceptible when they became adults (8-10 weeks after infection). Fairweather and Boray (2011), citing Boray and De Bono (1989), stated there were rafoxanide and closantel resistant *F. hepatica* on sheep farms in endemic areas of Australia; these retaining their resistance status in cattle. (However, Kelley and others (2015; see Table 1), cite Novobilský and Höglund (2015; cattle, Sweden) as the one confirmed/reported case of **closantel** resistant *F. hepatica.* Perhaps it was, for cattle?). (In the Novobilský and Höglund (2015) case, a topical (pour on) formulation containing closantel and ivermectin was used. The authors state that, according to pharmacokinetic data, **closantel** in this topical formulation achieves higher concentrations in plasma than when administered orally or subcutaneously, 'confirm(ing) that administration route cannot be associated with closantel failure in this study, but also observe that, for closantel, no comparison between the efficacy of per oral, subcutaneous and topic formulations has yet been available (2015). They further note that **oral applications of TCBZ** are often superior to pour-on applications (Hutchinson et al., 2009; Martin et al., 2009). (See also Sargent and others on variable efficacy of pour-on formulations between seasons (winter, spring, summer).

TCBZ resistance: The first reported case of **TCBZ resistance** in the world was from Victoria (Pyramid Hill), Australia (Overend and others, 1995). TCBZ resistance of *Fasciola hepatica* has been reported (peer-reviewed reports) in sheep and cattle in Australia. Before 2011, there were peer-reviewed reports of TCBZ resistance in livestock on only six properties in Australia, Scotland, Wales, The Netherlands, Spain, and the Republic of Ireland. Since then, TCBZ-resistance has been reliably reported in sheep or cattle on a further 24 properties in Northern Ireland, Scotland, Wales, Australia, New Zealand, Peru, and Argentina (Kelley and others, 2016). According to Elliot and others (2015), TCBZ resistance is now 'widespread in cattle in southeastern Australia'. In a study of 15 dairy farms in Gippsland (a major dairy area), Victoria, these authors found 6 ex 15 farms had cattle infected with liver fluke. One farm had TCBZ resistance but clorsulon or oxyclozanide successfully removed TCBZ-resistant adult flukes.

In Australia, apart from flukicide **resistance** to **TCBZ**, resistance, according to Fairweather and Boray (1999), has also been found to **closantel** and **rafoxanide** (both salicylanilides), with cross-resistance to the phenol, **nitroxynil** (manifested in immature fluke, rarely in adults), but no resistance to oxyclozanide, although it is a salicylanilide. (Note that, nitroxynil is usually only registered with a claim of efficacy against adult fluke). According to the UK's Veterinary Medicine Directorate, there are still **no reports of resistance to oxyclozanide** (as of Sept., 2016). Boray (2005b) states that, when TCBZ-resistance occurs, it is exhibited by all ages of fluke (2 weeks old and older) in the host.

Human infections with TCBZ-resistant *F. hepatica* have been reported in The Netherlands, Chile, Turkey, and Peru (Kelley and others, 2016).

Prenatal infections: Liver fluke have been found in Victoria in calves as young as 1-3 weeks old, which suggest prenatal infection had occurred (Rees and others, cited by Cole VG, 1986).

Liver fluke age groups. Early immature: 1–4 weeks, (migration stage, in the parenchyma ('meaty' or functional part) of the liver; **late immature:** 6–8 weeks (prepatent stage in bile ducts); **adult**: 12–14 weeks (bile duct stage) (Wood and others, 1995). There are obvious gaps in these definitions. Also occasionally authors refer to 10 and even 8 week old fluke as adults, with the younger age (8 weeks) generally/seemingly most often in the context of discussing liver fluke in small ruminants.

Nitroxynil: This is still registered as Trodax®) for subcutaneous use in cattle in Australia, but is not currently available. However nitroxynil is available as a component of two injectable cattle products in combination with clorsulon in one of these products, and in combination with ivermectin + clorsulon, in the other. Both of these products are effective against fluke 2 weeks old and older. Nitroxynil is not effective if given orally (it is degraded by ruminal microorganisms), so is given by injection (Merck).

Combinations and synergy: Use of combinations, whether or not there is synergy between the individual actives, is believed to be helpful in managing resistance (at least in nematodes of sheep). As to synergy, Fairweather and Boray (1999) reported Australian studies on synergy between various unrelated actives against liver fluke. (Synergy: the cooperative action of two or more drugs, resulting in a different or greater response than that of the individual drugs).

Combinations for which Fairweather and Boray (1999) or Fairweather (2011) reported synergy:

- TCBZ + clorsulon, or closantel, or luxabendazole, or artemether, or artesunate, or oxfendazole.
- Nitroxynil + clorsulon, or closantel.
- Closantel + clorsulon, or luxabendazole (strong synergy), or oxfendazole (moderate synergy).

No synergy was found with closantel plus albendazole or closantel plus fenbendazole or TCBZ plus OZ78.

Fairweather (2011) also reports synergy of TCBZ plus ivermectin (IVM), and clorsulon plus IVM, with IVM not acting directly on fluke but as an inhibitor of P-glycoprotein (Pgp)-linked drug efflux pumps, over-expression of which has been linked to resistance to some anthelmintics.

Some drugs are no longer available (luxabendazole (Hoechst)) or are experimental (the artemisinins, artemether and artesunate; and OZ78). OZ78 (and MT04) are synthetic peroxides (Meister and others, 2013).

More information on flukicides online

There is more information (including PDF documents) on synergism and efficacy of flukicides, summarised in two tables, here: https://wormmailinthecloud.wordpress.com/2017/04/10/wrml-2017-04-10-flukicides-summaries-efficacies-synergistic-combinations-resistance-management/

Short link: http://wp.me/pRGJe-1Jd

Figure 7. Metacercariae (infective cysts) encysted on blade of grass

Image credit / source: Boray JC, 2017

Table 2. Diagnostic tests for liver fluke and their characteristics

Test	Sensitivity %	Specificity %	Comments
Field investigation			History, clinical signs and necropsy (post mortem examination)
Detailed liver examination at abattoir	99[1]	98[1]	Too costly for routine use. In this study[1], 32% of animals were positive with the detailed test, and 29% with the routine test.
Routine liver examination at abattoir	63.2[4]		Based on one Swiss study (Rapsch and others, 2006). This may vary between abattoirs / countries.
Faecal egg count	58 – 81[1] 30 - 70[2,3]	99[1] ~100[2,3]	Only detects adults. Little indication of size of fluke burden. High specificity with skilled operator (who reliably distinguishes liver fluke from stomach fluke eggs etc.)
sELISA	72-94[1] 86 -100[2,3]	76-89[1] 83 – 96[2,3]	sELISA = serum ELISA. Various antibody ELISAs. Detects early infections, from 4 weeks post-infection. Antibody to liver fluke may be detectable for up to 12 weeks or more after a fluke infection is cleared. Cross-reactions with stomach fluke (*Calicophoron*).[1]
Bulk milk ELISA			Various antibody ELISAs; milk. Commonly used in dairy herds around the world. Indicates fluke burdens are low, medium or high.
cELISA	77 – 81[1] high	99[1] high	cELISA = Coproantigen ELISA. Detects antigen (a protease) in faeces from fluke in bile ducts. No cross-reaction with stomach fluke (*Calicophoron*).[1]
Liver enzymes in serum			GGT and GLDH: non-specific indicators of liver damage.

Notes: **Sensitivity**: ability to detect true positives (high sensitivity means few false negatives). **Specificity:** ability to detect true negatives (high specificity means few false positives). **References**: [1]Mazeri and others, 2016 (study on cattle; Scotland); [2]Williams and others, 2014; [3] Woodgate and others, 2016; [4]Rapsch and others, 2006, cited by Mazeri and others, 2016. **ELISA**: enzyme-linked immunosorbent assay. **Study by Mazeri and others, 2016**: This study was done on 619 naturally infected cattle, of various breeds, and ages (369-1121 days old; mean 720 days old). These were slaughtered at a large Scottish abattoir, at three different periods (summer 2014, winter, 2014 and autumn 2014). Tests included detailed liver examination (necropsy) including gall bladder egg count (overall, 32% were positive), faecal egg counting, a commercially available coproantigen (c) ELISA (Bio-X, Belgium), and an in-house serum excretory/secretory antibody (s) ELISA, and routine abattoir liver examination (overall, 29% were positive). Their results suggested the cELISA, unlike the sELISA, did not cross-react with stomach (rumen) fluke (*Calicophoron daubneyi*) parasites. In livers where fluke were found, the number of fluke ranged from 1 to 86, mean 8.5, median 4.

References and more information

Barger IA, Dash KM and Southcott WH, 1978. Epidemiology and control of liver fluke in sheep. *The Epidemiology and Control of Gastrointestinal Parasites of Sheep in Australia*. Eds, Donald AD and others, CSIRO, Melbourne, p. 66.

Boray JC, 1969. Experimental fascioliasis in Australia. Advances in parasitology, 75: 95.

Boray JC, 2005a. Essay: Strategic control of fasciolosis caused by a very sophisticated and resilient liver fluke, *Fasciola hepatica*. https://wormmailinthecloud.wordpress.com/2016/11/03/wrml-boray-on-fluke/ (Accessed April 2017).

Boray JC, 2005b. Essay: Drug resistance in *Fasciola hepatica*. https://wormmailinthecloud.wordpress.com/2016/11/03/wrml-boray-on-fluke/ (Accessed April 2017).

'Boray on fluke'. Accessed April 2017 at https://wormmailinthecloud.wordpress.com/2016/11/03/wrml-boray-on-fluke/ (short link: http://wp.me/pRGJe-16N) and http://www.dpi.nsw.gov.au/__data/assets/pdf_file/0015/121128/turning-the-worm-19.pdf

Boray JC, 2017. *Liver fluke disease in sheep and cattle*. Primefact 446 (February 2007; revised 2017), NSW Department of Primary Industries. (555 kb, PDF, 10 pages). Retrievable from http://www.dpi.nsw.gov.au/animals-and-livestock/sheep/health/internal-parasites/liverfluke-disease-sheep-cattle Accessed April 2017.

Boray JC and De Bono D, 1989. Drug resistance in *Fasciola hepatica*. In Advances in Veterinary Science, ed. P.M. Outteridge & R.B. Richards, pp. 166–9. Australian Veterinary Association; cited by Fairweather and Boray, 2011.

Boray JC and Enigk K, 1965. Laboratory studies on the survival and infectivity of *Fasciola hepatica* and *F gigantica* metacercariae. Institute of Veterinary Medical Zoology, Veterinary College, Hanover (or Z Tropenmed Parasitol. 1964 Oct;15:324-31). Cited by Williams and others, 2014.

Bowman D, 2003. Georgi's Parasitology for Veterinarians, 8th Edition. Published by Saunders. ISBN 0-7216-9283-4.

Brockwell YM, Spithill TW, Anderson GR, Grillo V and Sangster NC, 2013.Comparative kinetics of serological and coproantigen ELISA and faecal egg count in cattle experimentally infected with *Fasciola hepatica* and following treatment with triclabendazole. Veterinary Parasitology, 196.

Brockwell YM, Elliott TP, Anderson GR, Spithill TW, and Sangster NC, 2014. Confirmation of *Fasciola hepatica* resistant to triclabendazole in naturally infected Australian beef and dairy cattle. International Journal for Parasitology: Drugs and Drug Resistance. Volume 4, Issue 1, April 2014, Pages 48–54.

Cole VG, 1986. *Helminth Parasites of Sheep and Cattle. Animal Health in Australia*, Volume 8. Australian Agricultural Health and Quarantine Service, Department of Primary Industry, Canberra, p.255.

COWS, 2016. Controlling liver and rumen fluke in cattle. http://cattleparasites.org.uk/guidance/manual/COWS%20Controlling%20liver%20and%20rumen%20fluke%20in%20cattle.pdf Accessed April 2017.

Elliott TP, Kelley JM, Rawlin G and Spithill TW, 2015. High prevalence of fasciolosis and evaluation of drug efficacy against *Fasciola hepatica* in dairy cattle in the Maffra and Bairnsdale districts of Gippsland, Victoria, Australia. Veterinary Parasitology 2015 Apr 15;209(1-2):117-24.

Flanagan A, Edgar HWJ, Gordon A, Hanna REB, Brennan GP and Fairweather I, 2011.Comparison of two assays, a faecal egg count reduction test (FECRT) and a coproantigen reduction test (CRT), for the diagnosis of resistance to triclabendazole in *Fasciola hepatica* in sheep. Veterinary Parasitology. 176, 170-176.

Kelley JM, Elliott TP, Beddoe T, Anderson G, Skuce P and Spithill TW, 2016. Review: Current threat of triclabendazole resistance in *Fasciola hepatica*. Trends In Veterinary Parasitology 2016 Jun; 32(6):458-69.

Fairweather I and Boray JC, 1999. Fasciolicides: efficacy, actions, resistance and its management. Veterinary Journal. 158, 81–112.

Fairweather I, 2011a. Reducing the future threat from (liver) fluke: realistic prospect or quixotic fantasy? Veterinary Parasitology 180 (2011) 133– 143.

Fairweather I, 2011b. Raising the bar on reporting 'triclabendazole resistance'. Veterinary Record. 168.

Fanke J, Charlier J, Steppin T, von Samson-Himmelstjerna G, Vercruysse J and Demeler J, 2017. Economic assessment of *Ostertagia ostertagi* and *Fasciola hepatica* infections in dairy cattle herds in Germany using Paracalc®. Veterinary Parasitology.

Gordon DK, Zadoks RN, Stevenson H, Sargison ND, and Skuce PJ, 2012. On farm evaluation of the coproantigen ELISA and coproantigen reduction test in Scottish sheep naturally infected with *Fasciola hepatica*. Veterinary Parasitology, 187.

Haydock LAJ, Pomroy WE, Stevenson MA and Lawrence KE, 2016. A growing degree-day model for determination of *Fasciola hepatica* infection risk in New Zealand with future predictions using climate change models. Veterinary Parasitology 228 (2016) 52-59.

Hutchinson GW, 2009. Nematode parasites of ruminants: Australia and New Zealand Standard Diagnostic Procedures, February 2009. Accessed March 2017 at https://wormmailinthecloud.files.wordpress.com/2015/12/hutchinson-gw-anzsdp-ruminant_nematodes-scahls-issued-may-2009.pdf Also available at http://www.agriculture.gov.au/animal/health/laboratories/procedures/anzsdp/nematode-parasites-ruminants

Hutchinson GW, Dawson K, Fitzgibbon CC, and Martin PJ, 2009. Efficacy of an injectable combination anthelmintic (nitroxynil plus clorsulon plus ivermectin) against early immature *Fasciola hepatica* compared to triclabendazole combination flukicides given orally or topically to cattle. Veterinary Parasitology 162, 278-284.

Laboratories: see below.

Lane J, Jubb T, Shepherd R, Webb-Ware J, Fordyce G, 2015. Priority list of endemic diseases for the red meat industries. Final Report B.AHE.0010. Meat & Livestock Australia, 2015. Cited by Woodgate and others, 2016.

Lloyd J, Boray JC and Campbell N (revised by Love S), 2017. Identifying liver fluke snails. NSW DPI Primefact 476, 3rd edition.

Lloyd J, 2007. Stomach fluke (paramphistomes) in ruminants. NSW DPI Primefact 452.

Love S and Hutchinson GW, 2003. *Pathology and diagnosis of internal parasites of ruminants* in Gross Pathology of Ruminants, Proceedings 350, Post Graduate Foundation in Veterinary Science, University of Sydney, Ch. 16, pp. 309–38. Formerly retrievable from NSW DPI's Vet Lab Manual (http://www.dpi.nsw.gov.au/__data/assets/pdf_file/0003/34608/lh-pathol-int-para.pdf) but no longer available following an update (2016/2017) of the website. Now available here: https://wormmailinthecloud.wordpress.com/2017/04/20/wrml-2017-04-20-love-and-hutchinson-2003-pathology-and-diagnosis-of-internal-parasites-of-ruminants/ (Accessed April 2017). Among other things, this document preserves some of the valuable information – more or less timeless - from Cole VG, 1986 (no longer in print).

Love S, 2017. https://wormmailinthecloud.wordpress.com/2017/04/10/wrml-2017-04-10-flukicides-summaries-efficacies-synergistic-combinations-resistance-management/ Higher resolution PDF versions are available here. Accessed April 2017.

Martin PJ, Chambers M, and Hennessy DR, 2009. Efficacy against *Fasciola hepatica* and the pharmacokinetics of triclabendazole administered by oral and topical routes. Australian Veterinary Journal 87, 200-203.

Martínez-Vallada M and Rojo-Vázquez FA, 2016. Loop-mediated isothermal amplification (LAMP) assay for the diagnosis of fasciolosis in sheep and its application under field conditions. Parasit Vectors. 2016; 9: 73.

Mazeri S, Sargison N, Kelly RF, Barend M de C Broonsvort and Handel I, 2016. Evaluation of the performance of five diagnostic tests for *Fasciola hepatica* infection using a Bayesian No Gold Standard approach in cattle. PLOS ONE http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0161621. Accessed December 2016

Meister I, Duthaler U, Huwyler J, Rinaldi L, Bosco A, Cringoli G, Keiser J,2013. Efficacy and pharmacokinetics of OZ78 and MT04 against a natural infection with *Fasciola hepatica* in sheep. Veterinary Parasitology, 2013 Nov 15;198(1-2):102-10.

Merck Veterinary Manual. Accessed October 2016 at http://www.merckvetmanual.com/

Novobilský A, Averpil HB and Höglund J, 2012. The field evaluation of albendazole and triclabendazole efficacy against *Fasciola hepatica* by coproantigen ELISA in naturally infected sheep. Veterinary Parasitology,190.

Novobilský A and Höglund J, 2015. First report of closantel treatment failure against *Fasciola hepatica* in cattle. International Journal of Parasitology: Drugs and Resistance. 5 (2015) 172-177. (This case was in cattle in Sweden, and related to a topical (pour on) formulation containing closantel and

ivermectin. The authors do not believe route of administration was a factor. Boray and De Bono (1989) reported resistance to closantel and related flukicides in 1989).

Olah S, van Wyk JA, Wall R and Morgan ER, 2015. FAMACHA©: A potential tool for targeted selective treatment of chronic fasciolosis in sheep. Veterinary Parasitology, 212 (2015)188-192.

Overend DJ and Bowen FL, 1995. Resistance of *Fasciola hepatica* to triclabendazole. Aust. Vet.J. 72, 275–276.

Ponder WF, Hallan A, Shea M and Clark SA, 2016. Australian Freshwater Molluscs. Accessed March 2017 at http://keys.lucidcentral.org/keys/v3/freshwater_molluscs/

Radostis OM, Gay CC, Hinchcliff KW and Constable P (Editors), 2007. Veterinary Medicine: a textbook of the diseases of cattle, horses, sheep and pigs. Tenth edition. 2,065 Pages, Published 2007 by Saunders/Elsevier. ISBN: 978-0-7020-2777-2.

Rapsch C, Schweizer G, Grimm F, Kohler L, Bauer C, Deplazes P et al, 2006. Estimating the true prevalence of *Fasciola hepatica* in cattle slaughtered in Switzerland in the absence of an absolute diagnostic test. International Journal for Parasitology. Cited by Mazeri and others, 2015.

Rees JB and others, 1975. Prenatal infection with *Fasciola hepatica* in calves. Australian Veterinary Journal, 51, p. 497.

Sargent RM, Chambers M, and Elliott T, 2009. Seasonal differences in the efficacy of pour-on formulations of triclabendazole and ivermectin or abamectin against late immature liver fluke (*Fasciola hepatica*) in cattle. Veterinary Parasitology 161 (2009), 133-137.

RVC: Royal Veterinary College / FAO Guide to Veterinary Diagnostic Pathology: Flotation fluids – general purpose. Accessed March 2017 at http://www.rvc.ac.uk/review/parasitology/Flotation/Flotation_fluids/General.htm

Taylor MA, Coop RL, and Wall R, 2016. Veterinary Parasitology. 4th edition. Wiley Blackwell. ISBN 978-0-470—67162-7.

van Wyk JA and Barth GF, 2002. The FAMACHA system for managing haemonchosis in sheep and goats by clinically identifying individual animals for treatment. Veterinary Research, 33:509-529.

Veterinary Medicine Directorate, UK. Document on oxyclozanide accessed March 2017 at http://www.vmd.defra.gov.uk/productinformationdatabase/SPC_Documents/SPC_1027977.DOC

Williams D and others, 2014. Liver fluke - overview for practitioners. British Cattle Veterinarians Association. Accessed 3 Nov 2016 at http://www.cattleparasites.org.uk/papers/bcva2014liverfluke.pdf (*Be aware of regional differences, including climate, host snails etc.*).

Wood IB, Amaral NK, Bairden K, Duncan JL, Kassai T, Malone JB Jr, Pankavich JA, Reinecke RK, Slocombe O, Taylor SM, et al., 1995. World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P.) second edition of guidelines for evaluating the efficacy of anthelmintics in ruminants (bovine, ovine, caprine).Veterinary Parasitology, 1995 Jun;58(3):181-213.

Woodgate R, Cassidy T and Love S, 2016. Laboratory detection of *Fasciola hepatica* in live sheep. District Veterinarians of NSW Annual Conference. Accessed November 2016 at http://www.flockandherd.net.au/sheep/reader/fasciola-detection-live-sheep.html

Wormboss. Accessible at wormboss.com.au. (Australian Wool Innovation and Australian Sheep Industry Cooperative Research Centre).

WormMail Newsletter, 2014-11-14 (Love S, Ed.). Discussion of flukicide resistance and resistance testing protocols including a summary of Brockwell and others, 2014. Accessed March 2017 at https://wormmailinthecloud.wordpress.com/2014/11/24/wrml-2014-11-24-triclabendazole-resistant-liver-fluke-in-new-england-nsw-etc/

Laboratories and other providers

NSW DPI. http://www.dpi.nsw.gov.au/about-us/services/laboratory-services/veterinary

Charles Sturt University Veterinary Diagnostic Laboratory, Wagga Wagga. List and costs of tests: https://www.csu.edu.au/__data/assets/pdf_file/0007/256597/91020-VDL-Submission-Form_WEB.pdf

Professional service providers listed at WormBoss: http://www.wormboss.com.au/tests-tools/professional-service-providers.php

Experienced and expert advisers: these are scattered across various sectors (public, private, including commercial companies, university etc.) and organisations. In NSW, veterinary advisers include Local Land Services District Veterinarians and private practitioners with a particular interest in parasitology.

Acknowledgments and revision history

This third edition (May 2017) is a light revision of the previous edition, the new material being a short discussion of targeted selective treatment (page 7), partly stimulated by the paper Olah, van Wyk , Wall and Morgan (2015).

The second edition (April 2017) was a major revision of the first, 'Liver fluke – the basics' (Primefact 813, 2008, by the current author), and included information from papers published in the last several years, but still drawing heavily on material published by eminent veterinary parasitologist, Dr JC Boray.

The first edition ('Liver fluke – the basics', 2007) was essentially a summary of the publication by Boray ('Liver fluke disease in sheep and cattle', Primefact 446, revised March 2007, and 2017).

One aim in the second and third editions was to include answers to many of the questions I have had about liver fluke, but could never find in one or even several text books or other sources of information.

My thanks to those who reviewed this Primefact: **Dr Sue Hatcher** (Principal Research Scientist, NSW Department of Primary Industries, Orange NSW; Associate Editor - Animal Production Science) and **Leonie Martin** (formerly Farm Chemicals Officer, NSW Department of Primary Industries, Orange NSW). Thank-you also to colleagues, friends, associates and primary producers who have more generally provided constructive feedback, assistance and support.

For updates go to www.dpi.nsw.gov.au/factsheets

ISSN 1832 6668

Reference number (3rd edition, May 2017): (RM8) INT17/64609

Reference number (2nd edition, April 2017): (RM8) INT17/58446