

# FASCIOSIS IN LIVESTOCK

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## INTRODUCTION

Infection by members of the genus *Fasciola*, commonly known as liver flukes, may be responsible for morbidity and mortality in most mammal species, but are of particular importance to sheep and cattle livestock producers. The two species of the greatest veterinary importance are *F. hepatica* and *F. gigantica* and amphibious snails are their intermediate hosts. Adult parasites are found in the bile ducts, and the immature flukes in the liver parenchyma, of infected final hosts. Clinical disease is usually characterized by weight loss, anaemia and hypoproteinaemia. Fasciolosis is a worldwide zoonotic disease and the incidence is reported to be increasing in certain regions of the world and creating a serious public health concern in areas such as the Andean rural regions, Nile delta regions and some regions bordering the Caspian sea (e.g. Northern Iran).

Generally most aspects of the biology of the two species of flukes, epidemiology and of the disease caused by them, in cattle and sheep, are fairly similar. Most published material focuses on *F. hepatica* infection in sheep. This article follows this same trend and where necessary differences will be pointed out.

## LIFE CYCLE

Eggs are passed in the faeces of the mammalian host, hatch and release motile miracidia. Hatching may take nine days and the optimal temperatures required are

22-26° C. Miracidia have a short life span and must locate a suitable snail within three hours. In infected snails sporocysts, redial stages and cercaria develop. Snails pass the motile cercaria which then attach themselves to plant material, where they encyst and become the infective metacercariae. A minimum of 6-7 weeks is required for miracidia to form metacercaria. Under unfavourable circumstances this may take several months. Following on infection of one snail with one miracidium over 600 metacercariae may be produced. Final hosts ingest the metacercaria and these excyst in the small intestine, followed by migration through the intestinal wall, crossing of the peritoneum and penetration of the liver capsule. The immature flukes migrate through the parenchyma (6-8 weeks period), entering the small bile ducts and finally migrating to the larger bile ducts (and occasionally the gall bladder).

Generally the life cycle of both fluke species is the same. The prepatent period of *F. hepatica* is 10-12 weeks and one entire life cycle of *F. hepatica* may be completed in a minimum of 17-18 weeks. *F. hepatica* may live for years (up to 20) in untreated sheep but in cattle it is usually less than one year. For *F. gigantica* most phases of development take longer and the prepatent period is 13-16 weeks.

These parasites are hermaphrodites, and one adult *F. hepatica* in a bile duct may produce 20 000 eggs per day establishing patent infestation.

## EPIDEMIOLOGY

*F. hepatica* is mostly encountered in temperate areas, and in cooler areas of high altitude in the tropics and subtropics, whilst *F. gigantica* predominates in tropical areas. Snails are their intermediate hosts. Amphibious snails of the genus *Lymnaea* sp are widely distributed throughout the world and *L. trunculata* is the most common of them all. In South Africa the most common intermediate hosts are *L. trunculata* (*F. hepatica*), *L. natalensis* (*F. gigantica*) and *L. columella* (*F. hepatica* and *F. gigantica*).

Other important *Lymnaea* vectors of *F. hepatica* are *L. tomentosa* (Australia, New Zealand), *L. columella* (North America, Australia, New Zealand), *L. bulimoides* (Southern USA and the Caribbean), *L. humilis* (North America), *L. vector* (Southern America), *L. diaphena* (South America). Other important *Lymnaea* vectors of *F. gigantica* are *L. auricularis* (Europe, USA, Middle East, Pacific islands), *L. rufescens* and *L. acuminata* (India, Pakistan) and *L. rubiginosa* (Malaysia).

Large numbers of metacercariae will usually be produced when there is optimal availability of suitable snail habitats, optimum temperatures and optimum moisture is present. This frequently results in seasonal patterns of emerging disease in certain parts of the world for e.g. in Britain metacercariae may appear on pastures from August to October and also in May to June.

Suitable snail habitats will include all areas where snails may survive in clear water or mud such as the edges of streams, ponds, rivers and vleis (permanent natural habitats); or temporary man-made depressions filled with water (tractor tracks etc). A slightly acid environment may be more optimal. Temperature requirements are mean day/night temperatures of 10° C at which both the snails and the flukes will propagate.

Below 5° C all activity will stop and above 15° C significant increase in both snails and fluke larval stages may be seen, with the optimum being 22 -26° C.

Moisture levels are described as optimal when rainfall exceeds transpiration and when field saturation is achieved.

## PATHOGENESIS, PATHOLOGY AND CLINICAL SIGNS

Pathogenesis depends primarily on the two different stages of development of the parasite in the liver of the host, the level of parasitaemia, and if it is an acute, sub acute or chronic infection. Acute and sub acute disease is seen in sheep, and occasionally occurs under conditions of heavy challenge in young calves. The chronic form of the disease is by far the most important in sheep, and specifically in cattle.

The clinical signs of acute disease are characterised by sudden acute deaths, weakness, anaemia and dyspnoea. Sub acute and chronic fasciolosis is characterized by progressive loss of condition, anaemia, hypoalbuminaemia, emaciation, pallor of the mucous membranes, submandibular oedema and ascites. Anaemia is hypochromic and macrocytic and an accompanying eosinophilia is usually present.

In milder infections clinical signs may or may not be readily observed, however, a decreased appetite and interference with post-absorptive metabolism of protein, carbohydrates and minerals, may have a significant effect on production.

Acute disease is associated with mostly immature flukes, and usually seen in autumn and early winter, 2-6 weeks after ingestion of metacercariae in large numbers (> 2000). Immature flukes migrate through the liver parenchyma and create migratory tracts, which results from direct trauma, coagulative necrosis and release of toxic excretions from the flukes (eg Catephsins). Lesions may vary from mild (low infestations) to severe in heavy, or repeat infestations. The liver may be enlarged and haemorrhagic with fibrinous to fibrous exudates on the capsular surface (usually the ventral lobes). The migratory tracts may be visible as dark acute haemorrhagic streaks to, more yellowish white streaks typical of post necrotic scarring and granulation. Sometimes flukes may be seen in the migratory tunnels. If severe haemorrhages are present it may result in large subcapsular haemorrhages, which in turn may rupture with severe intra-abdominal haemorrhage and acute haemorrhagic anaemia as consequence.

In some heavy and repeat infestations acute lesion of multifocal pinpoint serosal haemorrhages and fibrinous peritonitis, to more chronic fibrous peritonitis may be present.

**Figure 1: Severe necrohaemorrhagic tracts in liver due to migrating immature flukes**

digital image kindly supplied by Dr. J.A.C. Steyl, Department of Pathology, Faculty of Veterinary Science, Onderstepoort).



Sub acute disease is usually seen during late autumn and winter, and 6-10 weeks after ingestion of smaller numbers (500-1500) of metacercariae. At this stage some parasites may have reached the bile ducts whilst others may still be migrating through the parenchyma. Sub capsular haemorrhages may be present but usually these do not rupture.

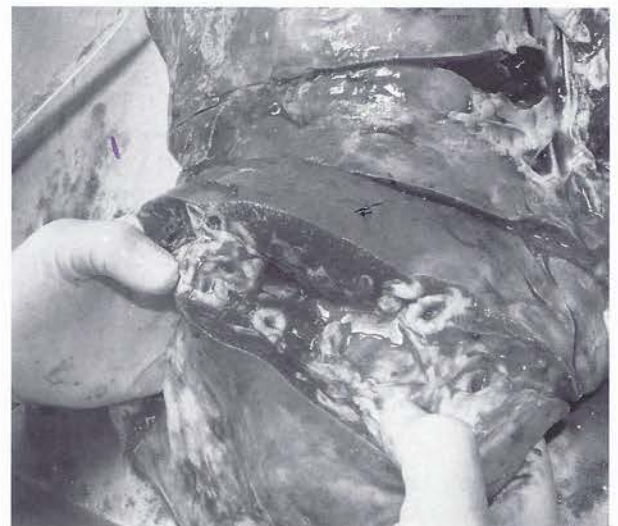
Chronic fasciolosis is associated with mature flukes, and seen mainly in late winter/early spring. It is usually 4-5 months after ingestion of moderate numbers (200-500) of metacercariae. Mature flukes, which are present in the bile ducts, cause necrosis and ulceration of the epithelium giving rise to peribiliary inflammation and severe hyperplasia of the epithelial layer. Mechanical irritation by their scales, and suckers, biliary retention and the production of toxic or irritant products by the flukes may contribute to lesions. Anaemia and hypoalbuminaemia are the most important consequences contributing mostly to the pathogenesis. More than 0.5 ml blood per fluke can be lost per day. Plasma protein may be lost through the bile ducts into the intestine due to the increased permeability of the hyperplastic bile duct epithelium, and

loss of plasma proteins through the fluke's digestive tract. Bile ductular distention in sheep, swine and horses may be more mechanical due to accumulation of parasites and bile, whereas in cattle the inflammatory lesion associated with erosions and granulation seems more prominent.

On post mortem the liver may have an irregular outline, and be pale and firm. The ventral lobe is most commonly affected and reduced in size. The liver pathology of chronic disease is characterized by hepatic fibrosis and hyperplastic cholangitis. Several different types of fibrosis may be present and includes post-necrotic scarring (mainly in the ventral lobe and associated with healing of fluke tracts), ischaemic fibrosis (infarction as consequence of damage and thrombosis of large blood vessels, and peribiliary fibrosis (damage by flukes in the small bile ducts). Fluke eggs may sometimes stimulate a granuloma-like reaction with obliteration of the affected bile ducts as consequence. In bovines calcification of bile ducts, enlargement of the gallbladder and aberrant migration of the flukes is more common. Encapsulated parasites are often seen in the lungs. If adult cows are reinfected, parasitic migration to the foetus and resultant prenatal infection has been reported.

**Figure 2: Chronic fasciolosis – chronic fibrosing cholangiohepatitis**

(digital image kindly supplied by Dr. J.A.C. Steyl, Department of Pathology, Faculty of Veterinary Science, Onderstepoort).



## ECONOMIC SIGNIFICANCE

Fasciolosis is of great economic significance worldwide with losses estimated to exceed 2000 million dollars yearly, affecting more than 600 million animals, in articles reported a decade ago. This would probably not accurately take into account losses due to the implications and consequences of zoonotic disease, and it has been reported that 2.4 million humans are affected. Studies of the effect of *F. hepatica* infection on live weight gain in cattle have produced results ranging from no significant effect to severe weight loss and death.

Where effects have been observed the depression of live weight gain has been reported as from 0.07 kg/week to 1.2 kg/week depending on the level of the infection. Belgian White Blue bulls aged from 10 to 12 months and weighing  $365 \pm 9$  kg showed an average daily gain of  $1.975 \pm 0.120$  kg in negative (based on faecal fasciolosis examination) animals as opposed to  $1.661 \pm 0.140$  kg in positive animals while positive treated animals showed adg of  $1.960 \pm 0.085$  kg. It is reported that animals on lower planes of nutrition show greater losses in performance.

In animals suffering from smaller fluke burdens, the clinical effect may be minimal and the loss of productivity is difficult to determine, or to differentiate from other cause such as nutritional deficiency. The effect of fasciolosis is not limited to daily gain and it is reported that infected heifers have a delayed first oestrous cycle and that heifers treated for trematodes as well as nematodes had higher condition scores and weight gains at pregnancy diagnosis as well as higher pregnancy rates. In cows a reduction in milk yield and quality, especially of the solids-not-fat component may be present, during winter months.

The level of liver condemnation at abattoir meat inspection worldwide varies according to the season and is reported to be as low as 0.26% but in areas where it occurs more commonly the average rate following dry summers is reported to be 5% but can be as high as 10% to 20% following wet summers. In the KZN and East Griqualand areas unofficial reports of condemnations due to liver fluke infections varied from

6.7% to about 40% during the autumn of 2002.

The condemnation rate at Cato Ridge Abattoir during September to November 1994 was 24% (unpublished data) and the condemnation rate of livers at the privately owned feedlot abattoir at Wartburg KZN during the autumn of 2002 was 17%. Data was accumulated for animals that were slaughtered between 3 September 2002 and 6 January 2003 and analysed to evaluate the effect of the liver fluke damage on performance in animals. Slaughter data from animals processed between September 2002 and January 2003 were accumulated and correlated with the data from the feedlot records to evaluate the effect of liver fluke on performance. Liver damage was subjectively classified as between 1 and 3 where grade 1 showed slight visible scarring of the bile ducts, grade 2 showed moderate scarring of bile ducts with occasional parasites present and grade 3 had severe scarring with parasites consistently present.

The average daily gain for normal calves was  $1.55 \text{ kg} \pm 0.37$ , calves with grade 1 liver damage ( $n=38$ ) was  $1.48 \pm 0.46$ , grade 2 liver damage ( $n=656$ ) was  $1.54 \text{ kg} \pm 0.35$  and grade 3 liver damage ( $n=243$ ) was  $1.44 \text{ kg} \pm 0.39$ . It was concluded that grade 3 liver damage caused a significant reduction in live weight gains. The 95% confidence interval for the difference in average daily gain was  $0.11 \pm 0.047 \text{ kg}$  less for animals with grade 3 liver damage than the normal population and the infected animals were fed  $7 \pm 4$  days longer. (unpublished data)

## IMMUNITY

It has been reported that sheep and cattle do not develop strong immunity to infection with *F. hepatica*, or to re-infections, and this lack of resistance in ruminants is believed to be associated with the inability of their macrophages to produce nitric oxide.

Several studies suggest liver flukes elicit immune responses typical of the Th2-type, with eosinophilia, IgE and IgG1 antibody production. It is thought that helminths are able to withstand the effects of some components of the Th2 arm of the host immune response, and although these

responses cause pathological damage to the host it can be tolerated over a long and sustained period.

Migrating and adult *F. hepatica* seem to secrete substances that may include Th2 responses, and products such as cathepsin L proteases appear to actively lessen Th1 responses. Adult flukes in bile ducts are thought to be immunologically safe, and can survive for many years at these sites, although they still secrete antigens, which may be responsible for maintaining a Th2-immune response during chronic fasciolosis.

From various vaccine trials with cathepsin L proteases a correlation between antibody responses, and protection has been reported. Naturally-infected animals produce high IgG1 antibody titres, controlled by the Th2 cytokine IL4, but little or no IgG2 at all. In contrast, both IgG1 and IgG2 are produced in vaccinated animals. The titre of IgG2 antibodies seem to be, in general, directly correlated with the fluke burden of animals and it was found that in vaccinated groups animals with the lowest liver fluke numbers had the highest IgG2 levels. This may inactivate parasitic enzyme activation, block parasite migration and feeding. It may also be detrimental to the parasites as they may also activate complement and enhance phagocytosis by macrophages. Sheep macrophages do not produce nitric oxide following binding to sera from infected animals, but they may become activated in the presence of IgG2. Eosinophils and neutrophils may also contribute to killing already damaged flukes.

It has been seen, however, that *F. hepatica* and *F. gigantica* have different immunomodulation and strategies to evade the host immune responses and it seem that in practice *F. gigantica* homologues of antigens with protective properties against *F. hepatica* may not necessarily protect animals against *F. gigantica*. This may therefore require presentation of such antigens in different adjuvant formulations, or administration regimes. ELISA has also very successfully been employed to study the serum antibody type response (total IgG, IgG1, IgG2 IgM and IgG in *F. hepatica* and *F. gigantica* infected sheep and cattle,

in a recent study, which may be worthwhile article to read (Phiri et al).

## DIAGNOSIS

Apart from the presence of typical clinical signs, suggestive haematological and biochemistry findings, typical macroscopic and histological findings the laboratory confirmation may be depend mostly of faecal sedimentation tests, serology tests (ELISA) and possibly in some regions of the world PCR tests. An ELISA test, produced by the Institut Pourquier, employing "F2" antigen purified from *Fasciola* extracts, is currently available for routine diagnostic use in South Africa.

It has been validated for the use on ovine and bovine serum, and bovine milk. Pooled and individual serum samples, and milk tank samples may be used, and the tests results/values may be indicative of the level of infection in herds. Many of the different ELISA's developed will be based on the use of different secretory and excretory products of flukes as antigens (e.g. Catepsin L cysteine). In some studies of *F. hepatica* infection in cattle a significant correlation was observed between the intensity of the infection, and the ELISA values obtained.

A very recent article published in 2008 by De Meulemeester et al reports on the findings of their quantitative and qualitative evaluation of sediment flotation technique, a copro-antigen ELISA and two indirect serum ELISA's. The sensitivities and specificities of these tests; and the influence of the level of infection and season, on the result were evaluated which makes for very good reading. Many other articles were published on the use of ELISA in other species such as humans, donkeys and water buffaloes.

Many recent advances were made in the field of molecular diagnostics. Polymerase chain reaction (PCR) tests were developed for the identification of the different snail species, which would be of great advantage in epidemiological investigations. PCR tests, including multiplex PCR and real-time PCR, were developed to diagnose/ identify flukes and fluke antigens, and used to study many different aspects of the immunology and epidemiology.

These findings were published in several articles e.g. identification of *F. hepatica* in *L. columella* and *L. viatrix* in snails, distinguishing between *F. hepatica* and *F. gigantica*, identification of *F. hepatica* in formalin fixed and wax embedded tissues of *L. viatrix*, and the measurement of interferon-gamma and (INF) interleukin-4 (IL-4) expression in *F. gigantica* infection in calves.

**CONTROL**

Treatment of infected animals will largely depend on the correct use of appropriate and registered anthelmintics. Fasciolosis may be controlled by reducing the populations of the intermediate snail host, or by appropriate anthelmintic treatment. Long-term snail control can be achieved by drainage of the habitat, but permanent destruction of snail habitats may be expensive and ecologically sensitive, or controversial, especially in widespread habitats.

their development; or to remove fluke populations (tactical treatment) at a time of heavy fluke burdens, or at periods of nutritional and pregnancy stress to animal. Use a liver fluke product group that works against the immature flukes, for any treatment of sub acute or acute disease as shown in the table below.

\* Subcutaneous injection. All the other products are given by the oral route

Pasture management can also be used in various seasonal, and minimal or maximal rotational systems, with separate or mixed grazing by sheep and cattle.

Control by means of vaccination has also been extensively investigated. Huge impetus is given to this research by the need to control zoonotic disease in humans, as human diseases is mostly associated with local endemic animal fasciolosis, and the spread of drug resistant liver flukes. In some parts there may be overlap, and concurrent *Shistosoma* sp infections may be seen. Some efforts are therefore also directed at producing vaccines, which could produce cross reaction between *Shistosoma* sp and *F. hepatica*.

During the past few years a number of proteins have been identified and investigated as potential candidates for vaccine production. These were tested in various different combinations and trials were conducted in many species of animals including mice, rats, rabbits, cattle and sheep. In some of these trials fairly high levels of protection was seen in sheep and cattle.

These proteins are fatty acid binding proteins (nFh12, rFh15, Sm 14), cysteine peptidase (cathepsin L1 and cathepsin L2), leucine amino peptidase, glutathione-S -transferase. More recent candidates are thioredoxin peroxidase, NK-lysin like molecule, cathepsin L3, cathepsin B cysteine proteases, thioredoxin reductase and enolase, which are under investigation.

It was found that many of these vaccines not only provided protection against the parasites but also resulted in reduction of the faecal egg counts, or production of non or poorly embryonating eggs.

Liver fluke product group	Recommended dose (mg/kg)		Minimum age in weeks at which the product is > 90% effective against the different stages of the fluke	
	Sheep	Cattle	Sheep	Cattle
Oxyclozanide	15	13-16	12	>14
Nitroxylnil	10*	10*	8	10
Rafoxanide	7.5	7.5	6	12
Closantel	7.5-10	3*	8-6	>12
Albendazole	4.75	-	>12	-
Clorsulon	-	2*	-	>12
Triclabendazole	10	12	1	1

When snail habitats are small and localised fencing of such areas, or annual treatment with a molluscicide, may be more feasible. Registered fluke anthelmintics may be used prophylactically (strategic treatment) to reduce contamination of pastures by fluke eggs at times most suitable for

Egg production seems vulnerable to the immunological response induced by vaccination. It is not clearly established to what extent reduced egg production would have on the transmission of eggs and this still needs to be mathematically investigated.

No articles on field studies of an effective vaccine have been reported so far. Two articles on the topic of vaccination, by G V Hillyer; and DP Mc Manus and JP Dalton, which make for excellent reading, are listed below in the references.

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