An Abattoir Survey to Determine the Population Profile in the Autumn of Fasciola hepatica in Condemned Bovine Livers from Ireland and the United Kingdom

F. MacGillivray[a]*

T. de Waal[b]

D. Maguire[b]

M.A. Taylor[c]

V. Boughtflower[c]

R. Daniel[d]

T. Jenkins[d]

B. Rice[a]

A.B. Forbese

[a]Merial Animal Health Ltd., Sandringham House, P.O. Box 327, Harlow, Essex CM19 5TG, United Kingdom
[b]School of Veterinary Medicine, University College Dublin, Dublin 4, Ireland
[c]Food and Environmental Research Agency, Sand Hutton, York, YO41 1LZ, United Kingdom
[d]Animal Health and Veterinary Laboratory Agency - Carmarthen, Job's Well Road, Johnstown, Carmarthen, SA31 3EZ, United Kingdom
[e]Merial SAS, 29 Avenue Tony Garnier, Lyon 69007, France
**Corresponding author, fiona.macgillivray@merial.com*

KEY WORDS: *Fasciola hepatica*; cattle; housing; developmental stages; flukicides

ABSTRACT

A study was conducted in three abattoirs, two in the United Kingdom and one in Ireland in the autumn of 2010 in order to determine the numbers of *F hepatica* in condemned bovine livers, and to classify the parasite stages present based on their length. The grazing and anthelmintic treatment history of the cattle sampled was unknown. In total, 126 condemned livers were processed, and of these, 108 had evidence of active infection with *F hepatica*, of which the majority were in the late immature or adult stages. Only a small proportion (<5%) were early immature. The weather patterns in 2010 and estimated risk of fasciolosis in the UK and Ireland did not indicate that the year was atypical, so the observations that adult fluke were the dominant age class in the fluke population at this time should be included in the assumptions behind recom-

mendations for the treatment of cattle for fasciolosis at housing.

INTRODUCTION

Liver fluke (*Fasciola hepatica*) infections are common in grazing ruminants in the United Kingdom (UK) and Ireland, and though traditionally more common in the wetter regions of the western seaboard, the disease appears to be spreading and is now found in most regions (McCann et al., 2010; Pritchard et al., 2005). Control options are limited by the absence of appropriate vaccines and the difficulties in managing infection in the intermediate host (*Galba truncatula*) and the free-living stages on pasture. Hence control relies heavily on the use of anthelmintics (flukicides). On epidemiological grounds and for reasons of convenience, cattle are commonly group-treated at housing or during the winter (Torgerson and Claxton, 1999; Webster et al., 2008). Animals that are housed during winter months are typically brought inside between October and December and during these months. Infections with *F hepatica* are expected to consist of both mature and immature stages, based on the assumption that the cattle have been grazing infected pastures up to the time of housing (Mitchell, 2002). Because of the assumed presence of this heterogeneous parasite population, the choice of flukicide has traditionally been determined by the products' spectra of activity in terms of efficacy against immature as well as adult stages of *F hepatica* (Torgerson and Claxton, 1999). If a product without activity against immature fluke is used, then advisors commonly recommend that administration is delayed for several weeks after housing based on the assumption that this should ensure that adult fluke comprise the majority of the parasite population when the cattle are treated. Alternatively, cattle treated with products of limited spectra at housing can be sampled 8-12 weeks later, when any remaining infections should be patent, to determine if fluke eggs are present and whether additional treatment is required.

The only flukicide currently available in the UK and Ireland with consistent efficacy against early immature (<6 weeks of age) fluke is triclabendazole (TCBZ). Although there are clear advantages in using a broad spectrum flukicide in fluke control, one of the most important clinical benefits of activity against juvenile fluke is in the treatment of acute and sub-acute fasciolosis. This occurs when large numbers of young fluke migrate through the liver parenchyma causing severe pathology and anaemia. This is a serious, not uncommon, disease of sheep but is rare in cattle.

Possibly as a consequence of its widespread popularity, treatment failures with TCBZ are apparently quite common in sheep in both the UK and Ireland and at least some of these failures are due to anthelmintic resistance (Fairweather, 2011; Sargison and Scott, 2011a). When present, TCBZ-resistance leads to difficulty in the treatment of acute and sub-acute disease (Sargison and Scott, 2011b). Thus, in order to avoid over-dependence on TCBZ and to maintain a refugia of fluke unexposed to TCBZ in ruminant populations (Sargison and Scott, 2011b), it is important to consider the use of flukicides other than TCBZ, particularly in cattle in which acute fasciolosis is rare.

The aims of this study were to identify the intensity of fluke infection in cattle in the autumn/winter and to determine the fluke stages present by post-mortem examination of condemned cattle livers in order to rationalise the use of flukicides in cattle at housing.

MATERIALS AND METHODS

Three laboratories University College Dublin (UCD), Food and Environmental Research Agency (FERA) and Animal Health and Veterinary Laboratories Agency (VLA), Carmarthen participated in this study. The livers were sourced from cattle entering three abattoirs, one each in Ireland, South Wales, and Northeast England. The livers sampled were opportunistically selected from livers condemned at meat inspection. In the abattoir from which livers were sourced by FERA, the gall bladders had

Table 1. *F. hepatica infection populations in condemned livers*

	VLA, Carmarthen	UCD, Dublin	FERA, York	Total
No. of livers	40	49	37	126
No. infected (%)	37 (92.5%)	48 (98%)	23 (62.2%)	108 (85.7%)
Infection stages				
Early immature <5mm			0	0
Early immature <7mm	36	46		82
Late immature 5-10mm			6	6
Late immature 7-19mm	203	352		555
Adult >10mm			218	218
Adult >19mm	1091	865		1956
Total	1330	1263	224	2817

been removed shortly after the animals were slaughtered as a standard procedure. The age of cattle slaughtered at these abattoirs ranged between 18 months to adult, with the majority being younger cattle. No information was available on their previous grazing management or treatment history.

Fluke were extracted from the liver in a two-stage process. Initially the gall bladder (where present) and larger bile ducts were dissected and opened and any fluke present were removed. The liver was then cut into slices 1 cm thick, squeezed and washed in saline or water. The slices were in turn cut into smaller pieces, squeezed or placed in a muslin cloth and macerated by hand, then washed in saline or water, and finally any remaining liver tissue was discarded.

The saline was passed through a wire mesh screen (aperture 0.5mm) in order to recover, count, and classify the fluke. The procedure took between 2.5 and 3.5 hours per liver examined. The process was based on a standard operating procedure developed by the Veterinary Laboratories Agency (MAFF, 1986).

The total number of fluke recovered from each liver was recorded, as was their size. If flukes were fragmented during processing, each head was considered to represent one fluke. Classification of fluke stages was made on the basis of their measured length. Fluke were classified as:

- early immature
- late immature
- adult

The participating laboratories used slightly different size criteria for classifying the age of the fluke stages: UCD and VLA used a threshold of ≤7mm to differentiate early immature fluke from the late immature/adult stages, based on data generated on artificial *F hepatica* infections in sheep (Boray, 1967; Soulsby, 1965), while FERA used a threshold of ≤5mm for early immatures, >5 - 10mm for late immature, and >10mm for adult stage fluke, based on a pooled summary of several data sources for sheep (Behm and Sangster, 1999).

RESULTS

A total of 126 condemned cattle livers were collected during the months of August to December 2010. Of these, 108 livers had a fluke burden. The total number of fluke recovered was 2,817, which equates to a mean intensity of 26 fluke per infected animal. Table 1 shows the numbers and categories of fluke recovered by each laboratory. Combining the flukes classified as "early immature" (≤7mm and ≤5mm), of the 2,817 flukes recovered, 82 (3%) were in this category and the remaining 2,735 (97%) were late immatures or adults. There appeared to be regional differences in the proportion of condemned livers that harboured fluke: 62%

of livers were parasitized in the Northeast of England compared with 98% in Ireland and 92.5% in South Wales. This may reflect regional climatic differences affecting fluke prevalence, or may have been an artefact in that some (adult) fluke burdens could have been completely removed when the gall bladder was excised at the abattoir supplying FERA. Amongst the infected cattle:

- 42.6% had 1-10 fluke per liver
- 31.5% had between 11 and 30 fluke
- 25.9% had >30 fluke present.

The greatest number of fluke recovered from a single liver was 172, of which 165 were categorised as adults (>19mm in length).

DISCUSSION

The ”classical” epidemiology of fasciolosis in northern Europe is that animals become infected while grazing contaminated pastures, with periods of risk in UK and Ireland in the spring (May and June), as a result of the over-winter infection of snails, and autumn (mid-August to November) as a result of the summer infection of snails (Ollerenshaw, 1959; Soulsby, 1965). The highest levels of metacercariae on pasture typically occur during the autumn months, particularly following a warm wet summer (Daniel, 2004). This pattern of infection has led to the assumption that at housing in the autumn, cattle have a fluke burden comprising a high proportion of immature fluke as well as adults – and this assumption is the basis for treatment recommendations (Mitchell, 2002). The objective of this study was to test this assumption.

The methodology for recovering flukes from the liver varied slightly amongst the participating laboratories and similarly, the fluke were characterised using slightly different length criteria and categories. Standards for fluke age and length relationships were derived from literature on *F hepatica* infections in sheep, whereas data from cattle experiments show similar trends, but slightly different age/length associations (Ross et al., 1966). The categories such as “early immature,” ”late immature,” and ”adult” based on measured length are approximate, as studies have shown that fluke length varies intrinsically following artificial infections, where the age of infection is known (Boray, 1967; Ross et al., 1966). In addition to counting fluke, one laboratory (UCD) also collected eggs from the bile of 45 infected livers; 41 of these samples were positive for fluke eggs, providing corroboratory evidence of the presence of sexually mature, adult fluke in those livers.

Regardless of the limitations of the methodology, whether the results are considered lab-by-lab or collectively, the conclusions are the same: the majority of fluke present in this survey in the autumn, at the time when cattle are commonly housed, were late immature stages or adults. However, two factors preclude drawing general conclusions from the results of this study. One is that the grazing history of these animals was unknown, so some may not have been grazing prior to slaughter. Given that the survey was conducted in regions where cattle farming is strongly pasture-based, it seems unlikely that this would apply to the majority, nevertheless it is an unknown.

The second factor is that only condemned livers were examined, and it is possible that apparently healthy livers could have harboured burdens of immature fluke. There is no way of knowing if this was the case. The enormous resources required for such a study precluded examining large numbers of livers at random, hence only condemned livers were examined. However, in a study conducted in 2006 in Belgium, immature fluke were recovered from randomly selected cattle livers in the autumn, and they comprised only 10% of the fluke population at that time (Charlier et al., 2008), so this indicates that, despite its acknowledged limitations, the results of the current study may indeed indicate that immature fluke are in a minority in cattle at this time of year.

A further consideration in putting the results of this study into context is whether the year 2010 was typical or otherwise in terms of fasciolosis. In Ireland, the risk of disease in 2010 had been estimated as high for most regions, and surveillance reports showed that liver fluke caused significant losses in 2010 (AFBI/DAFF, 2011), though the proportion of fatal liver fluke infections in cattle was slightly less in 2010 (6.5%) than in 2009, when fasciolosis accounted for 9% of deaths in adult cattle. In Great Britain, a moderate risk of disease had been forecast for autumn/winter 2010, based on the lower rainfall experienced during spring and summer months that year. Reports of disease detected in cattle in the period October to December 2010 showed a significant rise in the percentage of diagnosed cases of fasciolosis, compared to the same period in 2009 (VIDA, 2009). Collectively, this information does not suggest that autumn 2010 was a particularly low risk period for fasciolosis in Great Britain and Ireland.

The mean intensity of infection in this study was 26 fluke per infected liver examined; 42.6% of these livers hosted 1-10 fluke. A cull cow study in Ireland reported that 70.2% of infected livers harboured 1-10 fluke (Murphy et al., 2006) and in a Belgian study 49-60% of infected livers had low levels (1-10 fluke) of infection (Charlier et al., 2008). In the current study, 26% of infected livers carried more than 30 fluke. A virtually identical result was reported in the Belgian study in which 28-29% of infected livers harboured >30 flukes (Charlier et al., 2008). It has been proposed that the threshold of infection associated with production losses in cattle is >30 fluke per animal (Vercruysse and Claerebout, 2001). However, based on the association between the intensity of infection and the extent of liver pathology, Charlier et al (2008) proposed that the threshold for poor performance be lowered to 10 fluke per liver. Thus, although the cattle in this study appeared healthy at the time of slaughter, it is likely that the majority (57.4%) had experienced some degree of sub-optimal performance because of fasciolosis.

The primary objective of this survey was to establish the proportion of fluke present in the bile ducts (late immatures/adults) during the autumn months. This would help identify whether flukicides with activity against these stages could be used at housing to remove the majority of infection. In terms of the activity of flukicides against various stages of fluke, the transition from parenchymal to bile-duct stages that occurs between 6 and 8 weeks after infection seems to be important because claims for those products with activity against "(late) immature" fluke frequently span this period. This could be a consequence of the pharmacokinetics of the compounds permitting greater access to the flukes in the bile ducts, or the fluke may be more susceptible at this time when they have a phase of accelerated growth (Ross et al., 1966) and, presumably, high rates of metabolic activity. In this study 97% of the flukes recovered were classified as late immature/adults. Thus the majority of parasites would have been sensitive to flukicides with activity against these stages, of which several are currently available, including nitroxynil, closantel and clorsulon, for example (Fairweather and Boray, 1999; Mitchell, 2002).

To mitigate the risk of TCBZ resistance, reported in both cattle and sheep in the UK (Sargison and Scott, 2010) and sheep in Ireland (Mooney et al., 2009), the use of TCBZ in cattle could be reduced by using flukicides with different modes of action. The results of this and other studies suggest that recommendations for housing treatments may not need to be adjusted according to spectrum of flukicidal activity as early immature fluke may be present in relatively small numbers in cattle in the autumn.

REFERENCES

1. AFBI/DAFF 2011. All-island Animal Disease Surveillance Report 2010, p. 64.
2. Behm, C.A., Sangster, N.C., 1999, Pathology, Pathophysiology and Clinical Aspects, In: Dalton, J.P. (Ed.) Fasciolosis. CABI, United Kingdom, pp. 185-224.
3. Boray, J.C., 1967, Studies on experimental infections with *Fasciola hepatica*, with particular reference to acute fascioliasis in sheep. *Ann Trop Med Parasitol* 61, 439-450.
4. Charlier, J., De Meulemeester, L., Claerebout, E., Williams, D., Vercruysse, J., 2008, Qualitative and

quantitative evaluation of coprological and serological techniques for the diagnosis of fasciolosis in cattle. *Vet Parasitol* 153, 44-51.

5. Daniel, R., 2004, Control of Bovine Fasciolosis. *Cattle Practice* 12, 161-166.
6. Fairweather, I., 2011, Raising the bar on reporting 'triclabendazole resistance'. *Vet Rec* 168, 514-515.
7. Fairweather, I., Boray, J.C., 1999, Mechanisms of Fasciolicide Action and Drug Resistance in *Fasciola hepatica*, In: Dalton, J.P. (Ed.) Fasciolosis. CABI, United Kingdom, pp. 225-276.
8. MAFF, 1986, Manual of Veterinary Parasitological Laboratory Techniques. *Her Majesty's Stationary* Office, London, 160 p.
9. McCann, C.M., Baylis, M., Williams, D.J., 2010, Seroprevalence and spatial distribution of *Fasciola hepatica*-infected dairy herds in England and Wales. *Vet Rec* 166, 612-617.
10. Mitchell, G., 2002, Update on fasciolosis in cattle and sheep. In Practice 24, 378-385.
11. Mooney, L., Good, B., Hanrahan, J.P., Mulcahy, G., de Waal, T., 2009, The comparative efficacy of four anthelmintics against a natural acquired Fasciola hepatica infection in hill sheep flock in the west of Ireland. *Vet Parasitol* 164, 201-205.
12. Murphy, T.M., Fahy, K.N., McAuliffe, A., Forbes, A.B., Clegg, T.A., O'Brien, D.J., 2006, A study of helminth parasites in culled cows from Ireland. *Prev Vet Med* 76, 1-10.
13. Ollerenshaw, C.B., 1959, The ecology of the Liver Fluke (*Fasciola hepatica*). *Vet Rec* 71, 957-965.
14. Pritchard, G.C., Forbes, A.B., Williams, D.J., Salimi-Bejestani, M.R., Daniel, R.G., 2005, Emergence of fasciolosis in cattle in East Anglia. *Vet Rec* 157, 578-582.
15. Ross, J.G., Todd, J.R., Dow, C., 1966, Single experimental infections of calves with the liver fluke, *Fasciola hepatica* (Linnaeus 1758). *J Comp Pathol* 76, 67-81.
16. Sargison, N.D., Scott, P.R., 2011a, Anthelmintic resistance: potential benefits of 'over-diagnosis'. *Vet Rec* 168, 646-647.
17. Sargison, N.D., Scott, P.R., 2011b, Diagnosis and economic consequences of triclabendazole resistance in *Fasciola hepatica* in a sheep flock in south-east Scotland. *Vet Rec* 168, 159.
18. Soulsby, E.J.L., 1965, Textbook of Veterinary Clinical Parasitology. Volume 1: Helminths. Blackwell Scientific Publications, Oxford, England, 1120 p.
19. Torgerson, P., Claxton, J., 1999, Epidemiology and Control, In: Dalton, J.P. (Ed.) Fasciolosis. CABI, United Kingdom, pp. 113-149.
20. Vercruysse, J., Claerebout, E., 2001, Treatment vs non-treatment of helminth infections in cattle: defining the threshold. *Vet Parasitol* 98, 195-214.
21. VIDA 2009. Veterinary Investigation Surveillance (VIDA) Report, 2009 (Veterinary Laboratories Agency).
22. Webster, R., Knox, K., Berger, F., Delaveau, J., Forbes, A., 2008, Comparison of the time required to administer three different fluke and worm combination products to commercial beef cattle at housing. *Vet Ther* 9, 45-52.