FLUKES: OLD PARASITES BUT NEW EMERGENCE

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1. INTRODUCTION

Flukes of ruminants are flatworm (trematodes) parasites living in the liver, fore stomachs or blood vessels, of which 40 different species may be present. Many other trematodes are parasites of fishes, poultry, carnivores and man. The liver fluke, *Fasciola hepatica*, was first described in France by Jean de Brie (1379), in his treatise on wool production and sheep management for the king Charles V. He made mention of the disease “liver rot” in sheep but did not actually describe the appearance of the worm in this book. He did not associate the liver fluke with the disease but thought that this was a consequence of the liver being affected by toxic substances produced by certain plants eaten by the sheep (Dalton, 1998). Since these times, flukes of cattle have been extensively studied: *Fasciola hepatica* and *Dicrocelium lanceolatum* in the liver, *Paramphistomum daubneyi* in the fore stomach are widespread in countries with temperate climates. In this paper, tropical species will not be covered, examples of these include *F. gigantica* and *Paramphistomum explanatum* of the liver, *Eurytrema pancreaticum* of the pancreas, *Cotylophoron* sp, *Carmyerius* sp etc from the upper alimentary tract, *Homalogaster* sp of the large intestine. *Schistosoma bovis* and *S. nasale* etc are living in blood vessels. Worldwide, 700 millions of cattle are exposed to liver flukes, annually and the annual cost is 3,2 $10^9$ US$ (WAAVP 2005).

The effects of clinical fasciolosis are well known but those of sub clinical fasciolosis are often unnoticed, leading to marked economic losses: reduced live-weight gains, milk yields and fertility. It has been calculated that bovine fasciolosis represents a median loss of 299 € per infected cow (Schweizer et al. 2005).

A very new aspect of the subclinical pathogenicity was uncovered with research on immunomodulation of host-cell response. Experiments in mice on concomitant infection with *Bordetella pertussis* and *F. hepatica* clearly demonstrated a suppression of antibacterial immunity during *F. hepatica* infection as a consequence of bystander down regulation of the *B. pertussis*-specific Th1 cells by the parasite-specific Th2 cells. Moreover, excretory-secretory components of *F. hepatica* may also exert direct immuno-suppressive effects on antibacterial immunity, independent of Th2 cells, through the activity of proteinases on immune molecules (Brady et al. 1999; Dalton et al. 2005). We are deeply persuaded that this mechanism of interference by parasites must be a significant impediment to achieving efficient immunization of animals against bacterial
and viral diseases, as the development of the Th1 protection is impaired by the Th2 pathway, induced by ectoparasites and helminths. When the reaction is triggered by parasites to IL-4 production (Th2) it has been demonstrated that the INFγ pathway (Th1) cannot be activated and then reactive against bacteria and virus (Maizels, 2004).

Until the late sixties, it was very difficult to fight against flukes because there were few drugs and the most were of low efficacy and high toxicity (carbon tetrachloride). Since that time efficient drugs have been developed and it is possible to expel these parasites. Nevertheless, despite of repetitive treatments, the prevalence of *F. hepatica* remains very high, *D. lanceolatum* and *Paramphistomum daubneyi* are more numerous and more frequently found in cattle. Why these old parasites are more prevalent? What are the causes of this new emergence? Some answers developed in this paper will give tentative explanations and also the means of planning efficient control of these infections while avoiding drug resistance, which has been described in several countries.

2. EPIDEMIOLOGY

Global market, constraints of European Common Agricultural Policy and societal demands force production systems to change. Sustainability is one of the major trends expected in the future. The growth of organic farming, extensification and the increasing opportunity for grazing all year round in some regions, may explain the considerable modifications of the parasite fauna and consequently the epidemiology of parasitic infections. Sometimes, the massive use of drugs induced the development of resistance, which will be of great concern if it spreads. Moreover, in many countries, the awareness of farmers concerning this economically important parasite is low. In Switzerland, 72.2% of the farmers are unaware of the fasciolosis in their animals (Schweizer et al. 2005).

The life cycles of flukes are always indirect, involving one or two intermediate hosts before invasion of definitive hosts. They are narrowly dependent of their close environment (nature of the soil), and of the climatic conditions for the survival and multiplication of the intermediate hosts and also for the survival and evolution of larval stages (miracidium, sporocyst, redia, cercaria and metacercaria). In the future, climatic change may have serious consequences on some parasites such as *D. lanceolatum*, which is more prevalent in dry areas than in wet, while the opposite pertains for *F. hepatica* and paramphistomes.

2.1 *Fasciola hepatica*

Snails of genus *Galba* (*Lymnea*) are amphibious and allow the fluke’s larval evolution. Due to clonal multiplication inside the snail from one miracidium entering the snail, 300 to 1772 cercariae may be produced. The first cercarial shedding takes place 7 to 8 weeks after snail infection. Not all snail populations are able to sustain the complete larval development of *F. hepatica*: snail mortality, prevalence of snail infection and number of cercariae produced are very variable according to the location (Dreyfuss et al. 2006). There is a 6 to 8 days periodicity in cercarial shedding: the number of cercariae per shedding wave peaks at the second wave and subsequently decreases up to the fifth wave (Vignoles et al. 2005). The number of *cercariae* of cattle origin are higher than those of nutrias, rabbits or sheep origin (Vignoles et al. 2004).

Great variations in prevalence of fasciolosis appear, from surveys in different countries: prevalence is very high in wet countries with mild temperatures, as in Ireland, but in other areas it is difficult to know the true level of infection. In central France, between 1990 and 1999, 11.3 to 25.3% of the cattle faeces was positive for liver fluke eggs (Mage et al. 2002): there has been no apparent decrease but annual variations occur. Significant variations have been observed in south-western France after the severe drought in 2003. Comparison of prevalence of *F. hepatica* by epg in cattle
sampled, either in the cattle hospital at the Veterinary School (Toulouse) or in a veterinary practice close to the Pyrenees mountains (Pamiers, Ariège) showed significant differences before and after drought. Prevalences for 2002 (oceanic weather), 2003 (very hot and dry) and 2004 (hot and dry weather) were respectively 8.9, 1.6 and 1.6% (p < 0.001).

Some of the apparent variations in prevalence may be related to the method used to detect flukes. In a survey performed in the 15 regions of France where most of the cattle are found, 1303 heifers or recently calved young cows were submitted to faecal examination and specific ELISAs were performed on sera. The results indicated that cattle shedding eggs were present in 20% of the herds and specific antibodies in 93%. This survey confirms that liver fluke is present and actively circulating in herds.

Information about the prevalence in several countries is given by Genchi et al. (2000) who were working on the one and half million beef cattle annually imported into Italy for fattening, mostly of France (one million of 6-8 months calves, prevalence of infection from 3 to 40%). The Charolais breed is more frequently infected (30-40%) then Limousine or cross breeds (Donn & Maricci, 1996; Genchi et al. 1997, 2000). The highest prevalence is observed in heifers coming from Austria (> 90%), the lowest from Poland and Hungary (1 to 7%) (Pietrobelli et al. 1995; Genchi et al. 1997). In dairy cattle in Switzerland, the prevalence of infection is over 16% (Schweizer et al. 2005). In the north central region of Portugal, the mean herd prevalence is up to 48% (Conceicao et al. 2004).

In countries where the reason why livers are condemned is given to the owners, the percentage due to liver fluke infection is important information. In The Netherlands, between 1958 and 1970, the percentages were 25 to 45%, it decreased sharply from 1970 to 1985 and since that time it is close to 10%. These results explain why it is difficult to eradicate liver fluke in dairy cows: Dutch breeders are very well experienced and they benefit from a highly developed forecasting system for liver fluke as developed in others countries in Europe: Britain and Ireland (O’Brien, 1998). Breeders obtain a forecast for their region, nevertheless it seems impossible to reduce dramatically the prevalence of F. hepatica in cattle.

Clinical fasciolosis has been found in cattle in East Anglia, with signs including weight loss, diarrhoea, decreased milk yield and occasionally death, during the winters of 2001 to 2003: 14 cases during winter 2001/02 and 22 the year after (Pritchard et al. 2005). In this region, a drier region of Britain, the emergence of fasciolosis was attributed to recent higher summer rainfall which favoured the intermediate host snail and the free-living stages associated with the increased influx of sheep from endemic fluke areas for seasonal grazing. With an ELISA applied to bulk-tank milk, prevalence in dairy herds in England was estimated to be 48% and in Wales 86% (Salimi-Bejestani et al. 2005).

It may be presumed that the survival of liver flukes in many areas may be related to several factors: Extensification of breeding and the use of lowlands where snails may actively multiply. With a shortage of farm labour and pressure of work, drains and ditches are not cleaned as in years ago; they overflow onto pastures and create many areas favourable for snails. The danger is highest close to organic farms because water borne metacercariae may be transported onto neighbouring pastures where herds are under strict fasciolosis control regimen.

There are more and more wild ruminants: roe deer, red deer, rabbits, hares and nutria, which are reservoirs of flukes. Their true role is not often taken into account: for example in western parts of France 55% of Myocastor coypus (Nutria) and 34% of Oryctolagus cuniculus are infected and nothing is done to control them (Ménard et al. 2000).
Drugs are used extensively but in the most of herds the weight of animals is not accurately checked and animals are frequently under dosed. The efficacy of drugs is often calculated on geometric means that is to say the true efficacy is not as high as claimed: even after an effective treatment i.e. more than 95% of a given parasite eliminated (WAAVP guide lines), a small burden is still present and egg laying with obvious consequences!

No drug is 100% efficient even in absence of resistance. Adulticide drugs do not expel the small population of immature flukes, even if this percentage is low (5% in our experience), this parasitic burden ensures the survival of the infection.

The efficacy of drugs may be modified by the damage to the liver parenchyma caused by *F. hepatica*. This effect on drug pharmacokinetics have been clearly demonstrated.

Finally, it has to be stressed that drugs are effective, (except in few areas where anthelmintic resistance exists), but there is no quality assurance with their use: calibration of the drenching gun or of syringe, weighing of the animals, epg to monitor the efficacy etc and nothing is done to avoid quick re-infection of cattle after treatment.

### 2.2 *Dicrocoelium lanceolatum*

As opposed to *F. hepatica*, this worm has xerophilic intermediate hosts: snails and ants. Usually adult worms are very prolific and numerous eggs are found in faeces. Its pathogenicity is limited as the worms are not blood suckling and their tegument does not carry spines. Moreover, immature worms do not migrate through the liver parenchyma during the pre-patent phase.

For decades, this has been consider an important parasite in sheep and goats but was not considered to be pathogenic for cattle. Nevertheless, an increasing number of vets are reporting clinical signs related to infection with *D. lanceolatum*. An extensive study on cattle slaughtered in South western France between 1994 and 1996 indicated that 11 to 32% of the livers were infected by *D. lanceolatum* (Alzieu et al. 1998). According to Genchi et al. (2000), 10% of French beef cattle imported into Italy are infected. The apparent discrepancy between the results of Alzieu & Genchi may be related to the age of cattle. In the Charolais area, 7% of cattle samples are positive but most of them with less than 5 epg (Baudin et al 2005). In North western Canada, this parasite is spreading (Colwell, 2005).

Until now, there are very few papers on this topic but field observations are confusing and in the future particular attention should be given to this tiny worm living in small bile ducts causing liver lesions and altered blood parameters. Specific treatment with albendazole or with netobimin at high dosages clear animals and allow a return to normal blood parameters, normal growth rates and production: it is an indirect indication of the pathogenic role of *D. lanceolatum*.

### 2.3 *Paramphistomum daubneyi*

Prevalence of this trematode is increasing for twenty years in France. *P. daubneyi* has been identified in more than 30 Departments in France (Dorchies et al. 2000) and in central France 23 ± 10% of lactating cows and 37 ± 15% of dairy cows are infected (Smitz-Adigé et al. 2000).

The effects of paramphistomes on live-weight gains, milk yields and fertility are far less than those induced by *F. hepatica*. Nevertheless, many breeders and vets are convinced about the importance of this infection: bloat related to adult worms attached by their suckers to the wall of the reticulum and rumen is well known. Some cases of acute infection, with the death of calves have been described (Dorchies et al. 2000). Everywhere, the effects of paramphistomes are underestimated.
and they have to be considered, particularly when a risk of acute infection is present as the immature worms are very difficult to identify and there is no serological test available and during necropsy small lesions of abomasums and of ileum may be confused with other diseases. Adults are very prolific and many eggs are expelled: their number not being related to the parasitic burdens.

Some facts may explain the recent spread of paramphistomids:

The high numbers of available, *Lymnea truncatula*, snails, the intermediate host for both *F. hepatica* and *P. daubneyi*. This point has been discussed above for liver fluke.

There is no registered drug with claims for these flukes. Since the banning of bithionol oxide, the prevalence of paramphistomes has increased. Off label use of oxyclosanide, at a higher dosage (18.7 mg/kg), is indicated and effective against adult worms.

In conclusion, veterinarians should be aware of the diversity of the host range of trematodes. Up to now, it has been impossible to eradicate *F. hepatica* due to inappropriate usage of the available drugs and lack of action to help control the intermediate hosts. Extensification of breeding favours their spread. Other parasites are less pathogenic but have to be kept targeted for, even though acute infections by (*D. daubneyi*) or chronic infections by (*D. lanceolatum*) may have significant consequences in some areas there is no drug registered to control them.

3. **DIAGNOSIS**

Faecal egg counts are obviously the standard means of diagnosis of infection in cattle but these tests are useless when flukes are migrating and often then doing the most damage to the liver, also they are ineffective when the parasitic burden is too small for detection. Changes in hepatic enzyme levels may be an aid but changes in these parameters are not specific for parasitic infection. This is the reason why more immunological methods are being developed; but unfortunately many are experimental and not used in practice. Moreover, the presence of snails in wet areas of the pastures and identification of *parthenita* i.e. larval forms inside the snails may be useful in assessment of risks factors on a farm, this approach will be discussed later along with control systems.

3.1 **Faecal egg counts**

3.1.1 **Techniques**

Flukes have particularly heavy eggs and so do not float on water therefore it is necessary to use either liquids of high density for flotation techniques or to use sedimentation techniques.

McMaster technique (MAAF) is widely used, but sometimes modified according to the parasites being investigated. Modifications are related to the weight of the faecal sample, the specific gravity and the volume of the liquid (more or less dense) and on the surface examined on McMaster slide: one or two chambers, the whole surface or not.

Cringoli et al. (2004) compared 14 solutions of the highest specific gravity for validation of epg counting of gastro-intestinal strongyles and *D. lanceolatum*. The most reliable results were given by sucrose (specific gravity: 1.2 to 1.35) for gastrointestinal strongyles and iodomercurate of potassium (1.444) for *D. lanceolatum*. These results can be extrapolated to *F. hepatica* and Paramphistomids. Zinc sulphate solution (1.350) is not so reliable and if the specific gravity is increased to 1.440, the egg shape is distorted and colour modified and then it may be difficult to distinguish *F. hepatica* and *P. daubneyi*. Moreover it is not easy to read due to many artefacts. Cringoli stressed that the balance between the faecal sample and specific gravity must not be more than 1: 15 and egg counting must be done on the whole surface of the McMaster slide.
The sedimentation technique appears to be more accurate and sensitive than flotation techniques (Boray, 1969). In some cases, cup sedimentation using tap water is the simplest and cheapest but more time consuming compared to flotation techniques.

3.1.2 **Limits and reliability of coprological techniques**

There is a reluctance to use epg counts for identification of fluke infection due to limited accuracy, and false negative results related to low sensitivity (30% according to Happich & Boray, 1969). For *F. hepatica*, epg counts may be falsely negative:

- during the migration phase, immature worms passing through the parenchyma do not lay eggs,
- with low parasitic burdens the output is small and egg laying may be irregular and pass undetected.

Moreover, flukes are not prolific egg layers so the number of eggs is always very low (10 to 100 in cattle). It has been reported that if there are less than 20 flukes in bile ducts, the sensitivity of epg counting methods is too low to show eggs in the faecal sample.

Being aware of these limits, a positive result is the definite proof of an active infection and a focus of infection for the herd with eggs being released onto the pastures and infecting the snails.

Paramphistomids are very prolific and in many French herds counts of more than 1000 eggs per gram of faeces. Veterinarians should be aware of the variability of these results indeed sometimes a cow eliminating 2000 epg one day, may only shed 50 eggs the following one. There is not a true correlation between the parasite burden and the epg: according to Mage *et al.* (1998).

With *D. lanceolatum*, up to now, it is difficult to be definite about the significance of the counts because even with very low numbers of eggs (less than 50 epg). Practitioners report that they observe a clinical improvement after specific treatment! Nevertheless, this parasite is prolific and in sheep high faecal egg counts and high parasitic burdens are common, it is not usual in cattle.

Although faecal egg counts give a definitive positive diagnoses these should be used by vets in an epidemiological context. Moreover, coprological examination gives additional information on other parasites, which may be of great value in an overall approach to a Bovine Health Management Programme.

3.1.3 **Hepatic enzymes**

Before the development of immunological diagnostic techniques many researchers studied extensively damage to hepatic tissue and to bile duct epithelium and the effects of this on the hepatic enzymes.

Enzymes from hepatocytes are: glutamate deshydrogenase (GLDH) and glutamate-oxaloacetate aminotransferase. They indicate damage to hepatic cells, related to parasite migration. Their levels are increased during the migration of *F. hepatica*.

X-glutamyl transferase is present in the bile duct epithelium: its blood concentration increases after penetration of liver flukes into bile ducts, during the period from eight to twelve weeks after infection. This is followed by a decrease in this enzyme, frequently interrupted by brief increases,
between twelve to twenty weeks after infection (Wensvoort & Over, 1982). Several papers deal with the kinetics of these enzymes during fasciolosis: a good example is Gaasenbeek et al. (2001) illustrating the ongoing changes during experimental infection.

Despite the value of these tests, they are not usually used mainly because they are expensive and non-specific. Nevertheless, in some cases they may be helpful.

3.2 Immune diagnosis

Several methods are described, most of them relate to the ELISA test or variations but counter-electrophoresis, haemagglutination and indirect immunofluorescence tests may also be used. The majority of tests are applied to the detection of blood antibodies and the latest to the detection of antigen in faeces. Nevertheless, many cattle surveys are performed with the ELISA test alone without any confirmatory tests and under those circumstances false positive results may be considered as positive. According to Hillyer (1998) the need for immunodiagnostic tests for fasciolosis is much greater for humans than for livestock as “Definitive diagnosis of infection with F. hepatica is usually achieved parasitologically by finding the fluke eggs in faeces”, The question is therefore, “Why do we need immuno-diagnosis for liver flukes?”

There are several answers:

- due to the limitations of epg detection methods, it is compulsory to check if liver fluke is circulating or not in cattle. The risks related to wild animal spread are impossible to manage and their role in spread not known. Immuno-diagnosis may be useful in that situation,

- it may be necessary to identify the true period of infection and consecutively to treat animals with a flukicide efficient on very young immatures, late immature or on adult worms: the earlier animals cattle are treated, the lesser are the economic consequences of the infection,

- blood samples are taken for the diagnosis and follow up of several bacterial or viral diseases. It is a good opportunity to use these sera samples to look for other pathogens, if it is economically possible.

Only immunological tests for F. hepatica will be discussed in this paper as there are very few tests for other parasites. A specific ab-ELISA for paramphistomes has been studied in our lab without reliable results, Guillot (personal communication) had the same problem with an ag-ELISA. Colwell (2005) developed an ELISA for D. lanceolatum with crude ES antigen. Sensitivity and specificity were respectively 98% and 92%.

3.2.1 Serological tests for F. hepatica

An indirect haemagglutination test has been very well adapted to bovine fasciolosis (Levieux et al. 1998). It is reliable, cheap and easy to use. Moreover, it is applicable for sheep, horses, man, etc., because it does not necessitate a specific conjugate for each species. ELISA tests are currently used by the vast majority of investigators: this method is reliable, easy to do, cheap and also applicable on milk samples.

ELISA detection of antigens in blood

Detection of circulating antigens is a very good way to confirm the presence of living parasites in bile ducts. Several trial results have been published and they seem to be very promising. For example, Leclipteux et al. (1998) were able to detect the presence of infection in animals as early as
six days after infection. For unknown reasons, this test has not developed. A monoclonal antibody-based sandwich immunoassay (mAb sandwich ELISA) has been demonstrated to be a sensitive, specific and a suitable method for the detection of *F. hepatica* in serum in the diagnosis of active animal and human fasciolosis with monoclonal antibody ES 78 (Hybridome Laboratory, IPK) (Duménigo *et al.* 1999).

**ELISA detection of antibodies in blood**

Currently it is the most used assay with somatic f2-antigen (Institut Pourquier, Montpellier, France) with a crude excretory/secretory (ES) antigen or with purified/recombinant antigen.

ES products are produced by various methods. Briefly, *F. hepatica* freshly recovered from an infected animal are incubated at various temperatures, in saline or buffer, for a set time. It seems according to Hillyer (1998) that ES products are very similar whatever the mode of recovery.

Purified antigens obviate the occurrence of non specific positive reactions: Cathepsin L1, a cysteine proteinase from gut epithelial cells of flukes cleave immunoglobulin and inhibit antibody-mediated attachment of eosinophils to newly excysted juveniles. This enzyme is present in ES products and may be isolated (Smith *et al.* 1993).

ELISA antibody tests are reliable, with high sensitivity and specificity for example 95 and 98.2% respectively with f2-antigen (Reichel *et al.* 2005), 98 and 96% respectively with ES antigen containing predominantly fluke cysteine protease (Salimi-Bejestani *et al.* 2005). They allow detection of early infection (sometimes after the first week of infection) (Arias *et al.* 2006) and false positive results are limited when using purified antigen as shown in validation assays. On pooled sera, herd prevalence of infection as low as 5%, f2-antigen ELISA can be detected (Reichel *et al.* 2005). Nevertheless, positive results do not determine whether or not live flukes are present: detectable antibodies may persist long after treatment or after the natural death of liver flukes. A positive result indicates that the animal is, or has been, infected by the parasite, antibodies detected in natural infection may or may not be related to an active infection. Moreover it may be assumed that non-specific polyclonal stimulation of B lymphocytes induces production of specific antibodies: Abdel-Megeed & Abdel-Rahman (2004) described a *Toxocara vitulorum* antigen consisting of five polypeptides (137.7 kDa, 81 kDa, 75 kDa, 48 kDa and 21.6 kDa) which is cross reactive with *Fasciola gigantica*. Some years ago, cross reactivity between tuberculosis and fasciolosis was frequently observed in France.

Within these limits, detection of antigen, gives a very useful indication that animal is infected by living parasite.

### 3.2.2 Application of serological tests for *F. hepatica* to bulk tank milk

The detection of infected herds from bulk milk samples appears more difficult than by use of blood ELISA. Bulk milk ELISA results are consistently lower than the corresponding bulk serum ELISA results. Using a commercial kit with f2-antigen, Reichele et al 2005, demonstrated that only bulk milks from herds with infection prevalence of at least 60% were identified. Another test developed by salimi-Bejestani et al 2005 identified herds in which more than 25% of the cows were infected.

### 3.2.3 Detection of *F. hepatica* copro-antigens

The mAb sandwich ELISA described by Duménigo *et al.* (1999) also detects coproantigens, but, more recently an ultra sensitive capture method, using monoclonal antibody mAbMM3, produced by immunisation of mice with a 7 to 40 kDa purified and O-deglycosylated fraction of *F. hepatica*
ES (Mezo et al. 2004). The assay detected 100% of sheep with one fluke, 100% of cattle with two flukes and two of seven cattle with one fluke. Some false negatives were probably not detected because the flukes were immatures and so there were no ES products in the bile ducts. It appears that the copro-antigen concentration correlated positively with parasitic burden and negatively with the time after infection at which copro-antigen was first detected. Even in animals with low fluke burdens (1-36 parasites) the first detection of *F. hepatica*-specific coproantigens by the MM3 capture ELISA preceded the first detection in egg count by 1-5 weeks. After specific treatment, copro-antigen became undetectable from one to three weeks after treatment. This ultra sensitive method is also very specific with no cross-reaction even for animals infected by *D. lanceolatum*.

Unfortunately, mAbs are very expensive and not easy to purchase, production being limited to the need of the particular researchers. Sometimes, these trials are developed by PhD students and at the end of their experimental work, the method is not maintained for routine diagnosis.

It has to be stressed that the identification of antigen in sera or in faeces would be a very useful tool to control the parasitism allowing selective treatment of infected animals. Risks of anthelmintic resistance and residues would be decreased if these tests were available and inexpensive.

3.3 Conclusion: the best method of diagnosis of *F. hepatica*

Torgeson et Deplazes (2005) compared five methods of identification of infection: epg counts, egg determination in the gall bladder, ELISA for blood antibody and routine inspection of 1087 cattle in two abattoirs where the overall prevalence was 18.2%. Sensitivities were 69.6% (50.2-81.4) for epg, 93.7% (85.6-97.3) for eggs in gall bladder, 88.2% (80.5-92.6) and only (!) 64% (53.5-70.8) for routine inspection.

So, epg counts are reasonably acceptable in the case of chronic disease until a commercial Ag based ELISA becomes available!

4. CONTROL

Theoretically it is simple to teach control measures for flukes: don’t feed cattle with metacercariae! For a veterinary practitioner in the field it is much more difficult and it is necessary to:

- to select the best drug adapted to the epidemiological status of the herd,
- to identify the areas which are dangerous due to of the presence of the intermediate hosts, in order to avoid cattle picking up metacercariae,
- to convince the farmer that it is necessary to attempt simultaneously to control flukes and snails and not only use drugs to control the fluke in the cattle at that time!

4.1 Anthelmintics

4.1.1 *Fasciola hepatica*

In the EU, drugs available to fight *F. hepatica* vary according to different countries and the withdrawal period may be different for the same drug in two different countries. In France, some drugs are available for dairy and suckler cows but in most of countries drugs cannot be used in dairy cows.
Several benzimidazoles, salicylanilides, nitrophenols and halogenated carbons have been demonstrated as having a good efficacy either for liver adult fluke in the bile ducts, or on late immatures and adults or on all stages: early/late immatures and adults. Practitioners have to adapt their prescriptions to suit the system (beef cattle, dry cows, dairy or suckler cows) and to the period of infection: presence or not of early/late immature or adult flukes? All these concerns must be managed and it is not necessary to describe in detail drugs, their advantages and drawbacks. Only one emerging problem will be emphasised i.e. the anthelmintic resistance.

The problem for flukes is not as far advanced compared to resistance of nematodes to anthelmintics, and the emergence of relatively few cases of loss of efficacy is not yet a serious concern. In New South Wales, resistance is found to rafoxanide, closantel and nitroxynil, in western England and Wales resistance to salicylanilide has been described. Resistance is manifested against immature but rarely against adult flukes (Fairweather & Boray, 1999). This may be the reason why resistance to clorsulon has not yet been recorded. Loss of efficacy of triclabendazole (TCBZ) in naturally infected sheep has been reported in Australia (Overend & Bowen, 1995), in Ireland (Lane, 1998; O’Brien; 1998), in Scotland (Mitchell et al. 1998) and in the Netherlands (Moll et al 2000). Efficacy of TCBZ for the Dutch resistant strain is 10.8% and on a sensitive strain 99.8% (Gaasenbeek et al. 2001). As with nematodes, there is no reversion to susceptibility after switching to other drugs (Borgsteede et al. 2005). Mechanisms of TCBZ-resistance are not fully understood. It could be an enhanced biotransformation of TCBZ: *F. hepatica* can metabolise TCBZ sulphone into and relatively inert sulphone metabolite. This conversion is on average 20% greater in the resistant flukes compared with the susceptible flukes (Robinson et al. 2002, 2004). In order to gain some insight into the possible mechanisms of resistance, transtegumental diffusion of TCBZ parent drug and its sulpho-metabolites (TCBZSO and TCBZSO₂) into TCBZ-susceptible and TCBZ-resistant with an *ex vivo* device. It appeared that significantly lower (approximately 50%) concentrations of TCBZ and TCBZSO were recovered within the TCBZ-resistant flukes compared to TCBZ-susceptible ones. Moreover, as previously demonstrated by other researchers, the rate of TCBZ sulphonate metabolism into TCBZSO was significantly higher (39%) in TCBZ-resistant flukes. The development of resistance to TCBZ seems to be related to the altered drug influx/efflux and enhanced metabolic capacity (Alvarez et al. 2005).

### 4.1.2 Dicrocoelium lanceolatum

For cattle medicine, no drug is registered to treat dicrocoeliosis. Currently, vets use either albendazole (15 mg/kg) or Netobimin (20 mg/kg).

### 4.1.3 Paramphistomum daubneyi

No drug is registered but oxyclozanide is known to be effective either at 10.2 mg/kg or 18.7 mg/kg once or twice, two days apart (Alzieu et al. 1999). Farmers have to be informed that treatment induces a very severe diarrhoea, which lasts two or three days after administration.

### 4.2 Avoiding wet areas

Since years vets and breeders have been informed about the risk of infection in wet areas, along the banks of ditches, close to rivulets and drains. Despite many experimental works, development of forecasting systems and modelling epidemiology in order to predict outbreaks of fasciolosis even by Geographic Information System (Malone & Yilma, 1999) very little progress, if any, has been made in practice. Nevertheless, it is quite easy to locate the most dangerous areas of a pasture and to fence them off to avoid “feeding cattle with metacercariae”!
4.3 Snail recovery and confirmation of their infection

Snail recovery is easily achieved when they are active during spring and autumn. Identification of infection by immature stages of *F. hepatica* may be done by stereomicroscopy but also by specific PCR on snails (Losson *et al.* 2005; Crucher *et al.* 2006).

5. CONCLUSION

*Fasciola hepatica* had been a true plague for sheep farmers before the anthelmintic era. Currently, this infection seems to be under control in sheep and in cattle, although it remains a major problem in some areas, such as in the west of Ireland where serious losses occur annually. Nevertheless, the financial losses in dairy and lactating cattle are not well appreciated and an increase in productivity could be achieved with better control. Moreover, infection may have a negative effect on vaccination against bacterial or viral diseases. In most western countries, the actual prevalence of infection is very high, from 16 to more 90%. Until now, most of the drugs are still effective but anthelmintic resistance may spread. This is the reason why control of infection must include controlling the snail on pastures. Frescon was a highly effective molluscicide but is no available now although copper sulphate can be used with care and consideration for the environment in certain circumstances and if the best system of snail control is to fence areas where infection of cattle takes place or to use the area for alternative enterprises, however it is very difficult to convince breeders of this as anthelmintic have good efficacy and to treat because is less troublesome and time consuming. Other plagues are developing with *D. lanceolatum* and paramphistomes, their true impact is not very well appreciated but vets must be aware of the potential risks of infection by these worm.

6. ACKNOWLEDGEMENTS

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7. SUMMARY

The parasites *Fasciola hepatica* and *Dicrocœlium lanceolatum* both liver flukes and *Paramphistomum daubneyi* a fluke of the fore stomachs are frequently found in cattle in countries with a temperate climate. The effects of clinical fasciolosis are well known but consequences of sub clinical fasciolosis often go unnoticed, leading to major economic losses: reduced live-weight gains, milk yields and fertility. It has been calculated that bovine fasciolosis represents a median loss of 299 € per infected cow. Moreover, it has been clearly demonstrated that concomitant bacterial infection and *F. hepatica* induce a suppression of immune responses. Paramphistomes are less pathogenic but cases of acute infection may be severe and difficult to identify. *D. lanceolatum* has become more prevalent and sometimes has significant pathogenic consequences.

Traditionally, diagnosis of fasciolosis was made by faecal egg counts (epg) but has low sensitivity. Now several immunological methods are available. The Ab-ELISA systems are the most frequently used and give accurate indications of farm or herd prevalence but they do not give the individual current infection status. In the near future, Ag-ELISA must be developed to avoid drawbacks of Ab-ELISA. Immunological testing of bulk tank milk is used but its sensitivity is lower than serological tests.

Control of fasciolosis by means of flukicides has led in few cases in sheep to the development of drug resistance moreover no treatment is possible in milking cows in most of countries. Consequently, epidemiological assessment leading to judicious grazing systems and fencing or land drainage of infection foci, where the intermediate host snail abounds, must be employed.
No drugs are registered to control paramphistomes and *D. lanceolatum*, veterinary practitioners must adapt treatments safely, suitable for the particular enterprise and being aware of the dangers of drug residues.

8. KEY WORDS

*Fasciola hepatica*, *Dicrocœlium lanceolatum*, *Paramphistomum daubneyi*, epidemiology, diagnosis, control.

9. RESUME

*Fasciola hepatica* et *Dicrocœlium lanceolatum* dans le foie, *Paramphistomum daubneyii* dans le rumen et le réseau sont très fréquents dans les pays tempérés. Ils le sont aussi dans les pays chauds et humides cependant, cette présentation ne sera limitée qu’aux seuls problèmes rencontrés dans les pays tempérés. Les manifestations cliniques de la fasciolose bovine sont exceptionnelles alors que les conséquences de l’évolution de la forme chronique sont importantes et souvent méconnues par les éleveurs : baisse de croissance et de production laitière ainsi que de la fertilité. Il a été calculé que la fasciolose chronique provoque une perte économique moyenne de 299 € par vache laitière. Par ailleurs, il a été clairement démontré que lors d’infection bactérienne concomitante, *F. hepatica* induit une suppression de l’immunité antibactérienne.

Les paramphistomes sont moins pathogènes, mais les cas d’infestation aiguë sont sévères et difficiles à identifier. La petite douve devient de plus en plus fréquente et, dans certains cas, peut avoir des conséquences pathologiques qui rétrocèdent à un traitement anthelminthique.

Pendant de nombreuses années, le diagnostic de la fasciolose a reposé sur la coproscopie, cette méthode est de très faible sensibilité mais d’une spécificité absolue. Actuellement, plusieurs tests sérologiques sont utilisables, c’est l’ELISA-anticorps qui est le plus employé étant sensible, spécifique et peu onéreux. Il ne permet pas cependant de distinguer les animaux porteurs de parasites de ceux qui ont été traités quelques semaines ou quelques mois auparavant. Dans un proche futur, un test ELISA-antigène devrait permettre de n’identifier que les bovins infestés par des douves vivantes. Certains tests sont applicables aux laits de mélanges ce qui permet un dépistage de troupeau mais avec une très faible sensibilité.

La lutte contre la fasciolose est maintenant confrontée au développement de résistance aux douvicides dans un certain nombre de pays. En conséquence, la lutte contre la douve doit être associée à la clôture des zones dangereuses pour limiter le nombre de traitements et ainsi protéger l’avenir des molécules actives.

Jusqu’à présent, aucun médicament ne possède d’autorisation de mise sur le marché pour le traitement de la paramphistomose et celui de la dicrocoeliose. Les praticiens doivent adapter les traitements aux types de production pour éviter les problèmes de résidus.

10. MOTS CLES

*Fasciola hepatica*, *Dicrocœlium lanceolatum*, *Paramphistomum daubneyi*, épidemiologie, diagnostic, contrôle.

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