

Research Stimulus Fund

Final Report

Coping with Anthelmintic Resistance in Ruminants (CARES)

DAFM Project Reference No: 11/RD/EMIDA/2

Start date: 1/8/2012

End Date: 31/1/2016

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Collaborating Research Institutions and Researchers: Theo de Waal (UCD) CVRL (William Byrne)

Please place one "x" below in the appropriate area on the research continuum where you feel this project fits

Basic/Fundamental		→	Applied		→	Pre Commercial	
1	2	3	4	5	6	7	
			x				

Please specify priority area(s) of research this project relates to from the National Prioritisation Research Exercise* (NRPE) report;

Priority Area (s)	Sustainable Food Production and Processing
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Key words: (max 4)

Anthelmintic resistance, sheep, nematode

1. Rationale for Undertaking the Research

This section should outline the rationale for carrying out the research and identify the need / problem to be addressed

Irish lamb production is pasture-based with grazing sheep naturally exposed to gastrointestinal nematodes (GIN), which results in ill-thrift and occasional death. Infections with GIN are a major threat to ruminant health, welfare and productivity and thus to the viability of the ruminant livestock industries in Ireland and worldwide. For over 50 years, the administration of broad spectrum anthelmintics has been effective in controlling GIN in sheep but in recent years reports of anthelmintic resistance (AR) are increasing worldwide. Anthelmintic resistance can be defined as the inherited ability of worms to survive doses of drugs that would normally kill them and AR is the single-most important problem facing ruminant farmers today in relation to sustainable GIN control. Resistance to the benzimidazole (BZ) and levamisole (LEV) anthelmintics on Irish sheep farms had been previously reported. Resistance to macrocyclic lactones (ML) had been suspected but not confirmed. Therefore there was a need to establish the extent of AR on Irish sheep farms to the three commonly used anthelmintics, BZ, LEV and ML. If resistance to all three anthelmintic classes was established then whether multi-drug resistant GIN were present in Ireland also needed to be determined.

In addition to quantifying the problem of AR on Irish sheep farms sustainable strategies to prolong the lifespan of the currently available anthelmintics are required. The early detection of AR can facilitate the adoption of practices to delay the further development of AR and so studies were undertaken to identify molecular markers of BZ and ML resistance in *Teladorsagia circumcincta*, a common sheep nematode. Strategies that preserve an *in refugia* population of nematodes unexposed to anthelmintic have also been shown to delay the development of AR, however the effect of such strategies on lamb performance in Ireland needed to be quantified. The effect of part-flock anthelmintic treatment on lambs post-weaning was therefore examined. Additionally, weight was evaluated as a criterion for targeted selective treatment of lambs.

2. Research Approach

Specify the research methodologies employed, emphasising novel techniques and also outline any modifications from the original approved project proposal

In order to quantify the prevalence of anthelmintic treatment failure on Irish sheep farms a survey of 1446 anthelmintic treatments carried out as part of the Sheep Technology Adoption Programme (STAP) was evaluated. Treatments took place from 2013-2015 and anthelmintics

used were BZ (38%), LEV (22%) and ML (40%). In order to confirm if ML resistance was present on Irish sheep farms, 4 farms with suspected ML resistance were identified and visited in order to carry out an on-farm faecal egg count reductions test. Two farms were confirmed to have ML resistance and on both farms *T. circumcincta* was confirmed to be the only resistant species. Infected lambs were purchased from the farms in order to culture ML resistant nematodes. *In vitro* and *in vivo* studies were used to determine the anthelmintic resistance profile of the *T. circumcincta* isolates to BZ, LEV and ML.

In order to detect molecular markers of anthelmintic resistance, BZ resistant nematodes were recovered from four farms with BZ resistance. DNA was extracted from individual BZ resistant *T. circumcincta* nematodes and the isotype 1 β -tubulin gene sequenced in order to detect single nucleotide polymorphisms associated with resistance. In order to detect genes potentially associated with ML resistance, nematodes were exposed to ML and gene expression compared to nematodes unexposed to the anthelmintic in order to determine what biological pathways are activated in response to ML in resistant nematodes. This involved developing protocols for the extraction of RNA from single nematodes and creation and sequencing of cDNA libraries from these individual nematodes.

Finally part-flock anthelmintic treatment was evaluated as a tool to slow the development of AR. Leaving some animals untreated preserves an *in refugia* population of nematodes which can dilute any resistant nematodes that arise. The effect of part-flock treatment on productivity and levels of parasitism was quantified.

3. Research Achievements/Results

Outline main results achieved

This project achieved a number of aims. The extent of anthelmintic treatment failure in Irish sheep flocks was determined. Overall, only 51% of anthelmintic treatments administered by farmers in the study were effective (reduced faecal egg count by >95%). There was a significant difference in efficacy between the anthelmintic classes with 32%, 52% and 69% of treatments effective for BZ, LEV and ML respectively. The extent of treatment failure against *Nematodirus* spp was also established with 96% of treatments effective against *Nematodirus*.

This project also confirmed ML resistant sheep nematodes in The Republic of Ireland for the first time. ML resistant nematodes were identified on two farms and in both instances the resistant species was *Teladorsagia circumcincta*. Both resistant *T. circumcincta* isolates were tested *in vivo* (by means of a faecal egg count reduction test) and *in vitro* (larval development assay and larval migration inhibition assay) to determine if they were multi-drug resistant. Both

isolates were resistant to all three commonly used anthelmintics, BZ, LEV and ML. This study therefore identified the first multi-drug resistant nematodes in Ireland.

In order to ascertain the genetic mutations responsible for BZ resistance in *T. circumcincta* in Ireland, the isotype 1 β -tubulin gene of resistant nematodes was sequenced. For 89% of the BZ resistant worms the F200Y substitution was found which is known to be associated with resistance. In another 6% of worms an E198L substitution was identified. In the remaining 5% of worms no known mutation associated with resistance was identified and these worms may encode a novel BZ resistance allele.

In order to identify genes associated with ML resistance that could potentially be used as molecular markers, gene expression in ML resistant nematodes exposed and unexposed to ML was compared to identify biological processes that enable resistant nematodes overcome ML exposure. In total 1,367 differentially expressed genes were identified. ML exposure downregulated genes involved in normal cellular processes and metabolism and upregulated genes involved in pharyngeal pumping, feeding and eating behaviour and movement.

Finally the effect of part-flock anthelmintic treatment on lambs post-weaning was evaluated. Part-flock treatment was found to preserve a population of nematodes unexposed to anthelmintic. However, it had a negative effect on lamb performance with untreated lambs having significantly higher faecal egg count and significantly lower average daily gain than untreated lambs. Additionally, it was found that heavy lambs were not more resilient to being left untreated than light lambs.

4. Impact of the Research

A summary of the tangible impact of the research project should be provided under the 'outcomes' and 'outputs' heading below. In addition, please provide a short narrative synopsis of the benefits / improvements the research has made to the area under investigation particularly as regards end users, e.g. industry, consumers, regulatory authorities, policymakers, the scientific community, etc

This project has resulted in a large number of farmers being exposed to testing their anthelmintic treatment efficacy. The fact that only 51% of treatments administered were effective demonstrated the extent of the problem with anthelmintic treatment efficacy in Ireland and much of the treatment failure is due to AR. Awareness of AR in the industry has increased due to this study, which received substantial publicity in the farming media and has been covered in discussion group meetings. Additionally the study has resulted in the discovery of ML resistant and multi-drug resistant (MDR) sheep nematodes in Ireland for this first time. This has also

raised awareness of AR and the need for sustainable strategies to mitigate the risk of AR. The discovery of ML resistant and MDR nematodes has also highlighted the importance of biosecurity in a sustainable nematode control programme to ensure that farmers do not buy-in multi-drug resistant nematodes. The discovery of potential molecular markers will allow the future development of DNA-based tests for AR. The majority of BZ resistant *T. circumcincta* carry the F200Y allele and molecular tests for this allele are available and could be implemented in Ireland. Regarding ML resistance, up-regulation of genes encoding efflux pumps such as the P-glycoproteins (*pgps*) has been proposed to play a role in ML resistance. In this study we found no evidence for upregulation of *pgps* and resistant nematodes upregulated genes involved in pharyngeal pumping, and motility, processes which are inhibited by MLs. Part-flock anthelmintic treatment was also evaluated as a strategy to preserve the lifespan of the existing anthelmintics by preserving a susceptible *refugia* population of nematodes. While this strategy has benefits in terms of preserving a susceptible nematode population, it did result in a production penalty for untreated lambs. This evaluation of part-flock treatment will allow farmers make evidence-based decisions on part-flock anthelmintic treatment.

4(a) Summary of Research Outcomes

(i) Collaborative links developed during this research

This research project was part of an EMIDA ERAnet on Coping with Anthelmintic Resistance in Ruminants with partners from Denmark, France, Greece, Sweden, Canada and Guadeloupe. Additionally Teagasc, UCD and CVRL from Ireland were involved in the project. Therefore this project allowed us to establish links with eminent parasitologists throughout Europe and strengthened collaboration among veterinary parasitologists in Ireland. Teagasc also hosted one of the Consortium meetings attended by all partners which facilitated collaboration.

(ii) Outcomes where new products, technologies and processes were developed and/or adopted

This project developed protocols which allowed us to apply Illumina next generation sequencing technology to individual nematodes making this cutting-edge technology available for use in a range of species.

(iii) Outcomes with economic potential

(iv) Outcomes with national/ policy/social/environmental potential

This research project demonstrated that AR is widespread on Irish sheep farms with resistance to all three commonly used anthelmintics detected. Additionally multi-drug resistant nematodes were detected. This highlights the need for good biosecurity protocols and the implementation of strategies to delay the development of further resistance and limit the spread of resistance.

4 (b) Summary of Research Outputs

(i) Peer-reviewed publications, International Journal/Book chapters.

Acceptable Format: Walsh, D.R., Murphy, O., Cosgrave, J. (2008). Echinococcosis - an international public health issue. Research in Veterinary Science 774, 891-902.

Keane, O.M., Keegan, J.D., Good, B., de Waal, T., Fanning, J., Gottstein, M., Casey, M., Hurley, C., Sheehan, M. (2014). High level of treatment failure with commonly used anthelmintics on Irish sheep farms. *Irish Veterinary Journal*. 67(1):16.

Keegan, J.D., Keane, O.M., Farrell, L., Byrne, W., de Waal, T., Good, B. (2015).

Characterisation of ivermectin and multi-drug resistance in two field isolates of *Teladorsagia circumcincta* from Irish sheep flocks. *Veterinary Parasitology: Regional Studies and Reports*. 1-2: 3-9.

(ii) Popular non-scientific publications and abstracts including those presented at conferences

Keegan, J.D., Keane, O.M., Good, B., Gottstein, M., de Waal, T., Fanning, J., Hurley, C., Casey, M., and Sheehan M. The level of anthelmintic treatment failure detected by Sheep Technology Adoption Program (STAP) farmers. *Proceedings of the Agricultural Research Forum 2014* P102.

Keegan, J.D., Keane, O.M., Good, B., Gottstein, M., de Waal, T., Fanning, J., Hurley, C., Casey, M., and Sheehan M. High Level of anthelmintic treatment failure with commonly used anthelmintics on Irish Sheep Farms. *ISP BAVP EVPC Conference 12-13th June 2014, UCD, Dublin*.

Keegan, J.D., Keane, O.M., Lynch, C., Walsh H., and Good, B. Weight as a basis for targeted selective anthelmintic treatment of lambs *Proceedings of the Agricultural Research Forum 2015* P120.

Keegan, J.D., Keane, O.M., Good, B., Gottstein, M., de Waal, T., Fanning, J., Hurley, C., Casey, M., and Sheehan M. Widespread failure of anthelmintic drench tests on Irish farms in the sheep technology adoption programme Proceedings of the Agricultural Research Forum 2015 P125.

Keegan, J.D., Keane, O.M. and B. Good. Evidence for triple anthelmintic resistance in *Teladorsagia circumcincta* isolated from an Irish sheep farm. ISP Conference 26th June 2015, Queens University, Belfast.

Keegan, J.D., Keane, O.M., Good, B., Gottstein, M., de Waal, T., Fanning, J., Hurley, C., Casey, M., and Sheehan M. Drench Test Failure on Irish Sheep Farms WAAVP Conference 16-20th August 2015, Liverpool, UK 1A 209.

Keegan, J.D., Keane, O.M. and B. Good. First evidence for triple anthelmintic resistance on an Irish Sheep Farm. WAAVP Conference 16-20th August 2015, Liverpool, UK 1A 208.

Keegan, J.D., Keane, O.M., Lynch, C., Walsh H., and Good, B. Weight as a basis for Targeted Selective Treatment of Lambs at Weaning WAAVP Conference 16-20th August 2015, Liverpool, UK.

(iii) National Report

(iv) Workshops/seminars at which results were presented

Good, B., Keane, O., Lynch, C., Coll, T. Resistance to worm and fluke drenches in Sheep. Teagasc Sheep Open Day 6 June 2013 36-40.

Keane, O., Good, B. White drench (Benzimidazole) resistance in sheep roundworms: investigating the genetic basis for resistance. Teagasc Sheep Open Day 6 June 2013 p86.

Keegan, J.D. First evidence for triple anthelmintic resistance on an Irish sheep farm. Presentation to veterinary practitioners and stakeholders at Kilkenny RVL open day 29/10/15.

Keane, O.M. Assessment of the frequency of anthelmintic drench test failure on Irish sheep farms participating in the Sheep Technology Adoption Programme. Presentation to veterinary practitioners and stakeholders at Kilkenny RVL open day. 29/10/15

(v) Intellectual Property applications/licences/patents

None

(vi) Other

5. Scientists trained by Project

Total Number of PhD theses: 1

Please include authors, institutions and titles of theses and submission dates. If not submitted please give the anticipated submission date

Jason Devoy Keegan: Anthelmintic resistance in gastrointestinal nematodes of sheep: molecular characterisation and management. UCD. Submitted 16/3/2016. Successfully defended 1/6/2016.

Total Number of Masters theses: 0

Please include authors, institutions and titles of theses and submission dates. If not submitted please give the anticipated submission date

6. Permanent Researchers

Institution Name	Number of Permanent staff contributing to project	Total Time contribution (person years)
Teagasc	7	1.4
UCD	1	0.3
CVRL	4	0.55
Total	12	2.25

7. Researchers Funded by DAFM

Type of Researcher	Number	Total Time contribution (person years)
Post Doctorates/Contract Researchers	0	0
PhD students	1	3.3
Masters students	0	0
Temporary researchers	0	0
Other	0	0
Total	1	3.3

8. Involvement in Agri Food Graduate Development Programme

Name of Postgraduate / contract researcher	Names and Dates of modules attended
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9. Project Expenditure

Total expenditure of the project: €157,993.70

Total Award by DAFM: €149,666.00

Other sources of funding including benefit in kind and/or cash contribution(specify): €0

Breakdown of Total Expenditure

Category	Name Teagasc	Name UCD	Name CVRL	Total
Contract staff				
Temporary staff				
Post doctorates				
Post graduates	71,000			
Consumables	58,242.18			
Travel and subsistence	7829.90			
Sub total	137,072.08			
Durable equipment				
Other	1,100			
Overheads	19,821.63			
Total	157,993.71			

10. Leveraging

Summarise any additional resources'/funding leveraged by this award from other sources e.g. Additional Staff, National/EU funding secured, EI Commercialisation Fund, etc.

Contributions from Teagasc, UCD and CVRL for staff time, general lab reagents

11. Future Strategies

Outline development plans for the results of the research.

This project has been disseminated to end users via publication in peer reviewed journals (2 publications to-date with a further publication due for submission shortly). Additionally the results have been disseminated to farmers via the Teagasc advisory service. The results of this project were presented to Teagasc advisors at in-service training. The results have also been disseminated to veterinary practitioners who attended the Kilkenny RVL Open Day in 2015. The project has also resulted in the isolation of 2 multi-drug resistant isolates of *Teladorsagia circumcincta* generating valuable biological material for further studies on multi-drug resistance. The project has resulted in further collaboration between Teagasc and DAFM on coccidiosis and the prevalence of *Haemonchus contortus* infection on Irish sheep farms and further collaboration between Teagasc and UCD on the prevalence on anthelmintic resistance on Irish beef farms.