Liver fluke in alpacas

Jane Vaughan BVSc PhD MACVSc

Background

Liver fluke is the common name of the trematode, *Fasciola hepatica*. The parasite is found worldwide and is the only liver fluke found in Australia. Infection can lead to reduced productivity and death and costs millions of dollars each year in lost production (meat, wool, milk, liver condemnation, secondary infection, replacement stock requirements), stock deaths and costs of treatment and prevention. The fluke mainly affects cattle and sheep, but can also affect alpacas, goats, horses, pigs, kangaroos, wombats, rabbits and deer. Humans may also be infected, for example after eating watercress collected from fluke-infested creeks or following use of contaminated water on vegetable gardens. The adult fluke is a pale brown or grayish-brown flat worm about 1.5-4 cm long that lives in the bile ducts of the liver (Figure 1).

Figure 1. Adult liver fluke (15-40 mm long) (http://www.britannica.com/EBchecked/media/5519/Liver-fluke).

Liver fluke are typically found in areas of south-eastern Australia where the annual average rainfall is 600 mm or greater, or on irrigated country where the average rainfall is 400 mm, in and around swampy areas, springs and seepages, irrigation channels and shallow, slow moving creeks where the intermediate host snail of this parasite breeds (Figure 2). This includes:

- Most of Victoria and Tasmania
- South-eastern New South Wales
- Irrigation areas of NSW and Victoria
- Northern Tablelands and north coast of NSW
- Small areas of southern Queensland and South Australia

Western Australia is free of liver fluke and actively manages its fluke-free status using a system of drenching and liver fluke egg testing of faeces of stock being shipped westward (see www.agric.wa.gov.au for more information).

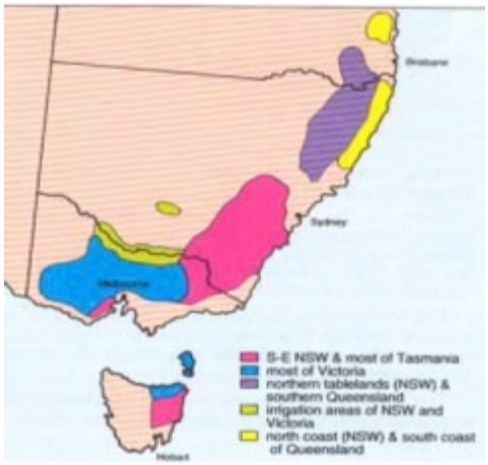

Figure 2. Distribution of liver fluke disease in different climatic regions (Boray 2007).

Lifecycle

The liver fluke requires two hosts: the definitive host, or alpaca, and the intermediate host, or lymnaeid snail, to complete its lifecycle (Figure 3). **Adult liver fluke** live in the bile ducts of the host species, such as the alpaca. The flukes produce eggs, which pass in the bile into the small intestine and into the external environment in the faeces. The eggs hatch in wet areas in warmer months (mean temperature above 10°C, typically mid-September to May) and release larvae called **miracidia**, which need to invade certain species of snails within 24-30 hours to survive. Once in the snail, they develop and multiply as **sporocytst, rediae and cercariae**. The motile cercariae leave the snails and swim onto vegetation and form microscopic cysts called **metacercariae** on grass, leaves and reeds. The definitive host, such as the alpaca, ingests these cysts from the pasture and they hatch out into **early immature flukes** in the small intestine. These burrow through the walls of the gut into the abdominal cavity, and make their way through the capsule of the liver into the bile ducts over some

weeks where they develop into egg-laying adult flukes.

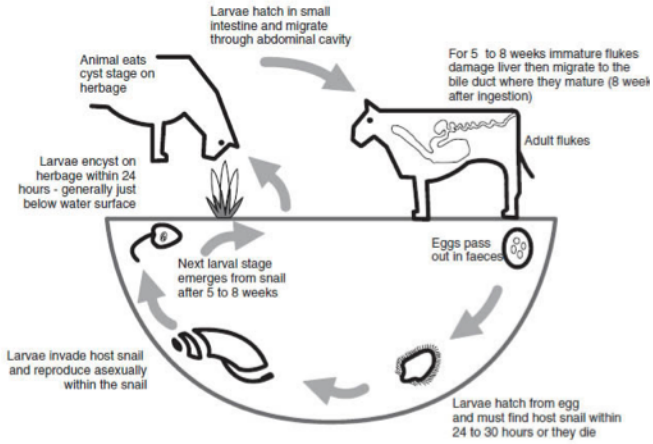

Figure 3. Lifecycle of liver fluke is similar in alpacas, cattle, sheep and goats (Muirson 2004).

Over-wintering infection. In winter, all stages of fluke development and snail reproduction cease when temperatures fall below 10°C overnight, but metacercariae produced in summer and autumn will survive in declining numbers on vegetation (especially if moisture is present) as will snails and dormant larvae. The early spring metacercarial cysts appear as a result of snails being infected by miracidia in autumn and completing the lifecycle when temperatures increase in spring.

Summer infection. It takes 2-3 months from eggs being laid on the pasture to encysting on vegetation and becoming infective to the definitive host in favourable conditions: it takes 21 days for eggs to develop into miracidia in summer, but up to 90 days in spring and autumn. Metacercarial cysts can survive on vegetation for many months if there is adequate moisture and temperatures stay below 20°C so there is usually high pasture contamination by late summer and autumn. Cysts die quickly in hot, dry conditions. Snails and fluke larvae in snails survive in mud during dry periods for about a year.

It takes 6-7 weeks from ingestion of metacercarial cysts until they enter the bile ducts, eating liver tissue and blood on their way. It takes another 2-3 weeks to reach sexual maturity and start laying eggs so it takes 8-10 weeks from ingestion to egg laying (pre-patent period). Each adult fluke lays 20,000 to 50,000 eggs per day, and each fluke egg can produce 4000 infective metacercarial cysts, rapidly contaminating pastures. However the eggs may enter the small intestine via bile secretions in an intermittent manner, so faecal shedding of eggs can be intermittent. Adult flukes live for about a year in cattle and may live for the lifetime of a sheep if they remain untreated. Longevity of flukes is unknown in alpacas. Egg production declines in cattle as they develop a natural resistance to chronic infections.

The lifecycle is dependent on the presence of one of three types of lymnaeid snail that breeds in sunny, open, wet areas where there is an abundance of aquatic plant growth and algae on which to feed. Therefore, alpacas are most at risk of ingesting cysts from vegetation when grazing around waterways. Also, long, wet seasons (2012!) are associated with a higher rate of infection. Bear in mind that alpacas may prefer to graze away from swampy areas when feed is plentiful, and may therefore become infected in dry times when forced to graze more closely in the marshy areas.

- *Lymnaea tomentosa* an indigenous, freshwater snail is the most important intermediate host in Australia and New Zealand. It lives near slow-moving, shallow creeks, swampy areas, springs and irrigation channels/drains and is summer-active. It is only rarely found in dams, water troughs and large creeks, but may be found in dam overflows after heavy rain. It can survive in dry mud for up to a year, and tolerates low temperatures. This snail is 6-12 mm long and has a clockwise thread when viewed from the point to the base of the snail.
- *Lymnaea columella* is an introduced snail from North America. It is less fastidious than *L. tomentosa* and may be found in deeper creeks and dams, and can survive in stagnant water. It is a small snail, 8-20 mm long with a dark grey body. The shell is thin, fragile and has a clockwise coil when viewed from the top. It is widespread in Western Australia.
- *Lymnaea viridis* has been recently introduced from various Pacific islands. It is a versatile snail, liking environments of *L. tomentosa* and *L. columella*, and has the ability to travel much further from water sources than the other snails. It is 4-12 mm long and its shell has a clockwise spiral too.

Clinical signs

Liver fluke disease can be acute, sub-acute or chronic depending on the size of infection and how quickly cysts are ingested. Clinical signs results from the damage caused by immature fluke migrating through the liver, and damage by adult fluke to bile ducts and blood ingestion. Sheep have no naturally acquired resistance to liver fluke so acute and chronic fasciolosis may occur at any age. Conversely, cattle develop resistance so clinical disease is more likely in young cattle.

1. Acute fasciolosis

This syndrome is seen in late spring and early summer following ingestion of massive numbers of metacercarial cysts from the pasture over a short period and many flukes develop at once. It is usually seen in specific climatic conditions combined with a

lack of fluke control measures when alpacas are forced to graze heavily contaminated wet areas as a result of over-stocking and/or drought. Animals may show some abdominal pain and/or jaundice but often present as sudden death. Death occurs within weeks of ingestion and is secondary to liver damage and blood loss caused by migration of immature flukes through the liver. Reduced liver function can be detected by elevated liver enzymes in the blood of affected animals 2 weeks after infection and can be useful in diagnosing acute fasciolosis. Elevated liver enzymes may persist in sub-acute and chronic cases for 16-18 weeks.

2. Sub-acute fasciolosis

This syndrome is characterised by liver damage, anaemia, jaundice and ill-thrift and is most common in late summer to late autumn. There is extensive liver damage and haemorrhage caused by the migrating fluke (Figure 4.). Liver failure and death occur in 8-10 weeks following reduced growth, fertility and wool production.

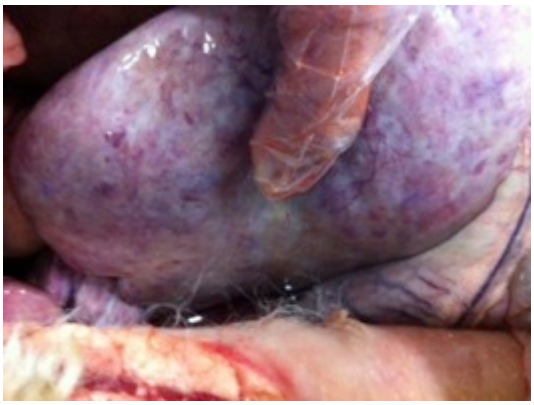

Figure 4. Liver of an alpaca with sub-acute fasciolosis.

3. Chronic fasciolosis

This is the most common scenario seen in sheep, goats and cattle. It can occur at any time but most commonly from autumn to spring. Parasites reach the bile ducts of the liver, and cause bile duct inflammation, enlargement and obstruction, hepatic inflammation and fibrosis and anaemia over a period of months. Animals gradually become inappetant, anaemic, reluctant to move and eventually may die. Chronic fasciolosis also provides the right conditions in the liver for the fatal clostridial disease *Clostridium novyi* type B, or **Black disease**. Clostridial bacteria multiply in areas of liver damage caused by migrating immature liver fluke and can lead to sudden death. This is one of the organisms contained in 5-in-1 clostridial vaccines so make sure vaccinations are up to date if alpacas are being run in a fluke-endemic area.

In alpacas other bacteria, such as ***E. coli,*** may be showered from the fluke-infected liver along the hepatic vein to the right ventricle of the heart, where it causes severe endocarditis and right-sided congestive heart failure (Figures 5 and 6; Links et al., 1992).

Severe production losses occur when immature flukes burrow through the liver even when obvious symptoms are not apparent. Death from fluke infection, Black disease and/or E.coli abscessation depends on the burden of fluke in the liver and stage of fluke infection.

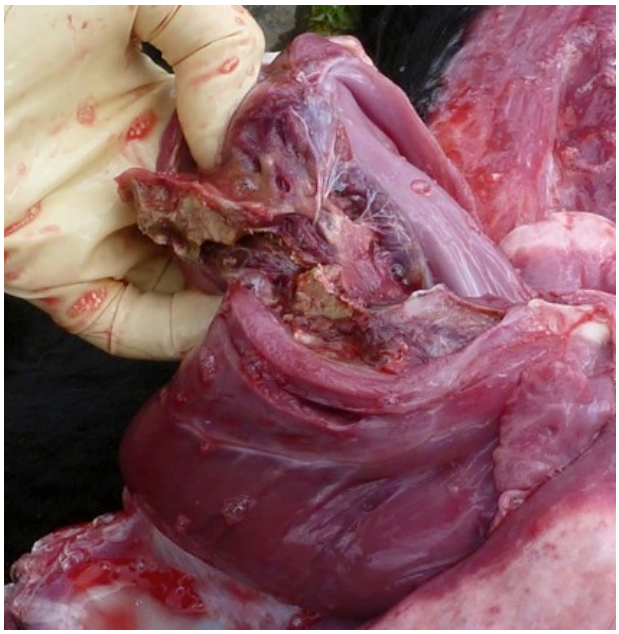

Figure 4. Valvular endocarditis secondary to fasciolosis.

Figure 5. Hepatic fibrosis secondary to liver fluke migration and congestive heart failure.

Diagnosis

Grazing history, clinical examination, serology, faecal egg counts, response to treatment and necropsy findings are usually used in combination to identify liver fluke infections in sheep, cattle and alpacas.

1. Dead alpacas. Adult and/or immature flukes ooze out of bile ducts and tissue parenchyma when the liver is cut open.

2. Faecal sedimentation test. Fresh samples of faeces should be collected directly from the rectum of approximately 10 alpacas in each mob using a gloved finger. 10-15 faecal pellets (30 g) should be collected from each animal and placed into separate freezer bags. Air should be excluded from the bag and bags placed into the refrigerator and kept cool during shipment to the laboratory. Collect samples early in the week so they do not get lost in transit over the weekend. Do not freeze faeces. Alternatively, move alpacas to a communal dung pile and hold them there for 10-15 minutes then collect warm samples from the dung pile in a similar fashion.

Fluke eggs are larger than strongyle (roundworm) eggs found in alpacas and require a different faecal test (sedimentation rather than flotation) to identify their presence (Figure 7). Make sure you specifically ask the laboratory for a fluke egg count. The eggs have a characteristic operculum at one end.

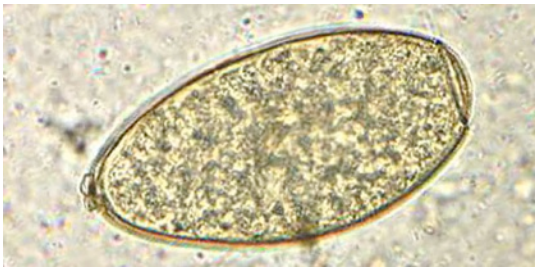

Figure 7. Fluke egg (150 µm long) (http://www.nhm.ac.uk/nature-online/species-of-the-day/scientific-advances/disease/fasciola-hepatica/index.html).

Sometimes alpacas will pick up massive numbers of larvae from the pasture and die before larval forms of fluke have had time to mature and begin egg laying in the bile ducts. Worm egg counts may be zero or low, but diagnosis will be made at autopsy by the presence of larvae in the liver.

Because there is a 12 week lag between ingestion of metacercarial cysts and fluke eggs appearing in faeces, false negatives occur in faecal tests between December and May. Between June and November, faecal testing for fluke eggs is more reliable. Intermittent shedding of eggs by adult flukes may also lead to false negative tests. Testing 10-12 animals in a herd rather than an individual animal is recommended to optimize potential for detecting fluke eggs.

3. Blood ELISA tests validated in sheep and cattle that test for antibodies made in response to flukes are **not validated in alpacas**. The test detects infection with both immature and adult flukes in a flock or herd (10 blood samples pooled in the laboratory into 2 groups of 5 prior to testing), but is not sensitive enough to diagnose infection in individual animals. Serum antibodies appear 6-8 weeks after infection in cattle, and 4-6 weeks after infection in sheep and remain high for at least 12 weeks after infection has been removed. Antibodies remain high in untreated subacute and chronic stages.

4. Faecal antigen tests are currently being validated in sheep and cattle in Australia. Commercial kits are available overseas, and they detect secretory-excretory antigens shed by both immature and adult flukes in the bile ducts. These kits should allow earlier detection of fluke prior to egg production by adult flukes.

Treatment

Once a diagnosis has been made, a zero-tolerance approach is recommended when treating and controlling liver fluke because even light burdens can cause significant damage and production losses. The treatment recommended will depend on the nature of infection – are immature or adult fluke causing disease? Not all flukicides (anthelmintics effective against liver fluke) are effective against immature fluke so are not recommended in acute fluke outbreaks (Table 1). They are also less efficient for strategic control of flukes. Even the best flukacides do not kill every fluke and an effective flukacide is described as being 90 % or more effective (compared with roundworm anthelmintics where effective drenches kill more than 95 % of target roundworms). Remember to test/treat introduced stock to reduce contaminating fluke-free areas.

Table 1. Efficiency of drugs available in Australia for the treatment of liver fluke in sheep and cattle (modified from Boray 2007).

Active ingredient	Safety index at recommended dose	Over 90% efficiency at the recommended dose rate (age of flukes in wks)						
		2	4	6	8	10	12	14
		Early immature		Immature		Adult flukes		
Triclabendazole	20	+	+	+	+	+	+	+
Closantel	5.3	-	-	-	+	+	+	+
Closantel + oxfendazole	5.3	-	-	+	+	+	+	+
Closantel + albendazole	5.3	-	-	-	+	+	+	+
Nitroxynil	4	-	-	-	-	+	+	+
Albendazole	6	-	-	-	-	-	±	+
Oxyclosanide + levamisole	4	-	-	-	-	-	±	+
Chlorsulon + ivermectin	20	-	-	-	-	-	+	+
Nitroxynil+chlorsulon+ivermectin	4	-	+	+	+	+	+	+

The most effective drug of choice against liver fluke is **triclabendazole** (E.g. Fasinex®, Flukare®, Tremacide®). While very specific for *Fasciola*, it is not effective against nematodes, cestodes and other trematodes. The dose rate for alpacas is estimated to be 15 mg/kg body weight orally (in comparison to sheep and goats 10 mg/kg po and cattle 12 mg/kg po). The oral route of administration ensures the active ingredient is absorbed from the gastrointestinal tract and delivered to the liver via the portal blood supply. Triclabendazole has been combined with oxfendazole (Flukazole S®) to improve efficiency through a synergistic effect. Note that different products contain different concentrations of the active ingredient so read the label carefully before use and contact your veterinarian for assistance with calculating drench volume if necessary. Remember no drugs are registered for use in alpacas.

Do not use any of the pour-on products containing triclabendazole (Martin et al. 2010) as absorption across the skin is impaired by hair growth (cattle groom themselves and each other and lick the drench off the skin!) and will likely be ineffective in alpacas.

Closantel is effective against liver fluke and *Haemonchus* (barber's pole worm). The drug is effective against young, mature fluke (6-8 weeks of age) but has reduced effect on immature fluke. It is an oral drench and has been combined with oxfendazole (Closicomb®) to improve efficacy against susceptible 4 week-old fluke.

Nitroxynil (Trodax®) is an injectable sheep and cattle flukacide that has activity against adult fluke. **Nitroxynil, clorsulon and ivermectin combination** (Nitromec®) is an injectable cattle flukacide reported to be effective against early immature fluke as well as immature and adult stages (Hutchinson et al. 2009). Unconfirmed reports claim that the nitroxynil, clorsulon and ivermectin combination may cause local injection site skin reactions in cattle.

Timing of fluke drenches will depend on region of Australia and rainfall and temperature pattern and may vary a little from the outline below. The programme needs to be developed in conjunction with your local veterinarian. In south-eastern Australia, pick up of larvae usually begins in spring and continues through summer when snail survival is favoured (wetlands/irrigation). Snail numbers tend to plummet in winter so pickup of immature flukes decreases markedly. Beware though that in mild winters snail activity and fluke pickup may continue.

1. Late April/early May. The most important treatment. Most fluke infections are picked up in summer and early autumn. After the first frost in southern Australia, snails become inactive so there is very little uptake from pastures once it gets cold. Drenching at this time will markedly reduce fluke egg output over winter and reduce pasture contamination. Use a drench that kills immature and adult fluke such as triclabendazole.

2. August. Even the best flukacides do not kill every fluke and some fluke would have been too young to respond to drenching in autumn, so there will be some fluke in the liver left after the autumn treatment. Some metacercariae may have been picked up over winter too. Use an adult flukacide, such as closantel+oxfendazole or nitroxynil, at this time to prevent spring pasture contamination before snails become active, as there will only be adult flukes in the bile ducts of the liver at this time of the year.

3. December/January. Drenching at this time will help reduce spring pick-up and to keep egg

production low if pastures are very contaminated. A drench effective against early immature fluke, such as triclabendazole, should be used. This drench is usually applied on farms that are heavily infected with fluke.

When using a drench that kills immature and adult flukes, egg production and pasture contamination will cease for about 10-12 weeks.

Treating more often than required is costly and may lead to the development of drench resistance. Not all of the above treatments may be required but treatment is essential when clinical disease is apparent. Aim to prevent disease and markedly reduce pasture contamination over the long-term. Remember to treat sheep and cattle if co-grazing with alpacas occurs.

Resistance of flukes to triclabendazole and closantel has been reported in Australia in various areas (Pyramid Hill in Victoria and New England Tablelands and Bega district in NSW) following 20 years of use. Resistance is not widespread and has been slow to develop because there are large refugia of susceptible fluke in the environment to dilute out resistant strains. Resistance is usually first seen as reduced effectiveness against immature flukes. Resistance to fluke drenches allows spread of fluke among properties via infected stock or snails. Consider rotating from a flukacide with activity against immature and adult fluke in autumn to an adult-specific drench in August. Closantel can be used successfully against triclabendazole-resistant fluke and vice-versa as they belong to different drench families. Nitroxynil, clorsulon and ivermectin combination drench may be useful to use in rotation with triclabendazole to reduce development of fluke resistance to triclabendazole. Limit the use of combination products that are effective against fluke and roundworms to when treatment of both is required.

Before you treat your animals, carefully read the directions for use of the selected drench. Shake the container so the drench is mixed evenly. Make sure you weigh some of the largest animals in the group and treat to the heaviest in the mob so that no animal is under-dosed. If the group has a wide range of weights, divide into lighter and heavier mobs so the smaller animals are not overdosed. Calculate the correct dose. Ensure drenching equipment, delivered via both injectable and oral routes, is calibrated to deliver the correct dose (and check throughout the day). Ensure drench is not spilled during drenching. If you are using an oral drench, place the drench gun over the back of the tongue and allow time for the alpaca to swallow. If injecting, place the needle subcutaneously. Do not hold drenched animals off water for too long after treatment.

To check how well your flukacide is working, do a fluke egg count on the day of drenching and again 28 days after drenching and calculate the percent reduction in faecal egg count. You need to wait 28 days for the fluke eggs to clear from the bile ducts of the liver.

Remember that no drugs are registered for use in alpacas and you should work in close consultation with your local veterinarian to obtain appropriate information about off-label use of drenches.

Control

A continuous and coordinated strategic control programme is required because flukes and snails reproduce at such a high rate.

- Use strategic fluke drenches. Eradication is almost impossible because it is usually not possible to prevent re-infestation of pastures and animals.
- Monitor worm burdens regularly in your herd by collecting fresh faeces and testing for fluke egg output in the herd. Worm egg counts are given as a measure of numbers of parasite eggs per gram of faeces. Remember that the routine faecal egg count method that detects roundworms/nematodes does not detect fluke eggs and a fluke egg sedimentation test must be requested.
- Participate in Q-Alpaca. Liver damage secondary to fluke will be detected at autopsy so a control programme may be implemented before too many deaths occur.
- Avoid introducing animals (alpacas, sheep, goats, cattle) with liver fluke onto your property by quarantining and drenching new animals that come from a liver fluke area with an effective, broad-spectrum flukacide.
- Do not forget about the intermediate hosts! It may be prudent to fence off swampy areas to prevent grazing where snails may reside or to drain wet areas to reduce snail habitat and numbers. Planting trees may reduce snails by increasing a dense cover of vegetation. Keep irrigation drainage channels clear of vegetation so water flows freely and snails and cysts do not accumulate in their vicinity. Chemical control of snails is not an option because they reproduce and repopulate areas so quickly and no molluscacides are registered for this use in Australia.
- Grazing management. Identify snail-infested pastures on the property and treat only those animals grazing these areas. Graze cattle on fluke-prone areas rather than alpacas. Limit grazing of ‘flukey’ country in autumn and early

winter (February to June), when pickup is greatest.

- Development of vaccines against fluke have been very disappointing to date as the level of protection is not high enough to warrant commercialisation. However, ensure alpacas are vaccinated against Black disease using a 5-in-1 clostridial vaccine.

References and further reading

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