LIVER FLUKE RELEVANCE AND CONTROL STRATEGIES (SMALL STOCK AND CATTLE) – INCLUDING PRACTICAL APPLICATION AND LIMITATIONS OF FLUKE SEROLOGY

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Introduction

Fasciolosis is one of the most important parasitic diseases of domestic ruminants throughout the world causing economic losses due to liver condemnation, mortality, reduction in milk and meat production, secondary bacterial infections, interference with fertility and the expense of frequent anthelmintic treatments (Boray)

We still need a lot of extension work to teach our farming community that the efficient control depends on correct and integrated control measures based on the epidemiology of the disease.

Control programmes should concentrate on reducing the numbers of parasites in the host, and thus the consequent pasture contamination, by regular strategic anthelmintic treatments, and correct farm management including pasture rotation. These measures can also be supported by the reduction of the number of intermediate host snails.

No acquired immunity to the disease develops in the sheep nor can it be induced by immunogenic manipulation, thus sensible farm management and the use of anthelmintics are still the most efficient control measures in the hand of the farmer.

There is no recent data showing the economic impact of fasciolosis. The results of tests carried out 20 or 30 years ago cannot be extrapolated to the current situation. The prevalence of infections and the number of parasites present have decreased: former trials showing weight gain or increased milk production no longer correspond to the current epidemiological situation or the performance of animals (which has increased over the last few decades). The indirect effects of liver fluke are the most difficult to assess since these parasites seems to circulate very actively through herds.

In cattle, fasciolosis is currently a subclinical infection that affects the quality and quantity of milk production, the fertility of cows (infected cows show a higher number of non-fertilising artificial inseminations), the health of calves from mothers infected with flukes (their colostrums is of poor quality), and the quality of carcasses after growing and finishing since infected animals produce fattier carcasses. The metabolism of drugs is also modified in infected cattle. (Philippe Dorchies)

Analysis of infection risk

In any given herd and for any age group, the relation between factors can be used to assess the level of risk: none, negligible, low, moderate or high. The potential health danger of

parasites explains their distribution across various risk levels. The levels are always related to individual factors (age, physiological state, immune-competence, associated infections etc.) and environmental factors.

The risk is always very high for liver flukes: despite the existence of effective anthelmintics, the possibilities of re-infection are raised by the absence of protection immunity.

Whether or not a farm has a problem with fasciolosis depends on a number of factors:

- The presence of snails on the farm.

- The presence of suitable wet, marshy areas or ponds or puddles which can support snail colonies – the water must be slow moving or still and its pH neutral or slightly alkaline.

- Rainfall is essential to help wash eggs out of the faeces and into the water and to maintain the water bodies where the snails develop.

- Temperature is also critical; the development of the egg and the stages found within the snail will only occur at ambient temperatures above 10°C. The warmer the temperature the more rapid the development, although if it is too warm there is a risk that conditions may be too dry.

The parasite and its life cycle

Two species of the genera *Fasciola* is present in South Africa ie. *F. hepatica* and *F. gigantica.* The most prevalent specie is *F. hepatica. F. gigantica* is present in the hotter bushveld areas of Limpopo, Mpumalanga and Kwazulu-Natal. Cattle and sheep are the most important hosts but many other mammals such as kudu and impala are susceptible to infection and may play a significant role in the epidemiology of the disease. The intermediate host essential for the completion of the lifecycle is a lymnaeid snail. *F. hepatica* is more prevalent at higher altitude in the summer rainfall region of South Africa.

The time taken for development of the parasite in the snail depends on the prevailing weather conditions through the summer but it usually takes about three months for the fluke eggs to develop and the stages in the snail to reach maturity. Then as the temperatures begin to drop in the autumn, the parasite development is stopped. Over the cold winter months the snails (*Lymnaea runcatula*) burrow deep into the mud and hibernate. As the temperatures rise in the spring, both the snail and the fluke inside, start to grow and develop again. This leads to a flush of fluke on the pasture in the early summer and can lead to summer infection in cattle. Figure 1 shows a quick recapitulation of the life cycle of *F. hepatica*.

The intermediate host

L. columella is the intermediate host for *F. hepatica* and *F. gigantica* while *L. natalensis* is the intermediate host for *F. gigantica*. An exotic snail *L. truncatula* has been introduced through the international trade in aquatic plants and this mud snail plays a big role as

intermediate host for *F. hepatica*. In Australia Boray has found that *L. tomentosa* was the most efficient intermediate host for *F. hepatica*. Boray found *L. tomentosa* to be susceptible to *F. gigantica*. As far as we know L. tomentosa does not occur in South Africa but could easily be introduced through aquatic plants.
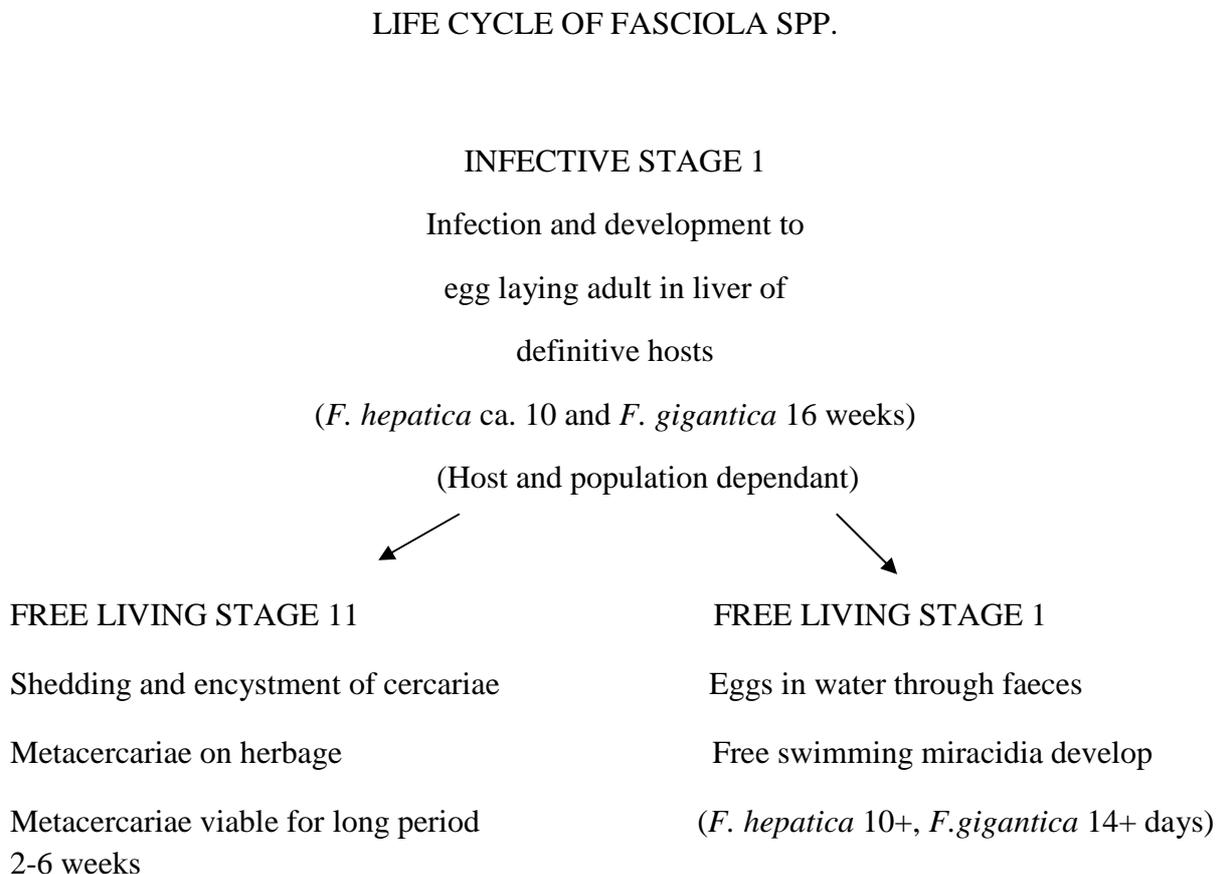
The definitive hosts

The most important hosts are sheep and cattle but horses, pigs, goats, game and rabbits may serve as alternative hosts. Significant differences exist in the development of the infection in sheep as compared to cattle. Very rarely does acute fasciolosis cause death in cattle whereas in sheep this is still a relatively frequent occurrence.

Cattle naturally exposed to fluke are repeatedly re-infected and age-intensity studies revealed that there was no evidence that cattle acquired resistance with age. When naturally infected cattle were challenged with an experimental infection, they were equally as susceptible as a group of naive cattle, again supporting the view that cattle do not acquire protective immunity.

This means that repeate drug therapy is required to reduce the build up of infection within a herd and within the environment.

Figure 1

LIFE CYCLE OF FASCIOLA SPP.

INFECTIVE STAGE 1

Infection and development to

egg laying adult in liver of

definitive hosts

(*F. hepatica* ca. 10 and *F. gigantica* 16 weeks)

(Host and population dependant)

FREE LIVING STAGE 11

Shedding and encystment of cercariae

Metacercariae on herbage

Metacercariae viable for long period
2-6 weeks

FREE LIVING STAGE 1

Eggs in water through faeces

Free swimming miracidia develop

(*F. hepatica* 10+, *F.gigantica* 14+ days)

(Temperature and moisture dependant)   (Temperature and moisture dependant)

INFECTIVE STAGE 11

Infection of Lymnaeid snails

Larval development in snails

(sprocysts, rediae, cercariae)

4-10 weeks

(temperature and moisture dependant)

Some obvious pathological differences between these two species following infection include the degree of calcification of the scar tissue, and hyperplasia of the bile ducts. Both of these reactions are more pronounced in cattle than in sheep. In sheep a low infection has been shown to survive for at least 11 years but a spontaneous recovery occurs in cattle. The recovery is due to the calcification of the bile ducts effectively starving the fluke (Keck and Supperer). This calcification is particularly noticeable from 19 weeks after infection in cattle (Dow et al). No similar findings have been found in sheep. Other hosts vary considerably in their susceptibility to fluke infections (Table 1)

Table 1 Some hosts of the liver fluke and their resistance status

Early resistance	Delayed resistance	Low resistance
Horse	Cattle	Sheep
Donkey	Buffalo	Goat
Wombat	Man	Hamster
	Red deer	Mouse
	Fallow deer	Rat
	Rabbit	Posum
	Hare	
	Kangaroo	
	Wallaby	
	Guinea Pig	

Many wild animals are also susceptible to the parasite and on pastures contaminated by livestock they may become infected. *F. hepatica* is regarded as one of the most pathogenic parasites of wild life (Presidente).

There is evidence that the role of the rabbit may be limited in the epidemiology of fasciolosis in Australia (Boray). Generally, domestic animals other than sheep and cattle together with wild animals as reservoir hosts may become more important in certain areas when eradication of fasciolosis will be attempted. However it was shown in epidemiological studies that the most important factor contributing to the continuous contamination of pastures is chronically and sub-chronically infected sheep with an average egg output of 2 000 000 per animal per day and most attention should be paid to that host in the general control of the disease.

Physiopathology of fasciolosis (Grace Mulcahy, University College Dublin)

The mechanisms underlying differential host susceptibility to *F. hepatica* infection are thought to involve differences in the fibrosity of the liver, the extent to which fluke migration through the liver parenchyma can be retarded by this and the extent of the host inflammatory response.

In cattle, experimental infection with a single dose of metacercariae results in a proportion of flukes developing to give rise to patent infection in bile ducts 8-10 weeks later, while others remain at various stages of immaturity, and some remain as small newly-excysted juveniles.

In sheep a similar experimental infection results in flukes developing at more or less the same rate, all reaching patency at about 10 weeks post-infection.

In cattle, even relatively high infectious burdens almost always give rise to chronic, rather than acute, disease. In sheep there is a strong relationship between numbers of fluke present and severity of disease. This results directly to the amount of hepatic tissue involved, together with the extent of traumatic blood loss.

<u>Pathological mechanisms</u>

Migration through the host tissue is accomplished with the aid of backwardly projecting spines on the parasite tegument and also secreted proteolytic enzymes. Anaemia with resultant iron deficiency is due to haemorrhagic tracts and hematophagous regimen of adult flukes.

Excretion and secretion products (eg. cathepsin –Ls, thioredoxin peroxidase (Tpx), proline) serve in aiding the parasite to digest host blood and tissue. Tpx are known to subvert host responses through various mechanisms including cleaving antibody molecules, reducing lymphocyte activity and inducing alternative activation of macrophages. The amino acid proline is produced by mature flukes in bile ducts and may be involved in inducing the hyperplasia of bile duct epithelium.

Host inflammatory and immune responses

F. hepatica infections are associated with induction of strongly polarized Th2 responses. There is no evidence that these are protective against re-infection. Large deposits of fibrin form on the surface of the liver and lead to adhesions developing.

Damage to hepatic parenchyma can be measured by serum levels of liver enzymes. Serum glutamate lactate dehydrogenase (GLDH) is used as a measure of the acute stages of infection while gamma-glutamyl transpeptidase (GGT) indicates chronic infection with bile duct damage.

There is evidence that *F. hepatica* renders animals more susceptible to infection with other pathogens (eg. *Salmonella* and *Clostridium* spp.). Evidence suggests that immune-regulation by flukes is involved such as polarization of the immune response to a Th2 and Treg (regulatory or suppressive) phenotype. *Clostridium novyi* has been isolated from livers in South Africa (Maryke Henton, personal communication).

Extensive hepatic lesions

In the acute stages of infection, the liver reflects the rapid migration of flukes with haemorrhagic tracts along the migratory routes.

As the infection progress, repair processes begin and the haemorrhagic tracts resolve into fibrotic tracts. Widespread scarring occurs resulting in contraction. Fibrosis occurs not only in the vicinity of the tracts but also more widely.

As the flukes begin to migrate into the bile ducts, hyperplasia of the bile duct epithelium takes place. In cattle, calcium deposits are layed down around the flukes in the bile ducts resulting in a pronounced stiffening of the ducts (cholangitis with calcification).

Microscopically, there is an infiltration of mononuclear cells, particularly eosinophils, into the affected areas together with extensive fibrosis. There is also a marked increase in the size of the hepatic lymph nodes.

Summary

Anaemia- haematophagia by adult flukes in dairy cows haematocrit below 26-27%

Hyper-leucocytosis and eosinophilia

Hypoalbuminaemia – in severe cases albuminaemia of less than 10g/l can cause decrease in oncotic pressure leading to oedema in lower regions of the body.

Globulinaemia – indicators of inflammation (IgG, IgA, IgM are sometimes increased)

GLDH increased – hepatic damage

GGT increased – chronic infection of the bile ducts (above 50 IU/l)

Immunity and permanent adaptation of the fluke (Diana Williams, Liverpool School of tropical Medicine, UK)

Some thirty years ago work at Glascow Veterinary School suggested that cattle could acquire a degree of resistance to re-infection with *F. hepatica*. The experimental data obtained in the early 70's led to the precept that cattle acquired immunity to *F. hepatica*. However this has

not been borne out by observations from the field. Cattle naturally exposed to fluke are repeatedly re-infected and age-intensity studies revealed that there was no evidence that older cattle had lower worm burdens, i.e. there was no evidence that cattle acquired resistance with age.

When naturally infected cattle were challenged with an experimental infection, they were equally as susceptible as a group of naive cattle.

Longitudinal studies of cattle exposed to high levels of infection showed that worm burdens fluctuated over time suggesting that adult flukes may be lost from the bile ducts but that cattle were also rapidly re-infected. Whether this apparent self-cure was due to an immune response or the senescence of the flukes is not clear. What does appear to be certain however is that cattle can be re-infected if exposed to *metacercariae* at any stage of their lives.

This means that repeated drug therapy is required to reduce the build up of infection within a herd and within the environment.

Helminth infections typically are controlled by what is described as Type 2 helper T cell (Th2) responses.

It is surprising therefore that following vaccination with specific fluke antigens a protective immune response characteristic of a Th1 response develops. These results suggest that flukes actively divert the host's immune system to favour their survival.

Cattle chronically infected with *F. hepatica* show a polarised response. However, *F. hepatica* has evolved a number of escape mechanisms. It secretes enzymes that can cleave antibodies bound to its surface and changes its surface antigens. This enables it to migrate through the liver parenchyma and, later, to survive in a hostile environment (i.e. bile ducts).

Good immune reaction but no vaccination in near future

Several antigens of *F. hepatica* have been investigated to determine if they can elicit a protective immune response. To date, variable levels of protection have been observed in vaccinated cattle and sheep, ranging from 57% to 72%. None of these antigens has so far reached the field trial stage, thus a commercially available vaccine against liver fluke is still a long way off.

Serology

Screening for fasciolosis can be achieved by; passive haemagglutination, indirect immunofluorescence or ELISA.

Individual serology is better than examinations performed on pooled samples that lose sensitivity due to dilution.

Positive test results from all or some of the animals are a sign of parasite exposure.

The antigens are made up of excretion/secretion (ES) products from flukes (Pourquier kit, Montpellier, France). For the last 40 years the presence of anti "f2" antibodies in humans has been considered as a serological evidence for the liver fluke (Biguet and Coli)

These tests are specific (95 to 98%) and sensitive (96-98%) and turn positive two to six weeks after infection. Antibodies persist for two to six months after the disappearance of adult parasites.

Serum and milk samples (individual or bulk) are tested with the ELISA method determining f2 antibodies. There is a correlation between the test result and infestation level when individual sera is used.

It must be remembered that a positive test indicates that an animal or herd is infected or has been infected, but may have been infected, but may have been treated in the two to six previous months.

It is possible that antibodies detected result from an infection that did not end up in the installation of adult flukes in the bile ducts.

Cattle susceptibility is fairly low. Only 10% of ingested metacercariae reach adulthood. The remainder is destroyed, thus freeing antigens, which can explain positive serology in animals that do not harbour liver flukes in their bile ducts.

These tests may be used on bulk milk samples but with a marked drop in sensitivity for example, using f2 antigen, infection is detectable when prevalence within the herd exceeds 60%.

The potential benefits of method for diagnosis have been screened by Torgeson and Deplazes (2005). These authors compared five methods in 1807 cattle for which the overall prevelance was 18,2%.

Sensitivities of the different methods were:

93,7% for examination for eggs in the gall bladder

88,2% for ELISA-Ab

69,6% for faecal examinations

64% for routine slaughterhouse inspection by health officials

See addendum 1: **Immunological diagnosis of Fasciolosis by Elisa method in serum and milk (Institut Pourquier).**

In a trial conducted at the Intervet/Schering-Plough Malelane Research Unit it was shown that the immune response can be detected between 7 and 14 days after infestation with 400 metacercariae of *F. gigantica*. The animals will become sero-negative after approximately 12-13 weeks after treatment with a drug that eliminates the liver fluke infestation.

Control measures

Netherlands (Fred Borgsteede, Division of Infectious Diseases, Animal Sciences Group, Lelystad, The Netherlands)

Distribution

Distribution of liver fluke positive farms based on faecal egg counts was mapped.

Results from liver condemnation are another useful parameter in determining the prevalence.

Analysis of parasitic risks

- Release of *miracidiae* from eggs occurs between June and October (summer) infecting snails.

- Development in the snail is temperature dependant. First *cercariae* are released 2 months later under favourable conditions and encyst as *metacercariae*. This is the autumn infection.

- Shedding of cercariae stops when the temperature drops below 10°C.

- Infected snails may survive the winter and starts shedding in spring when temperatures rise above 10°C.

- Autumn metacercariae may survive on the vegetation during the winter months, depending on climate condition, thus contributing to the spring infection.

Forcast of *Fasciola hepatica* infection

- Animal Health Services and Animal Sciences Group visit farms with a history of fasciolosis and harvest snails. The presence of larval liver flukes and their developmental stage is identified. This gives information of their shedding time and contamination of the pasture.

- A forecast formula that takes rainfall into account is used. A very wet day has much less influence than a period of rainy days with lesser precipitation.

Diagnosis and therapeutic strategies

- Common way to diagnose liver fluke is by means of faecal examination.

- Because of the long prepatent period of liver fluke blood samples for serological tests are taken from calves, heifers and cows in November.

Available molecules and recommended periods for treatment

- Only products for treatment of non dairy animals are available. It is forbidden to use flukicides in animals that produce milk for human consumption. Triclabendazole and clorsulon are registered for the treatment of liver fluke in cattle in the Netherlands.

- Treatment is usually given during the winter period. Dairy cattle are advised to treat at the start of the "dry" period.

<u>Resistance against flukicides</u>

1998/1999: Triclabendazole treatment was ineffective on a farm with mixed sheep and cattle population. Now the number of farms has risen to 20.

<u>Alternative measures for the control of liver fluke</u>

- Locate snail habitats and avoid grazing these areas during high risk periods.

- If possible fence these areas off.

- When there is a possibility to lower the ground water level, this may decrease the chances for survival of the snails.

- In the past, molluscicides had been used to control the snail population, but they are now banned due to the environmental damage.

- Vaccination could be a good alternative to the flukicides, but there is still a long way to go to reach that goal.

Anthelmintic treatment

Adulticide: The drug only affects adult parasites (8 to 10 weeks and older) living in the bile ducts

Larvicide: Affecting migrating larvae within the hepatic parenchyma as early as the sixth week

All stages as early as the second week

Halogenated phenols: nitroxynil

Nitroxynil, given by subcutaneous injection at a dose of 10mg/kg, is active against flukes between 6 and 8 weeks old. It inhibits two key enzyms, malate dehydrogenase and succino dehydrogenase. This product uncouples oxidative phosphorylation. In less than three hours, the liver fluke experiences spastic paralysis.

Salicylanilides: closantel, rafoxanide and oxyclozanide

These actives bind strongly to plasma albumin.

Salicylanalides interfere on oxidative phosphorylation which leads to metabolic changes. The initial effects of closantel affect glycolysis. Lesions are created in the tegument and the liver fluke experiensis spastic paralysis as a result of the penetration of calcium ions in muscle cells. In South Africa oxyclozanide has no milk withdrawal period.

Benzol-sulfamide: clorsulon

The active stops glycolysis which is the main metabolic pathway used by the fluke for energy production resulting in flaccid paralysis.

Benzimidazoles: Triclabendazole and albendazole

Benzimidazoles act specifically by fixing beta tubuline and subsequently inhibiting microtubule formation and modifying synthesis of proteins involved in RNA synthesis. They also induce lesions in parasite intestinal cells, cessation of feeding and egg laying by the fluke.

Triclabendazole

Triclabendazole differs in its chemical make-up from other benzimidazoles by the lack of a carbamate nucleus, by the presence of chloride ions and additional thiomethyl groups, and most of all in its non-planar spatial configuration which makes it more target specific. The drug is administered at a dose of 12,5 mg/kg in cattle, and is the only anthelmintic specifically active against liver flukes over two weeks of age i.e. against all intra-parenchymatous migrating larval stages as well as against adults within the bile ducts. Its activity is due to triclabendazole-sulfoxide, its main metabolite.

Albendazole

Albendazole is effective against adult flukes over 12 weeks of age at a dose of 10mg/kg.

Triclabendazole plus oxfendazole

This combination has a synergistic effect against 2-week old immature liver fluke (Boray, 1998).

Liver fluke resistance to anthelmintics have been described in sheep:

New South Wales – rafoxanide, closantel and nitroxinyl

Western England and Wales – salicylanilides

Australia, Ireland, Scotland and the Netherlands – triclabendazole

Resistance is seen primarily in immature forms and rarely in adults.

If resistance is suspected, it may be wise to ensure the efficacy of the anthelmintic by performing routine faecal examinations 3 weeks post-treatment.

The development of resistance may be delayed by alternating use of triclabendazole with the combination drug triclabendazole plus oxfendazole.

Overall control strategies

The liver fluke requires an intermediate host. Pevention of fasciolosis can only be effective if the parasite is attacked on two fronts: in the animal, via administration of fasciolicides and external control of known or potential habitats.

Basic principles

- Suppressive chemotherapy

Protective immunity does not develop following parasite elimination. Recontamination occurs rapidly and repeated treatments are required.

To optimize the efficacy of strict adulticides such as oxyclozanide, albendazole and clorsulon, 2 treatments 8 to 10 weeks apart are recommended. The adults present at the time of the first treatment are eliminated and the inhibition that they were exerting on the development of immature forms is removed. Thus, immature flukes that have matured into adults by the time of the second treatment are also eliminated.

- Knowledge and control of snail habitats

  Identify risk

  ➤ Water sources: Rivers, vleis, fountains, dams, leaking water troughs and pipes, irrigation.

  ➤ Serology, egg counts, necropsies.

  ➤ Mapping of potential snail habitats on the farm.

  ➤ Identify the risk of floating metacercariae.

  ➤ In identifying habitats, the presence of snails allows an estimation of risk level.

  ➤ Drainage is the most effective solution. It is not always a cost effective solution in pasture areas.

  ➤ The use of fencing around wet areas and snail habitats is often the easiest solution but requires an alternative drinking source. Cleaning water troughs at regular intervals should be done.

  ➤ The use of molluscicides

  ➤ Meteorological data

  ➤ The following data should be recorded: Rainfall data, minimum and maximum temperatures.

  ➤ Water snails become active when temperatures rise above 10°C.

  ➤ Basic data:

  Infected snails can overwinter thus causing spring shedding of cercariae.

Metacercariae formed in autumn can survive during the winter period if there is sufficient moisture available and infect animals when grazing in infested areas.

- Control snails

- Clean water troughs weekly, ducks, water birds and fish could help to keep the snail population down.

- Copper sulphate, salt???? Beware of the negative effects of copper and salt.

- In the past, molluscicides (sodium pentachlorophenate, dinitro-orthocresol, N-trityl-morpholine, calcium cyanamide at a dose of 500 Kg per hectare) had been used to control the snail population, but they are now banned in most countries due to the environmental damage.

- Buying in of animals (quarantine treatment)

- To prevent introduction of liver fluke, treat animals with triclabendazole before introducing them into the herd

- Flukicides

- Know the action of the drugs you are going to use (active against adults, juveniles?0

- Oxyclozanide is the only drug that is registered for use in dairy cows in milk.

- Strategic treatment

- Prevent the infection of water snails with miracidia i.e. treat animals with a drug controlling liver fluke before they come in contact with areas where the intermediate hosts are present.

- Tactical treatment

- When animals are grazing liver fluke infested pastures, treat them every 56 days with triclabendazole.
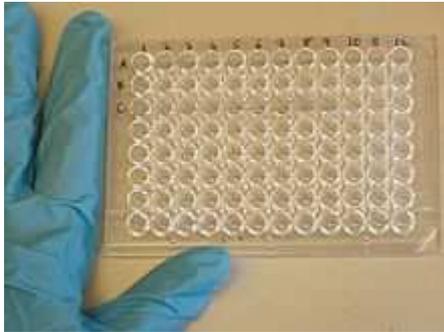
- Target selected treatment

- Treat only animals with clinical signs of parasite infestation: weight loss, bottle jaw, anaemia, drop in milk production, diarrhoea.

Addendum 1

Immunological diagnosis of Fasciolosis by Elisa method in serum and milk (Institut Pourquier)

ELISA

From Wikipedia, the free encyclopedia

A 96-well microtiter plate being used for ELISA.

Enzyme-linked immunosorbent assay (**ELISA**), also known as an **enzyme immunoassay** (**EIA**), is a biochemical technique used mainly in immunology to detect the presence of an antibody or an antigen in a sample. The ELISA has been used as a diagnostic tool in medicine and plant pathology, as well as a quality-control check in various industries. In simple terms, in ELISA, an unknown amount of antigen is affixed to a surface, and then a specific antibody is applied over the surface so that it can bind to the antigen. This antibody is linked to an enzyme, and in the final step a substance is added that the enzyme can convert to some detectable signal, most commonly a colour change in a chemical substrate.

Principle of the test

- The wells of the polystyrene microplate are coated with "f2" antigen (only the even-numbered columns are coated with the specific antigen).
- The samples to be tested are diluted and incubated in the wells. Any antibodies specific to "f2" antigen present in the serum or milk will form a "f2" antigen antibody immune-complex and remain bound in the wells.
- After washing, a Peroxidase conjugated anti-ruminant IgG antibody is added to the wells. This conjugate will bind to the immune-complex.
- After another washing, the enzyme substrate (TMB) is added to the conjugate, forming a blue compound becoming yellow after blocking. The intensity of the colour is a measure of the rate of antibodies present in the sample to be tested.

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