

Project number: 6605
Funding source: Department of Agriculture, Food & The Marine

Date: March 2020
Project dates: Oct 2013 – Sept 2018

Liver & Rumen Fluke; Prevalence, Prediction & Production in sheep, beef and dairy cattle (Flukeless)



Key external stakeholders:

Department of Agriculture, Food & the Marine (DAFM); Animal Health Ireland (AHI), The Irish Cattle Breeding Federation (ICBF); Dairy, Beef and Sheep Farmers; Veterinarians; Consumers

Practical implications for stakeholders:

This study has:

- Contributed to design of rapid testing chips using recombinant *F. hepatica* antigens.
- Generated national liver (*Fasciola hepatica*) and rumen (paramphistome species) fluke prevalence data in sheep flocks, dairy and beef herds.
- Completed a six-year longitudinal study of *F. hepatica* infection in Irish dairy herds using bulk milk ELISA results yielding a roadmap to fluke control.
- Identified and sequenced rumen fluke species on Irish livestock farms.
- Completed an evaluation of all four commercially available *F. hepatica* ELISA test kits on the market
- Contributed to elucidation of spatial patterns of *F. hepatica* and *Calicophoron daubneyi* infections in ruminants in Ireland and to modelling of *C. daubneyi* infection.
- Completed a field trial on a novel *F. hepatica* vaccine candidate and investigated the use of additional alternative strategies for fluke control such as targeted selective treatment.
- Provided the necessary data to undertake national multi trait genetic evaluations for parasite phenotypes.
- Contributed to the development of a long-term strategy for routine procurement of parasite data in dairy and beef cattle from Irish slaughter plants.

Main results:

- The importance of continuous monitoring for liver and rumen fluke has been highlighted in this project and strategies for achieving a reduction in liver fluke exposure are presented.
- Anthelmintics still have a considerable role to play in the control of fluke in Ireland but their use can be better targeted by use of routine diagnostics.
- National prevalence of *F. hepatica* in sheep flocks was 50.4% and co-infection with rumen fluke was observed in 40% of the studied population. Rumen fluke prevalence in sheep flocks was 77.3 %.
- Bulk milk serology highlighted a national *F. hepatica* prevalence of 55% in dairy herds in 2014, down from 75% in 2009. Prevalence was considerably higher in beef herds with over 90% of herds studied nationally recording positive herd status.
- A number of test kits have been identified to allow accurate monitoring of *F. hepatica* exposure.
- Heritability of *F. hepatica*-antibody responses was 0.13 and the coefficient of genetic variation was 20%.

Opportunity / Benefit:

F. hepatica has been estimated to cost Irish livestock farmers €25million annually. It is imperative, therefore, that Irish producers are provided with research-based strategies for sustainably controlling fluke. This project was unique in generating national liver and rumen fluke prevalence data in dairy and beef herds, and sheep flocks, in a single season. This yielded a baseline from which future progress can be measured post-implementation of control strategies. This project has also generated a testing platform to allow rapid serological testing for liver fluke. This will afford the opportunity for farmers and veterinarians to determine

fluke status of animals on farms allowing rapid and informed decision making, such as targeted animal treatments. This is important in ensuring responsible anthelmintic use. Considerable progress has also been made in investigating genetic resilience and resistance to liver fluke and a system for continuous collection of liver fluke data from Irish slaughter plants is now in place to allow further fine-tuning of current findings. FLUKELESS has highlighted that pharmacologicals will continue to play an important role in the control of liver and rumen fluke, but the alternative strategies researched in this proposal allow real and measurable progress to be made with regard to fluke control in Ireland, while actively promoting sustainable use of anthelmintics.

Collaborating Institutions:

Tyndall National Institute (TNI, UCC)

University College Dublin (UCD)

Zoetis

The Enfer Group

Teagasc project team: Róna Sayers; Donagh Berry; Noel Byrne; Ana Ibeas-Martinez; Maria Pia Munita-Corbalan

External collaborators: Alan O'Riordan (Tyndall), Grace Mulcahy (UCD), Annetta Zintl (UCD), Thomas Guerden (Zoetis), Michael Kennedy (The Enfer Group)

1. Project background:

Liver fluke (*F. hepatica*) continues to impact on dairy, beef, and sheep farming enterprises worldwide. The cost of liver fluke globally is estimated at €3billion with Irish estimates approximated at €25million annually. Levels are increasing worldwide and fascioliasis is also re-emerging as a human disease. The past number of years has seen the prevalence of liver fluke increase up to 12-fold across Europe. Current strategies, therefore, are not providing an optimal means of controlling liver fluke in Irish livestock. Anthelmintic resistance is also of major concern in that effective tools may be lost in the face of increasing prevalence. Additionally, in recent years, clinical disease due to *Paramphistomum* spp. (rumen fluke) in cattle and sheep has been reported for the first time in Ireland. Unlike liver fluke, clinical paramphistomosis is rare, although multiple mortalities have been reported on individual farms. Anecdotal evidence (Zoetis) would suggest that an over-emphasis on the control of rumen fluke in Ireland has distracted from effective control of liver fluke. It has also led to a significant increase in sales of oxcyclozanide-containing products due to its anti-paramphistome activity. Overuse of oxcyclozanide as a treatment for both flukes could lead to emergence of anthelmintic resistance. This project proposed to develop, evaluate, and apply innovative and sustainable technologies to the control of liver fluke in order to achieve effective control but also mitigate against anthelmintic resistance. National research on anthelmintic resistance is lacking and international opinion would suggest that although anthelmintic resistance is growing, it may be prevented or slowed through use of strategies which maintain an unselected refugium. With this in mind, the extensive knowledge base that exists amongst Irish researchers with regard to *F. hepatica* was exploited and expanded in FLUKELESS to develop Irish-applicable and sustainable fluke control protocols. Building on the innovative diagnostic capabilities of TNI-UCC, the unique immunological expertise of UCD's parasitological antigen team, and Teagasc's team of livestock researcher scientists, a novel, practical, effective, and sustainable approach to fluke control in Ireland was investigated. The project marked the coming together of a truly multidisciplinary team, spanning a broad range of research fields to the benefit of project stakeholders.

2. Questions addressed by the project:

This proposal investigated and developed tools (diagnostics, GIS, immunologicals, genomics) for use by multiple end-users including veterinarians, farmers, and policy makers. FLUKELESS provided the basis for a novel blueprint for on-farm parasite control methodologies, thereby, allowing farmers to rapidly intervene and correct parasite-related animal health issues. The tools are applicable to both cattle and sheep allowing effective decision-support at farm level, thereby, optimising the sustainable use of existing treatments and mitigating against anthelmintic resistance. Short term gains in production efficiency at farm level can be achieved through i) raising awareness of liver fluke and parasitic diseases in general, ii) applying innovative approaches to liver fluke control such as novel vaccines, GIS prediction models etc and iii) developing practical solutions for sustainable anthelmintic use. Longer term gains can be achieved through application of targeted breeding programmes based on project outputs.

3. The experimental studies:

- Workpackage 1: Aimed to develop a prototype point-of-care diagnostic tool for rapid diagnosis of liver fluke at farm-level using biosensor nanotechnology.

- Workpackage 2: Aimed to generate up-dated liver fluke prevalence data for Irish livestock and evaluate a web-based GIS decision support tool for use at farm level.
- Workpackage 3: Aimed to develop a blueprint for sustainable fluke control by developing and field trialing a novel liver fluke antigen vaccine and investigating targeted anthelmintic treatments.
- Workpackage 4: Aimed to evaluate genetic selection as part of a national strategy for improved resistance to parasites in dairy and beef cattle by evaluation of genetic parameters for resistance and resilience to liver-fluke. It also aimed to develop a long-term strategy for routine procurement of parasite data in dairy and beef cattle from Irish slaughter plants.

4. Main results:

- Components of a nanotechnology testing platform to allow rapid on-farm testing for liver fluke have been developed.
- National *F. hepatica* prevalence in dairy and beef herds was 55% and >90%, respectively, with farms in the west of Ireland at greatest risk.
- The national true prevalence of *F. hepatica* in sheep flocks was 50.4%. Co-infection with rumen fluke was observed in 40% of the studied population and correlated with increased *F. hepatica* egg counts (OR = 2.9; $P \leq 0.001$). Co-infection with *F. hepatica* and *C. daubneyi* was significantly associated with the presence of other livestock on the farm, the type of flukicide used and clinical fascioliasis.
- A national apparent rumen fluke flock prevalence of 77.3 % was determined. Several risk factors for rumen fluke infection were identified including type of pasture grazed, regional variation, and sharing of the paddocks with other livestock species. A novel relationship between the Suffolk breed and higher FEC was reported for the first time. The predominant rumen fluke species found in Irish sheep flocks was *C. daubneyi*.
- Annual and seasonal *F. hepatica* data can vary significantly within a herd and highlights the importance of continuous monitoring in achieving effective fluke control.
- Cattle had a higher likelihood of being infected with rumen fluke than sheep (OR = 3.134, $P < 0.01$) and infection with one parasite increased the odds of infection with the other in both host species.
- Flukicides active against both immature and mature *F. hepatica* were approximately half as likely to record a positive bulk milk result than a flukicide active against only the mature stage of the parasite (OR ~0.45; $P < 0.01$) in Irish dairy herds. Similar findings were highlighted in sheep. In general, across all species investigated, triclabendazole was the most effective flukicide, at reducing serum antibody levels, although certain individuals recorded no change in serological status post-treatment.
- Ildana, IDEXX and Bio-X *F. hepatica* test kits yielded 100% sensitivity and specificity and Svanovir yielded a sensitivity of 59% and a specificity of 96%. In flukicide-treated beef cattle, kits highlighted decreasing antibody levels 90 days post-treatment to variable degrees. Care should therefore be taken when using serological assays to assess the efficacy of treatment.
- Although further research is required to produce a viable vaccine against *F. hepatica*, good progress was made in determining a combination of antigenic components for additional field-trialing.
- Heritability of *F. hepatica*-antibody responses, when treated as a continuous trait, was 0.13 and the coefficient of genetic variation was 20%. Genetic correlations between parasite phenotypes and milk production traits were all close to zero (-0.14 to 0.10), as were the genetic correlations between *F. hepatica*-damaged livers and the carcass traits of carcass weight, conformation, and fat score evaluated in cows and young animals (0.00 to 0.16). This study provides the necessary information to undertake national multi trait genetic evaluations for parasite phenotypes.

5. Opportunity/Benefit:

FLUKELESS provides the basis for a blueprint for novel on-farm parasite control methodologies, thereby, allowing farmers to rapidly intervene and correct parasite-related animal health issues. The tools are applicable to both cattle and sheep allowing effective decision-support at farm level, thereby, optimising the sustainable use of existing treatments and mitigating against anthelmintic resistance. Pharmacologicals will continue to play an important role in the control of fluke for the foreseeable future. The alternative strategies to be researched in this proposal, however, allow real and measurable progress to be made with regard to fluke control in Ireland while actively promoting sustainable use of anthelmintics.

6. Dissemination:

Main publications:

- Munita, M.P., Rea, R., Martinez-Ibeas, A.M. Byrne, N., Kennedy, A., Sekiya, M., Mulcahy, G., Sayers, R. (2019). Comparison of four commercially available ELISA kits for diagnosis of *Fasciola hepatica* in Irish cattle. BMC Vet Res 15, 414.

- A.M. Martínez Ibeas, M. Munita, K. Lawlor, M. Sekiya, G. Mulcahy, R. Sayers. (2016) Rumen Fluke in Irish Sheep: Prevalence, Risk Factors and Molecular Identification of Two Paramphistome Species. BMC Veterinary Research 12, 143.
- A. J. Twomey, R. G. Sayers, R. I. Carroll, N. Byrne, E. O' Brien, M. L. Doherty, J. C. McClure, D. A. Graham, D. P. Berry, (2016). Genetic parameters for both a liver damage phenotype caused by *Fasciola hepatica* and antibody response to *Fasciola hepatica* phenotype in dairy and beef cattle, Journal of Animal Science, 94, 4109–4119

A further 6 peer-reviewed papers were also published.

Popular publications:

- Ana Martinez-Ibeas, Yris Bloemhoff & Riona Sayers (2014). A survey of parasite control practices on dairy farms in Ireland Its Your Field - Winter edition.
- Riona Sayers & Alan O'Riordan (2014). The Science Squad TV programme. Recorded 23/06/2014.

Other

International conferences

- M. P. Munita, R. Rea, A. Martinez-Ibeas, N. Byrne, R. Sayers, (2016). Six-year longitudinal study of *Fasciola hepatica* bulk milk ELISA testing in Irish dairy herds. In Proceedings of World Buiatrics Conference, Dublin.
- K. Delaby, E. Kennedy, J.P. Murphy, R. Sayers. (2016). Targeted selective treatment of gastrointestinal nematodes based on live weight gain variations in first season grazing dairy calves in Ireland. In Proceedings of World Buiatrics Conference, Dublin.
- A. M. Martinez Ibeas, M. Munita-Corbalan, N. Byrne, R.G. Sayers. (2016). First Report of *Paramphistomum Leydeni* in Irish Sheep Flocks. In Proceedings of World Buiatrics Conference, Dublin.
- Martinez-Ibeas, A., Byrne, N., Munita, M., Lawlor, K., Mulcahy, G., Sekiya, M., Sayers, R. (2015). Preliminary study of prevalence of rumen fluke in Irish flocks. In proceedings of BSAS conference April.
- Munita, M., Byrne, N., Lawlor, K., Martinez A., Mulcahy, G., Sekiya, M., Sayers, R. (2015). Prevalence of *Fasciola hepatica* in Irish sheep flocks in 2014. In proceedings of BSAS conference April.
- Naranjo lucena A., Munita Corbalán, M., Martínez-Ibeas A., McGrath, G., Murray, G., Casey, M., Good, B., Sayers, R., Mulcahy, G., Zintl, A. (2018). Spatial analysis of liver fluke and rumen fluke infections in Ireland. International Geographical Union Thematic Conference to the Centennial of the Institute of Geography of the Russian Academy of Sciences.
- J. McClure, A. Cromie, R. Sayers, D. Graham, N. Byrne & D. Berry (2014). Genetics of susceptibility to bovine viral diarrhoea virus, bovine herpesvirus type 1 and *Fasciola hepatica* in cattle 10th World Congress of Genetics Applied to Livestock Production, Vancouver.

National Conference

- R. Sayers, N. Byrne. (2015) National Dairy Conference- Workshop with focus on liver fluke control.
- D. Berry, J. McClure, A. Cromie, D. Graham & R. Evans (2014). Breeding a healthier herd Dairy Herd Health Conference

Open Days & seminars

Results presented at all Teagasc, Moorepark Open Days between 2013 and 2018. Results were also presented at the Teagasc Beef Open Day 2014 in Grange and at Sheep 2015.

Industry consultation days, farmer discussion groups

Presented and discussed at several industry meeting days and at numerous farmer discussion groups, farm walks and seminars.

R. Sayers & G. Mulcahy compiled an on-line continuing veterinary education module to promote fluke control and the Flukeless project amongst veterinarians. Recorded 25/11/2014.

Theses

This project directly generated two PhD theses which included four experimental chapters each. It also generated positions for 2 post-doctoral candidates and linked to projects yielding a further two PhD theses.

7. Compiled by: Dr. Riona Sayers