European Innovation Partnership (EIP) Wales

Improving the diagnosis and treatment of gastrointestinal round worms in cattle

Final report

Claire Reigate, Techion Ltd
Eurion Thomas, Techion Ltd
Diana Williams, The University of Liverpool
Alison Howell, The University of Liverpool
Tony Little, ADAS

July 2022

Cronfa Amaethyddol Ewrop ar gyfer Datblygu Gwledig: Ewrop yn Buddsoddi mewn Ardaloedd Gwledig
European Agricultural Fund for Rural Development: Europe Investing in Rural Areas
Llywodraeth Cymru
Welsh Government

Executive summary

The 'Improving the diagnosis and treatment of gastrointestinal round worms in cattle' EIP Wales project investigated how dairy farmers could improve the diagnosis and treatment of cattle roundworms without compromising performance. The project assessed the successes and challenges of implementing sustainable parasite control based on the latest industry recommendations.

The practical work took place on three dairy farms in Ceredigion, Wales. The key objectives of the Cattle Roundworm EIP Project were:

- Improving the detection of cattle roundworms
- Improved targeting of anthelmintics
- Determine anthelmintic efficacies
- Investigate roundworm species composition
- Reduce reliance on anthelmintics
- Improving growth rates of young stock
- Reduce environmental impact

The project ran for three years between August 2019 and July 2022. During this time a substantial amount of parasite and management data was recorded, and modifications were made to each farm's parasite control policy.

All the objectives were achieved.

KEY OUTCOMES:

- Regular monitoring of faecal egg counts (FEC) and growth rates enabled the better targeting of wormer treatments on each farm meaning wormers were administered when required rather than on a regular set treatment regime.
- The number of wormer treatments for R2 Cattle (2nd season grazers / yearlings) was significantly reduced on each farm.
- One of the three farmers also reduced treatments of R1 cattle (1st season grazers / calves), and changes to timing of treatments were seen on the other two farms.
- Treatment failures were detected on multiple occasions when the Group 3ML (clear) wormers were used. Both Group 1BZ (benzimidazole / White) and 2LV (levamisole / Yellow) were fully effective.
- The results of efficacy testing meant each farmer changed from relying solely on 3ML wormers to alternating between the three wormer classes. Some confusion was apparent about which active ingredients were present in different commercial wormers.
- The changes to treatment strategy did not result in a negative impact on performance (growth and condition).
- FEC's and FECRT's were useful tools in monitoring parasitic disease patterns when used correctly and when their limitations are understood.
- The frequency and efficacy of each treatment, irrespective of the active ingredient in each wormer, did not appear to impact growth rates in R1 and R2 calves on most occasions. However, it was difficult to assess this within the constraints of working on commercial farms as there are several possible confounding factors that could mask performance differences (*e.g.* intakes, grass availability, climate, co-morbidities).
- Prior to treatment, there was a mix of roundworm species present in each group of calves. *Cooperia oncophora* was the dominant worm species on most occasions with *Ostertagia ostertagi* also present in every sample.

- *Cooperia oncophora* was the principal species that survived the 3ML treatments. Infections with *C. oncophora* alone, are not normally associated with clinical disease in UK, although they may exacerbate disease caused by the more pathogenic *Ostertagia ostertagi.*
- Dealing with the risk of lungworm (and sometimes Type II ostertagiosis) was a factor that confounded treatment decisions. Lungworm vaccinations may benefit in further reducing reliance on anthelmintics.
- Technology advancements could further assist cattle roundworm control. Such initiatives include using models to predict pasture larval challenge and affordable, rapid diagnostics that provide information on the species present in the animals.
- Although not directly assessed in this project, the potential ecological benefits (*e.g.*, protecting dung beetles) was a real motivator for participating farmers.
- Changing farmer behaviour is challenging – but this project has demonstrated that with the correct support, significant changes are possible to achieve.

This project has generated a substantial amount of interest, and considerable effort has gone into communicating the project and its findings, including preparation of knowledge exchange materials; planned media campaigns (broadcast, print and social); presentations at conferences and events; and on farm open days.

Contents

1. Introduction

The UK cattle industry has largely relied on routine / regular anthelmintic treatments to control gastro-intestinal nematodes (GIN). The sheep sector has shown this strategy can lead to widespread anthelmintic resistance (AR) and consequently, uses tools like faecal egg counting (FEC) and treatment efficacy testing more often. The Cattle Roundworm EIP Project is a response to concerns from three dairy farmers in West Wales that parasitic GIN burdens in youngstock may be affecting growth rates and performance, and the approach of regular, routine anthelmintic treatments may need to be reviewed. The aim of this project was to evaluate sustainable control practices to improve the management of GIN in dairy youngstock.

In 2018 Eurion Thomas of Techion Ltd, approached local dairy farmer Eilir Evans (Farm E), about participating in a small student-led pilot study investigating GIN burdens in dairy heifers. The initial faecal egg count (FEC) unearthed a surprisingly high FEC in a group of calves given a moxidectin pour-on anthelmintic only 5 weeks previously. Moxidectin (MOX) is a long acting anthelmintic and claims to have a persistent effect in preventing re-infection by *Ostertagia ostertagi* for five weeks (https://www.noahcompendium.co.uk/), indicating a likely treatment failure. Eilir then wormed the calves again with an oral levamisole wormer (LEV) and subsequently recorded a drop in FECs, and an increase in growth rates. This got Eilir thinking about GINs and their impact on performance and he was keen to investigate more. Eilir spoke to two other Ceredigion farmers, namely, Irfon and Eurig Jenkins, Pentrefelin (Farm J) and Chris Mossman, Nantybach, (Farm M) who were operating similar systems. Conversations between the three dairy farmers formed the basis behind this Cattle Roundworm EIP Project. The objectives were to improve the management of roundworms in dairy youngstock (< 24mths) by: (1) exploring the use of regular FEC testing; (2) liveweight gain monitoring and analysis; (3) improve targeted wormer treatments; (4) treatment efficacy testing, and; (5) GIN speciation. As an extra, in partnership with researchers at Queens University Belfast (Dr. Christopher McFarland), grazing records, pasture larval counts (PLCs) and FEC results from Farm J were used to validate a mathematical model, GLOWORM, which maps parasite contamination risk for different pastures.

The operational group for the Cattle Roundworm EIP Project was made up of the three farmers and Eurion Thomas of Techion Ltd, with technical input from Steffan Vets, Tysul Vets and Prof. Diana Williams of the University of Liverpool, a leading parasitologist and advisor to the COWS group (Control of Worms Sustainably).

The project ran from August 2019 to Jun 2022. This report:

- Details the project outline
- Presents the results
- Discusses the results and limitations
- Reports on the knowledge exchange
- Highlights some of the lessons learned

2. Project outline

2.1 Participating farms

The practical work was based on three dairy farms in Ceredigion, that run similar farming systems and shared concerns that parasite burdens may be affecting the performance of their youngstock, and the need to review the use of routine anthelmintics. All three participating farms have spring calving dairy herds, rely heavily on grass for production, utilise extended grazing seasons with minimal supplementary feeding, and some out wintering of stock. All three farms already monitored grass growth and daily liveweight gains (DLWG) in first (R1) and second (R2) season grazers, and set targets for DLWG, bulling weights and dates.

2.2 Monitoring Parasite Burdens - Faecal egg counting

Faecal egg counting (FEC) is a test in which microscopic GIN eggs within a faecal sample are observed and quantified, and this gives an indication of worm burden within the gastrointestinal tract of the animal. FECs can be used to monitor GIN burden longitudinally and inform treatment-based decisions. In this project the FECs were carried out by Steffan Vets using the FECPAK^{G2} system (using a test sensitivity of 20 eggs per gram ((epg)). The farmers were tasked with regularly collecting composite faecal samples (see instructions below) from the R1 and the R2s and sending them to Steffan Vets. For each of the management groups on each farm, it was advised that FEC samples were taken at approximately 3-week intervals during the grazing period (April – October). This was to enable regular assessment of the parasitic GIN burdens in the youngstock and support treatment / management decisions.

Faecal Sampling Instructions Provided to the Farmers

- Please use the plastic zip lock bags and FECPAK^{G2} sampling scoops provided
- Samples can be collected from the ground (either in the field or yard)
- Samples must be fresh (deposited within last few hours)
- Ensure there is no contamination (older faecal samples, soil etc.)
- Once collected, expel the air from the back and seal securely.
- Keep in a cool dry place until delivered to the vet practice.
- If not taken to the vets that day, please store the samples in a fridge.
- Label the bags clearly and complete the submission form.

<u>Individual sample</u>

- Invert the plastic sampling bag over your hand, sample a 3-finger pinch from 3 different sites of the faecal pat.

<u>Pooled / composite sample</u>

- Using the sample scoop provided (2.5 ml volume) take level scoops from 3 different sites of the dung pat.
- Repeat this until samples from 15 to 20 animals are collected (minimum of 10 in smaller groups).
- Combine all samples into the same sample bag.

2.3 Anthelmintic treatments

Two anthelmintic dosing strategies have been proposed to control parasitic gastroenteritis (PGE) in ruminants. Targeted treatment (TT) in which whole-group treatments are administered based on evidence of infection, *e.g.,* FEC; and targeted selective treatment (TST) in which only specific individuals are treated based upon a specific factor *e.g.,* DLWG. Both strategies aim to reduce overall anthelmintic usage, and reduce the pressure on anthelmintic resistance (AR) development (Höglund et al., 2013). Theories such as

TST and TT have been proposed by parasitologists for over a decade, yet evidence of their feasibility in a truly commercial setting is scarce. In this project, the three cattle dairy farmers were able to undertake regular FECs in the R1 and R2s and make treatment decisions, (such as to only treat a select few or the whole group) based upon the FEC results.

2.4 Assessing anthelmintic resistance / efficacy - Faecal egg count reduction tests

Anthelmintics, the therapeutics used to treat 'worms' have been around since the 1950s, and are used to maximise livestock health and productivity. However, over reliance and misuse has led to the development of anthelmintic resistance (AR) to all drug classes and most livestock species (Kaplan, 2020). In simple terms, AR is when a proportion of targeted parasites survive a treatment (*i.e.,* they are resistant to treatment), which previously should have eliminated them. AR presents as a treatment failure, but it must be noted that treatment failures are not solely caused by AR, but can be the result of a range of variables including host factors (*e.g.,* gut transit, co-infections, co-morbidities, metabolism), improper treatment administration and interactions between drugs. The rate at which AR develops can be affected by a number of different factors including the parasite species, the frequency of administration, grazing practices, and the proportion of refugia (GIN that do not come into contact with a treatment, such as those on the pasture).

Faecal egg count reduction tests (FECRT) are the only field-based test used to assess anthelmintic efficacy. Because FECRTs are not only influenced by AR but multiple other factors (such as those mentioned above), variation in results is to be expected. A single FECRT can be informative, but the limitations must be recognised. Preferably longitudinal monitoring of treatment success / failure (even in a reduced form) gives a more holistic and broader picture of anthelmintic efficacy.

To assess the anthelmintic efficacy status on each of the farms a full FECRT was intended to be carried out at intervals during the project when high enough FEC levels were recorded to warrant treatment. This could only be completed on two of the farms (Farm E and Farm J) as the third farm (Farm M) never had high enough FECs to enable an FECRT.

These tests covered the key groups of anthelmintics:

- Benzimidazole - 1 BZ (White Drench)
- Levamisole - 2 LEV (Yellow Drench)
- Ivermectin - 3 ML (Clear – Pour on / injectables)
- Moxidectin - 3 ML (Clear – Pour on / injectables)

2.5 Animal performance – daily liveweight gains

Cattle were weighed at appropriate intervals throughout the project and measured against pre-set targets (including reaching a suitable weight at mating). Each of the farms used electronic weigh scales and EID ear tags to regularly monitor liveweights and ensure they met performance targets.

The liveweight data was cross-referenced to FECs, FECRTs, speciation tests and anthelmintic intervention records. The purpose of this was to assess the impact of improved FEC monitoring, and to determine if there are any effects on growth rates due to adaptations in parasite control management.

2.6 Animal performance – FECs and daily liveweight gains

During the process of completing the FECRTs, it was realised that individual data had been collected on FEC, liveweights and daily liveweight gain (DLWG) from the same individual animals on the same day as the first treatment. The R1 calves came in for treatment and in order to allocate the correct dose rate, each calf's weight was recorded. Subsequently, it was decided to compare the data to see if there was a correlation between pre-treatment FEC counts (as an indicator of overall parasite burden) and their growth performance over the previous few weeks.

2.7 Parasite speciation

The two main bovine GIN species endemic on UK cattle farms are *Ostertagia ostertagi* and *Cooperia oncophora*. In terms of pathogenicity, *Ostertagia* is considered to be the main danger and biggest contributor to Parasitic Gastroenteritis (PGE), the main consequence of which is sub-optimal performance through loss of appetite and reduction in absorption of nutrients. In older cattle this species can also lead to Ostertagiosis Type II disease which is caused by to the emergence of inhibited larvae harboured over winter from the previous season. Although Type II disease is not very common, it is important when considering a treatment strategy. *Cooperia oncophora* is generally considered to be a mild pathogen in calves although some studies associate it with inappetence and poor weight gains (Taylor et al., 2007). However, the impact of dual infections is higher than either of them individually (COWS, 2020). *Cooperia* spp. are also the dose limiting worm species for the 3ML class of wormers (Taylor et al., 2007)

It was deemed of interest to the project to identify which species are present on the project farms and in particular, which species (if any) survive anthelmintic treatment. FEC samples were collected when high FEC's were recorded and when FECRT's were undertaken. These were sent to University of Liverpool for culturing the eggs to L3 stage larvae. These were fixed and shipped to University of Calgary for detailed speciation using Nemabiome sequencing (Avramenko et al., 2015).

A third roundworm that also have severe consequences for grazing cattle is *Dictyocaulus viviparus*. It is a pathogenic lungworm, causing parasitic bronchitis and is mentioned in this report as the anthelmintics used to treat gastro-intestinal roundworm and lungworm are the same. Lungworm cannot be detected in a FEC test and therefore it was not possible to monitor their burdens in this project. Vaccinations are available for lungworm but none of the farms vaccinated as part of their routine parasite control plans.

2.8 Predicting future worm burdens – Pasture larval counts

The lifecycle of bovine GIN is direct. Briefly, adult roundworms live in the gastrointestinal tract, the females lay thousands of eggs which are shed in the faeces. Under suitable climatic conditions (temperature and moisture) these eggs develop and hatch as L1 in the faecal pat. The L1 migrate onto the pasture and undergo two more moults to become infective L3 larvae, which are ingested by the cattle, these migrate to the gut where they borrow into gastric glands (*O. ostertagi*) or the wall of the small intestine (*C. oncophora*), grow, moult and finally emerge and mature into adult worms. Pasture larval counts of L3s (PLCs) are a method used to predict pasture contamination levels and potential risk to grazing animals.

The FEC and grazing records from Farm J were used to validate a mathematical model called GLOWORM – FL, to predict pasture contamination risk. PLCs were completed to measure the success of predicting burdens. The aim of how the model could be used is to:

- Monitor pasture contamination and estimate the risk of exposure to infection for each field in the grazing rotation, and therefore identify which fields should be avoided / monitored more frequently.
- Estimate relative pasture contamination levels at the end of the grazing season in order to plan grazing for the following year, for example, deciding which fields can be safely grazed by susceptible animals and which should be cut for silage.

This part of the project was in collaboration with Queens University Belfast (QUB) and funding for any additional work in validating the model is covered by a separate BBSRC project run by QUB

3. Results

3.1 Participating farms

The practical work was based on three grass based dairy farms in south Ceredigion, West Wales.

Farm system background

- Three farms running similar systems
- Spring calving dairy herds
- All three farms rely heavily on grass and have extended grazing seasons
- There is minimal supplementary feeding for youngstock
- Some groups of youngstock outwintered

3.2 Faecal egg counting

3.2.1 Summary per farm

Table 1. A summary of the number of FECs (R1 and R2) submitted to Steffan Vet Practice for routine monitoring during each year of the Cattle Roundworm EIP Project. Samples collected during the FECRT tests were not included here.

	2019 (From Aug)	2020	2021	2022 (To end June)
Farm E	9	22	13	7
Farm J	17	32	35	11
Farm M	5	11	17	1

The figures illustrate good engagement from the participating farmers. Farm M only ran one group of R1 and R2 cattle, whereas Farms E and J ran two or more. These were often managed separately which explains the variation in sample numbers. Prior to the project, Farm J did a few FECs during a season. Farms E and M, however, did not.

3.2.1 Monitoring FEC results

The results from the monitoring FECs, pooled FECRT pre-treatment FECs, and FECRT post treatment FECs in R1s, and all R2 monitoring FECs submitted by each farm throughout the Cattle Roundworm EIP Project have been plotted on Figure 1. Farm E and Farm M R1 FEC results never exceeded 250 epg and 70 epg, respectively, throughout the project. Overall Farm J had much higher egg counts indicating a higher GIN challenge. Variation in FECS between years is also evident. R2 FEC results remained low (< 20 epg) for all three farms throughout the entire sampling period. This illustrates the variation between the three farms in the challenges they faced despite running similar systems. It also highlights why a standard blueprint to roundworm control cannot be implemented.

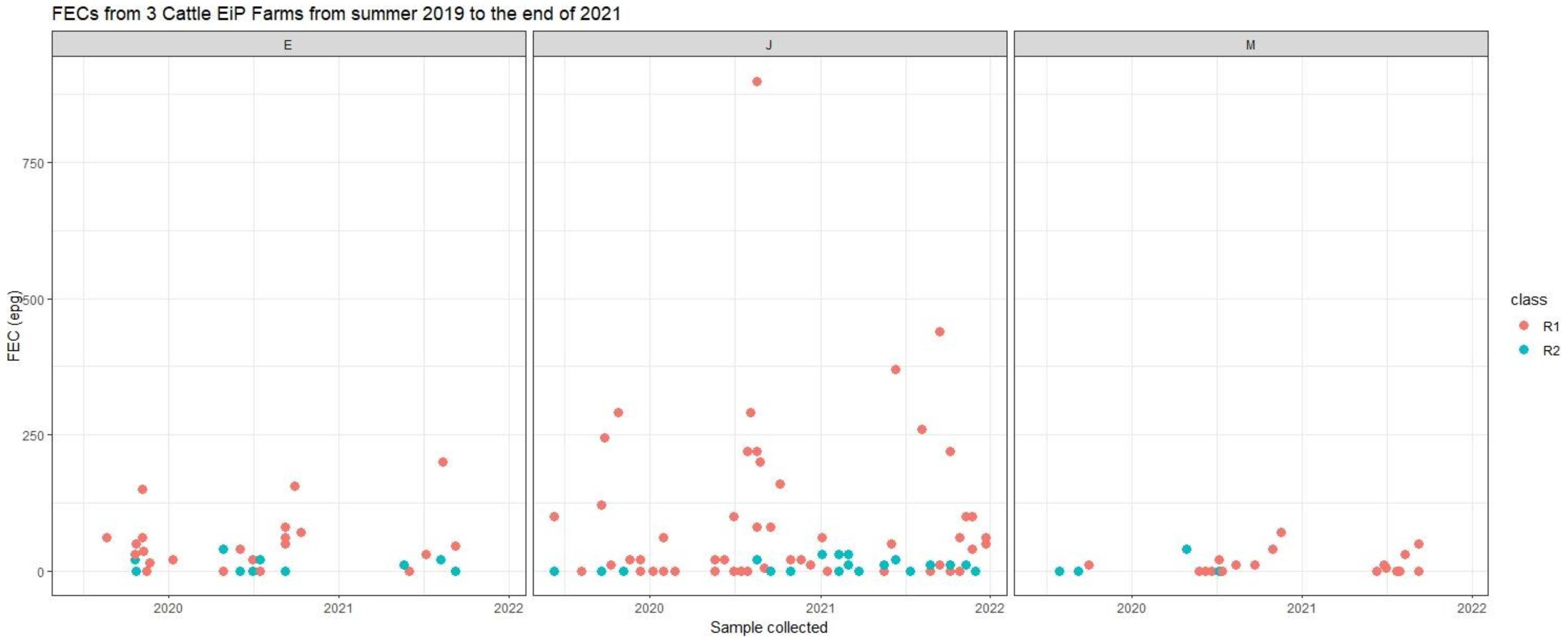


Figure 1: The FEC results from R1 (first season, red dots) calves and R2 (second season, blue dots) from Farm E, J and M. Regular FEC monitoring began in Summer 2019 and continued until the end of 2021. FEC; faecal egg count, epg; eggs per gram.

3.3 Anthelmintic treatment summaries

3.3.1 Farm E Anthelmintic treatment usage in cattle

Farm E comprises of two pasture-based spring batch calving dairy herds and their associated R1 (calves in their 1st grazing season) and R2 (heifers in their 2nd grazing season) followers. R1 (after weaning) and R2 cattle follow a rotational pasture grazing system on land separate to the adult dairy herd and separate to each other. Prior to participating in the Cattle Roundworm EIP Project, there were concerns regarding anthelmintic performance and suspicion of wormer resistance in the last few years

Summary of Farm E worming management / control prior to participation in the Cattle Roundworm EIP Project

Traditionally all R1 calves were dosed for worms 3, 8 & 13 weeks after turn-out and R2 grazers were dosed for worms a few times during their 2nd grazing season. Additional anthelmintic doses were given during the grazing season if:

- There were symptoms of gastrointestinal worms (scour)
- There were symptoms of lungworm (coughing)
- There were performance concerns (reduced growth rate)

All youngstock (R1 & R2) were given an additional worming dose around housing. Macrocyclic Lactones (ML) were predominantly used to dose both R1 and R2 cattle.

Summary of Farm E worming management / control following participation in the Cattle Roundworm EIP Project

Farm E did regular monitoring FECs throughout out the season. The objective was to reduce reliance upon regular worming, and aim to dose R1 and R2 cattle for gastrointestinal worms when indicated by an elevated faecal egg count. Additional strategic worming doses were given:

- During the grazing season if symptoms of lungworm are observed
- Around housing to control inhibited 4th stage larvae in housed R1 and R2 cattle
- During winter (depending under weather conditions) to control inhibited 4th stage larvae in out-wintered R1 cattle
- Due to concerns regarding the risk of lungworm several worming doses are still administered during the grazing season, especially to R1 cattle (see note below).

The FECRT highlighted reduced efficacy in IVM-inj, IVM-pour and MOX (Table 2). Post treatment sample sin 2019 were speciated and were >95% *C. oncophora* (See section 3.6.2*)*. As a result of the FECRT, BZ and LEV are now being used to dose R1 and R2 cattle.

Impact on Farm E following participation in the Cattle Roundworm EIP Project

Taking part in the Cattle Roundworm EIP Project has proven beneficial to Farm E by enabling investigations into suspected worm resistance by carrying out by FECRT over 2 years. Participation in the project also gave scope to adopting a more targeted approach to dosing the cattle for gastrointestinal worms which reduced anthelmintic usage and achieved:

- Financial gains from reduced anthelmintic purchases
- Reduced the time and labour associated with handling and worming cattle
- Promote responsible use of medicines
- Reduced risk of the development of worm resistance

In particular, there was a significant reduction in treatments of R2 cattle which demonstrated to the farmers that older animals could cope better with GIN challenge. One consequence of undertaking regular FECs is the increased labour associated with collecting faecal samples, however this is largely negated by reduced labour associated with treatments. Also the R1 calves are still frequently wormed due to concerns about lungworm infection as this cannot be monitored by the faecal egg counts used to monitor gastrointestinal worms. This was especially true in 2020 but further investigation showed another underlying health issue was confounding matters and mistaken as lungworm symptoms and this resulted in some unnecessary 'knee jerk' treatments. Vaccinating R1 cattle against lungworm would negate this risk and enable a more targeted approach to dosing for gastrointestinal worms.

QUOTE FROM FARM E

"This project has investigated so many aspects of parasite control that I couldn't have imagined to be possible. It has really opened my eyes to underlying health issues in my calf rearing system".

3.3.2 Farm J Anthelmintic treatment usage in cattle

Summary of Farm J worming management / control prior to participation in the Cattle Roundworm EIP Project

Prior to participating in the Cattle Roundworm EIP Project, Farm J followed a similar R1 cattle worming regime that is standard on most dairy farms. In 2017 and 2018 R1s were dosed routinely every 6-8 weeks from turnout to mid-February. Ivermectin was used mostly, except for when a combination product was used during winter presumably to cover liver fluke.

Summary of Farm J worming management / control during the Cattle Roundworm EIP Project

The Cattle Roundworm EIP Project began in 2019, and Farm J started the season treating all R1s with an IVM-pour, but in September 2019 switched to BZ (Albex) which was used up to February 2020. Albex is licenced for treating roundworm, lungworm and liver fluke. Farm J changed the worming strategy for 2020 R1 born calves in that different wormer groups were used (MOX and LEV), and the treatments were more targeted. Early in the season Farm J employed TST, in that only a proportion or specific individuals were treated (possibly based on their liveweight gain, but it is not clear from the treatment records), as opposed to treating all the animals. In October and twice through the winter all R1s were given Albex a combination used to treat fluke and roundworms.

In 2021-born R1s, Farm J moved away from IVM totally and used LEV and BZ wormers, other than a dose of Ivermec-Super (IVM-inj) given in November but this is a combination product that treats roundworm, lungworm, liver fluke, and some ectoparasites.

Impact on Farm J following participation in the Cattle Roundworm EIP Project

Participation in the Cattle Roundworm EIP Project has led Farm J to adopt a strategic approach to dosing the cattle for gastrointestinal worms. Most significantly, there has been reduction in the number of treatments to R2 cattle. Farm J no longer worms all R1s routinely throughout the season with an IVM-based product, but uses different products (BZ, MOX and LEV) and is more targeted. It is worth noting that Farm J is showing evidence of considerable parasite challenge on the block of land where the R1 calves are grazing and regular treatments are still warranted on the basis of high FEC results. There are other management strategies that could be used to reduce the pasture larval challenge (*e.g.,* grazing with non-susceptible stock), but it is difficult as it is a grazing block away from the main holding.

QUOTE FROM FARM J

"We will now be far more selective in the use of the clear group of wormers and we may make some changes to heifer grazing policy, with clean grazing provided on reseeds in the spring where possible. It is important that dairy farmers learn lessons from the sheep industry and the progress it is making on addressing anthelmintic resistance."

3.3.3 Farm M Anthelmintic treatment usage in cattle

Farm M is a pasture-based spring calving dairy herd which is currently comprised of 260 cross-bred cows and associated R1 calves and R2 heifers followers. R1 and R2 cattle followed a rotational pasture grazing system on land separate to the adult dairy herd and separate to each other.

Summary of Farm M worming management / control prior to participation in the Cattle Roundworm EIP Project

Traditionally R1 calves were dosed for worms 3, 8 & 13 weeks after turn-out and R2 grazers were dosed for worms once or twice during their 2nd grazing season. Prior to participation in the Cattle Roundworm EIP Project attempts were made to monitor worm FECs in-house but cattle were generally given a precautionary worming dose despite the result. Additional worming doses were given during the grazing season if:

- There were performance concerns (reduced growth rate)
- There were clinical signs of gastrointestinal worms (scour)
- There were clinical signs of lungworm (coughing)

All housed youngstock (R1 & R2) received an additional worming dose. IVM was the predominant wormer used to dose both R1 and R2 cattle.

Summary of Farm M worming management / control following participation in the Cattle Roundworm EIP Project

Faecal samples were collected from R1 and R2 cattle frequently throughout the Cattle Roundworm EIP Project during the season and also after housing. R1 and R2 cattle are now only dosed for gastrointestinal worms when indicated by an elevated faecal egg count. Although additional strategic worming doses were given during the grazing season if symptoms of lungworm are observed, at housing to control inhibited 4th stage larvae.

Ivermectin are still predominantly used to dose both R1 and R2 cattle due to their low price and convenience of use.

R1 cattle now adopt a leader-follower system on the main grazing platform with the adult dairy cows (where weather conditions and grass growth allow this practice). The FEC results were much lower on Farm M in comparison to the other two farms which indicated a much lower GIN challenge.

Impact on Farm M following participation in the Cattle Roundworm EIP Project

As a consequence of participating in this Cattle Roundworm EIP Project the number of treatments to both R1 and R2 cattle have reduced considerably. R1 calves only receive with one or two anthelmintic treatments a season, and this was largely due to the risks associated with lungworm infection. One small drawback is that reduced worming has led to a reduction in frequency of R1 and R2 animals being put through a handling system during the grazing season. This has led to a reduction in the number of times the animals were weighed.

Farm M's participation in the Cattle Roundworm EIP Project has led to a newly adopted strategic approach to dosing the cattle for gastrointestinal worms. Farm M no longer worms routinely, and only worms cattle in response to a FEC or possible lungworm risk. Consequently, there has been a large reduction in anthelmintic usage on farm which has led to:

- Financial gains from reduced anthelmintic purchases
- Responsible use of medicines and less promotion of worm resistance
- Improved animal health and welfare

Farm M has also decided to start vaccinating calves against lungworm as a result of engagement with this project (2022 born calves will be the first to be vaccinated). The aim is to further reduce reliance on anthelmintics and avoid lungworm related performance losses in calves.

QUOTE FROM FARM M

"I have been astounded by how much unnecessary wormer treatments we were giving to youngstock in the past. I am surprised by our low parasite burdens but it's a great outcome for us and performance hasn't suffered. Balancing production with protecting the environment is important for us on this farm so it is great that we are not overusing chemicals that could be harmful to dung beetles and other insects".

3.4 Faecal egg count reduction tests

Within this Cattle Roundworm EIP Project, five FECRTs were performed on two of the farms (Table 2).

3.4.1 FECRT protocols

There has been substantial debate over the years about appropriate protocols to determine treatment efficacy / anthelmintic resistance status. Techion Ltd and Liverpool University drew up the following robust protocols based on most recent guidance and experience. Due to the size of the herds, treatment groups of 15 R1 cattle were available to test multiple anthelmintics. Coinciding with the Cattle Roundworm EIP Project guidelines were being reviewed on a European level by COMBAR (Combatting Anthelmintic Resistance in Ruminants, www.combar-ca.eu) and worldwide level by WAAVP (World Association for the Advancement of Veterinary Parasitology, www.waavp.com) and the protocols met the criteria set out by both organisations.

- Group of youngstock below 18 months of age to be used (ideally below 12 months)
- Pre-test monitoring - The average pooled FEC must be > 100 epg minimum before starting but ideally, >140epg.
- Need 15 animals per treatment (TX) group
- Each animal treated according to weight band
- Individual faecal sample collected rectally and identified to tag number – collected and processed as individual FECs
- Same 15 animals sampled individually at post treatment visit – (7 days for LEV, 14 days all other TX groups)
- Additional pooled samples taken at all visits for faecal larval culture and speciation
- Vets carry out all sampling and treatment – under strict protocol.

- All FECs processed by Steffan Vets using FECPAKG[2] system, which has a detection sensitivity of 20 epg.
- Reduction percentage was calculated and analysed using 'RESOLOOT' (Brown et al., 2001) and 'Jeffreys Interval' (Dobson et al., 2012).
- If the reduction percentage was below the 95% threshold, then this shows a lack of efficacy of that treatment.

In November 2019, Farm E tested four products (Levamisole (LEV), Macrocyclic lactone / Ivermectin injection (IVM-inj), Macrocyclic lactone / Ivermectin pour on (IVM-pour), and Moxidectin (MOX)). In October 2020 Farm E tested three anthelmintics: Benzimidazole (BZ), IVM-inj and MOX.

Farm J performed an FECRT in Oct 2019 (BZ and IVM-inj), August 2020 (LEV, MOX and IVM-inj) and June 2021 (BZ and IVM-inj).

Table 2: Summary of faecal egg count reduction test results performed on first season grazing cattle (6 - 8 months old) on farms E and J farms over 3 years.

Farm	Anthelmintic	2019 Reduction (%)	2020 Reduction (%)	2021 Reduction (%)
Farm E	1BZ		96	
	2LV	98		
	3ML - IVM-inj	44	57	
	3ML - IVM-pour	8		
	3 ML MOX	86	40	
Farm J	1BZ	100		100
	2LV		98	
	3ML - IVM-inj	81	62	96
	3ML - IVM-pour			
	3ML - MOX		80	

BZ: Benzimidazole, LV: Levamisole, IVM-inj: Ivermectin injection, IVM-pour: Ivermectin pour-on, MOX: Moxidectin.

Note that for the 2019 test carried out on Farm E the statistical analysis showed low or medium confidence in the results due to low FECs on the day of treatment. And when the same data was considered against the new protocols published by COMBAR and WAAVP they also failed to meet the criteria. However, the failures detected for the 3ML group in 2019 were confirmed in the repeat test in 2020. All other tests had high confidence and met the new AR guidelines. In summary, the 1BZ and 2LV wormer groups showed good efficacy each time they were tested while the 3ML wormers showed treatment failures on multiple occasions, with one exception in the 2021 test.

3.5 Animal performance – daily liveweight gains

3.5.1 Summary of data collected

Liveweights and DLWG of R1 cattle from three farms over 3 years (2019-2021) were recorded. Figure 2 shows that overall, there was no particular trend across time in either liveweight or daily liveweight gain. A vertical dotted line is shown in October to aid annual comparison, as the date of sampling varies year-on-year. This data shows similar growth patterns and no obvious negative effects on performance as a result of changes to parasite control strategies implemented as part of the project.

Mean liveweights

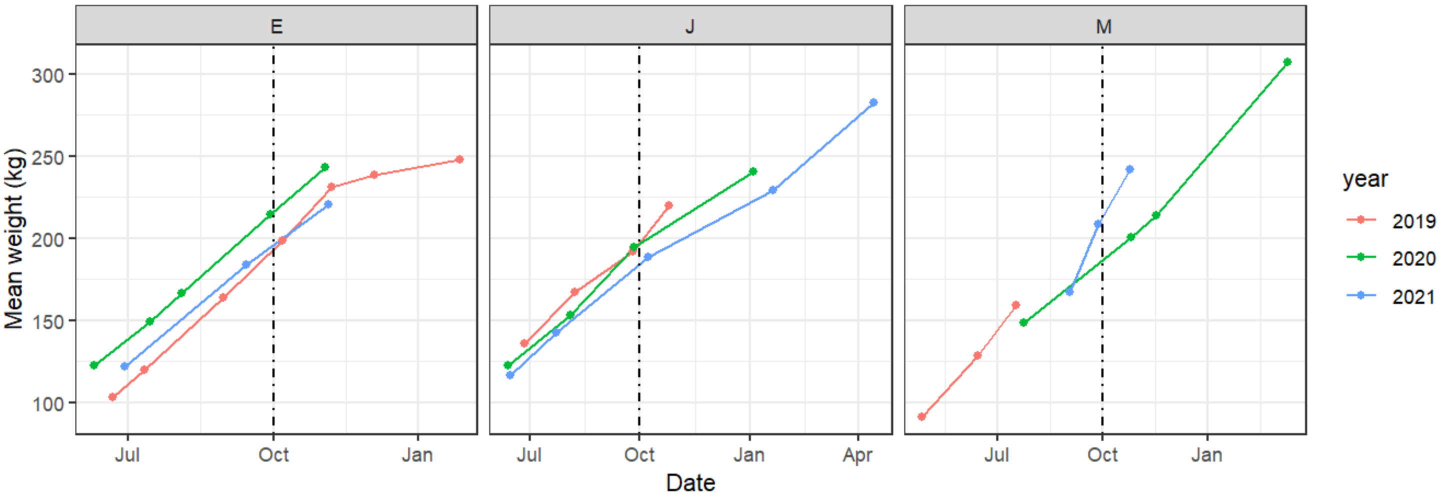


Figure 2: Mean liveweights (kg) of cattle from the three farms, for each year.

3.5.2 Farm E FEC and Liveweight analysis in 2019 and 2020

Farm E Pre-treatment summary

In 2019, all cattle in the low and high pre-treatment FEC categories met the target LW. Most cattle fell into the low FEC category and just under a third failed to meet the target LW (Table 3). There was no significant relationship between R1 pre-treatment FEC and either LW or DLWG (Spearman's rho, *p*=0.35, 0.56 respectively, Figure 3). In 2020, most cattle had high egg counts, and the majority met the LW target (Table 3). There was no significant relationship between pre-treatment FEC and either LW or DLWG (Spearman's rho, Figure 3).

Table 3: Number of cattle on Farm E in each pre-treatment FEC category that met the target liveweight in 2019 and 2020.

FEC category	Met LW target			
	2019		2020	
	no	yes	no	yes
Negative	0	6	2	0
Low (<100 epg)	15	37	2	12
High (>100 epg)	0	2	3	23

FEC: faecal egg count, LW: liveweight, epg: eggs per gram

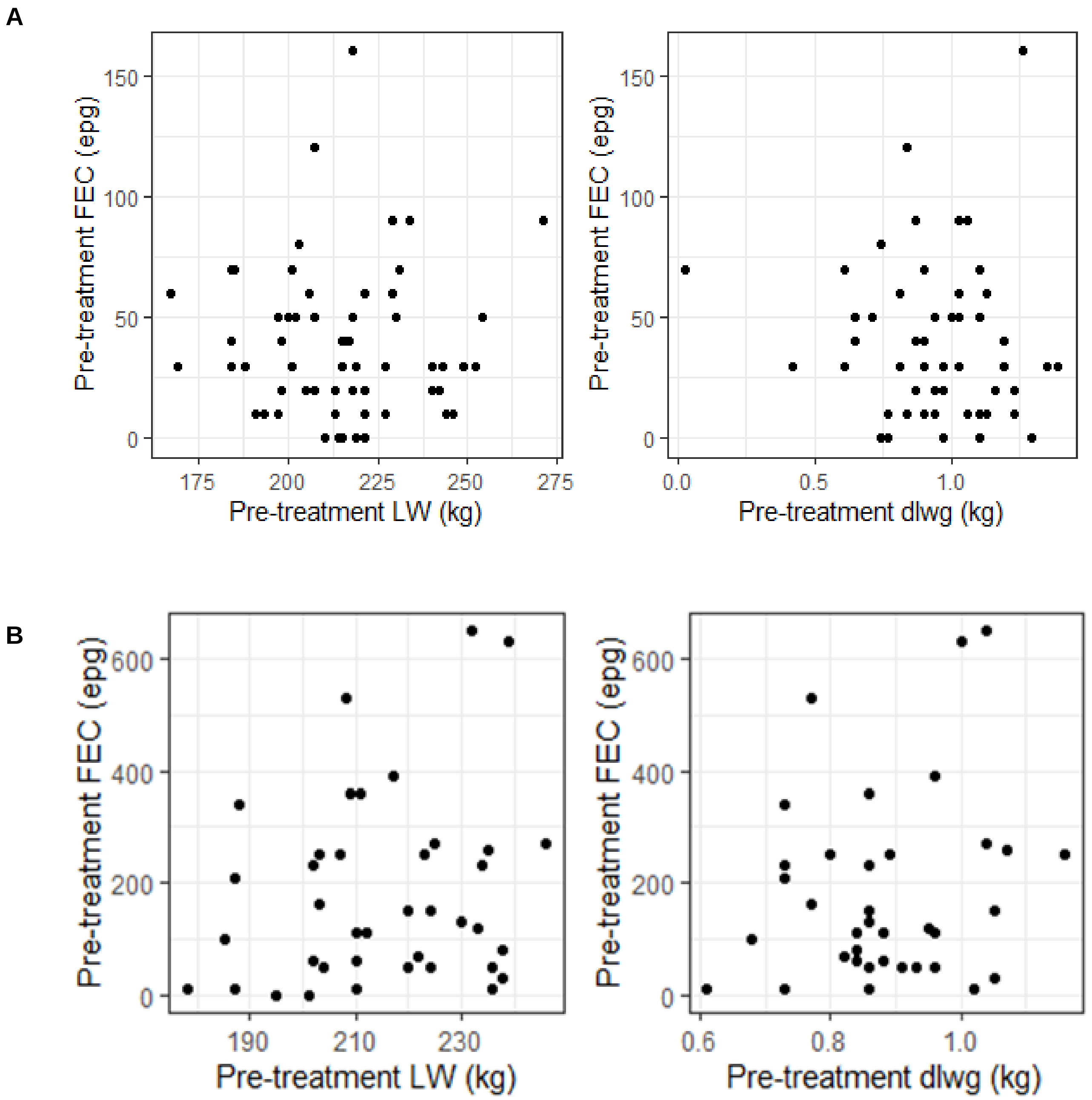


Figure 3: Farm E: There was no clear correlation between R1 (first season) cattle faecal egg counts (epg) and liveweight (kg) in (A) 2019, and (B) 2020 FEC - faecal egg count; LW – liveweight; epg – egg per gram.

In 2019, 60 8 month old calves on Farm E were divided equally between four treatment groups. Day 0 (treatment and sample date, plus pre-treatment weight measurement) was 8/11/19. Day 7 (sample collection date for LEV group) was 15/11/19. Day 14 (sample collection date for IVM-inj, IVM-pour and MOX) was 22/11/19. Post treatment weights were measured on 10/1/20.

The IVM-pour and LEV groups had a lower mean LW than the IVM-inj group (one-way analysis of variance with post hoc pairwise *t*-test with Bonferroni correction, p<0.05). There were no significant differences in pre-treatment FECs or in DLWG (Table 4, Figure 4).

In 2020, 15 R1 cattle were initially included, but one from each of the three treatment groups had a missing weight for one of the relevant dates, resulting in 14 R1s per group. Average pre-treatment FEC, LW and DLWG are shown in Table 4. Treatment was administered 29/9/20 and weights measured on 3/11/20. The DLWG and LW for the MOX group were significantly lower than for the other two treatment groups (Figure 4). The FEC were similar for all groups.

Table 4: Summary of Farm E pre-treatment FECs, mean LW and DLWG in R1 cattle, from 2019-2020.

	2019				2020		
Treatment group	IVM-inj	IVM-pour	LEV	MOX	BZ	IVM-inj	MOX
Number of calves	15	15	15	15	14	14	14
Median FEC (epg)	50	40	30	20	130	95	160
Mean LW (kg)	231.1	209.8	203.8	214.3	219.6	217.8	203.3
Mean DLWG (kg)	1.07	0.91	0.85	0.97	0.93	0.92	0.79

BZ: Benzimidazole, ML-inj: Macrocyclic lactone injection, ML-pour: Macrocyclic lactone pour-on, MOX: Moxidectin. FEC: faecal egg count, LW: liveweight, DLWG: daily liveweight gain, epg: eggs per gram, R1: first season calves

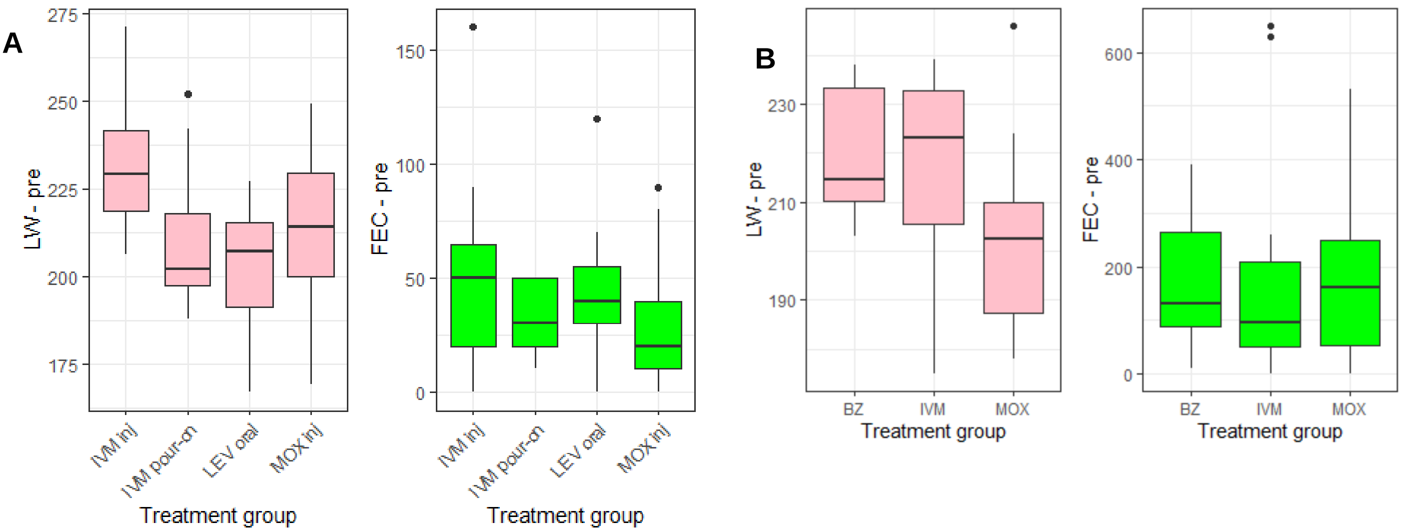


Figure 4: Farm E: Boxplots of R1 (first season) calves pre-treatment liveweights (LW, pink) and FECs (green) per treatment group in (A) 2019 and (B) 2020. LW-pre: pre-treatment liveweight, FEC – pre: pre-treatment faecal egg count, BZ: Benzimidazole, IVM-inj: Ivermectin injection, IVM-pour: Ivermectin pour-on, LEV: Levamisole, MOX: Moxidectin.

Farm E post treatment summary

Levamisole was the only drug in 2019 to result in an acceptable reduction in post treatment FEC in 2019 (See Table 2). There were no significant differences in DLWG between the pre and post treatment groups. DLWG post treatment were measured mid-January 2020, hence the decrease compared to pre-treatment DLWG. For LW compared to target, this was significantly lower for the IVM pour-on and LEV treatment groups compared to IVM-inj, both pre- and post-treatment (Figure 5A).

In 2020, the most effective wormer tested on Farm E in R1 cattle was BZ and the least effective was MOX (Figure 5B). The DLWG for the MOX treatment group was significantly lower pre-treatment (pairwise t-test with Bonferonni correction, p=0.005). Post treatment, DLWG for all groups were similar. LW compared to target was lower for the MOX group compared to BZ group both before and after treatment but this difference was only significant before treatment (pairwise t-test with bf correction, p=0.03). DLWG improved for the MOX group after treatment and became similar to the other two groups.

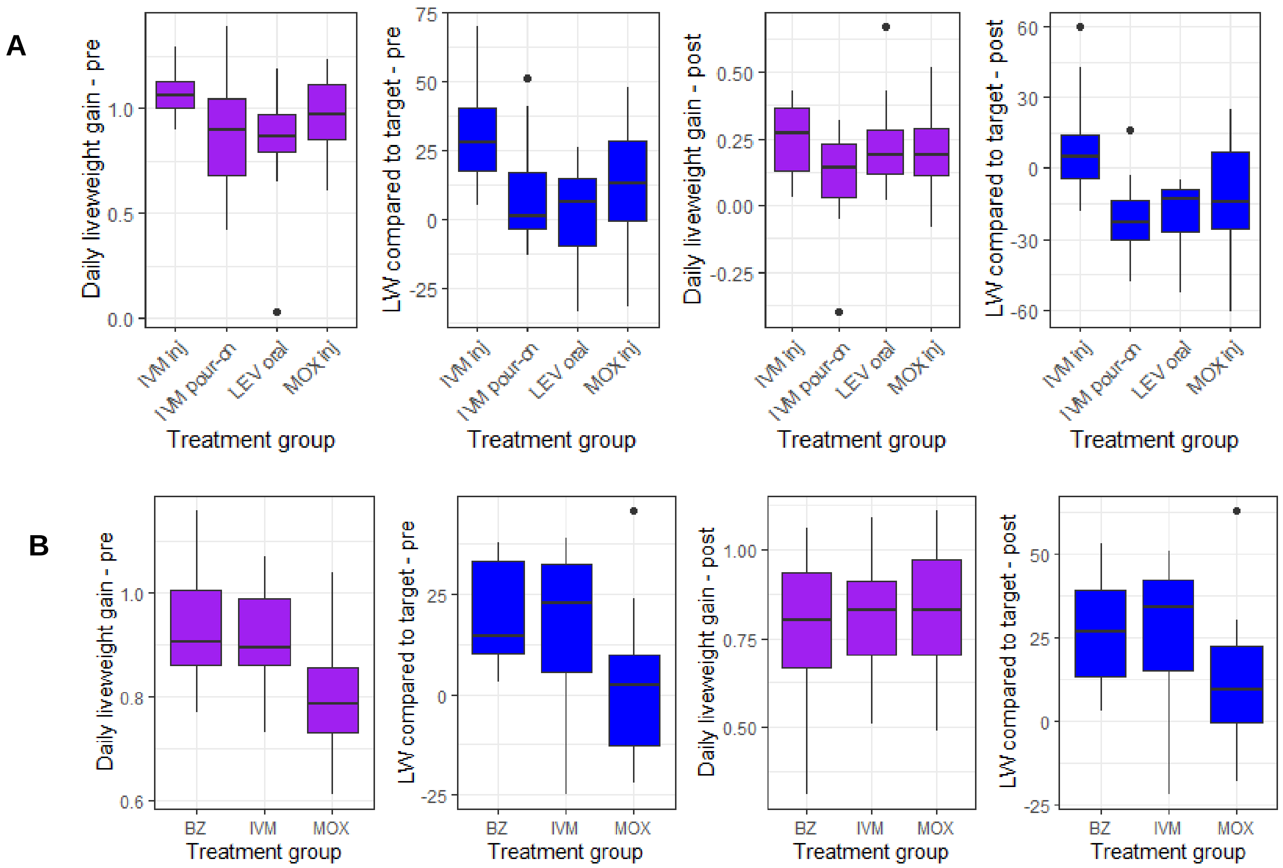


Figure 5: Farm E: Boxplots of the pre- and post- treatment daily liveweight gain (DLWG, purple) and liveweight (LW) compared to target (blue) for the different anthelmintic treatment groups in (A) 2019 and (B) 2020. LW liveweight, BZ: Benzimidazole, IVM-inj: Ivermectin injection, ML-inj: Macrocyclic lactone injection, LEV: Levamisole, MOX: Moxidectin.

Farm E pre and post treatment FECs and liveweight summary

In 2019, on Farm E there was no relationship between pre-treatment FEC and either DLWG or LW. Four treatments were compared. Although LEV performed much better than the other three, there was no effect on daily liveweight gain or LW compared to target.

The pre-treatment DLWG and LW compared to target of the R1 cattle in the MOX treatment group were significantly lower than the other two treatment groups (BZ and IVM). Post treatment the DLWG of the MOX group improved regardless of the poor treatment efficacy (40%, Table 2) became similar whilst the LW compared to target remained significantly lower post treatment.

It was difficult to assess if there was any treatment effect on liveweight gains post-treatment, as weighing took place 9 and 5 weeks post treatment in 2019 and 2020, respectively. Another confounding effect on post treatment performance in 2019 was that calves were also housed on day of treatment and the stress of housing and change in diet could have affected the calf growth rates.

3.5.4 Farm J – FEC and liveweight analysis in 2019, 2020 and 2021

Farm J pre-treatment summary

In 2019, 2020 and 2021 most cattle fell into the pre-treatment high FEC category but there was no significant difference in the number achieving target weight (Table 5). In each year, there was no significant relationship between FEC and either liveweight or daily liveweight gain (Figure 6).

Table 5: Number of cattle on Farm J in each pre-treatment FEC category that met the target liveweight in 2019, 2020 and 2021.

	Met LW target					
FEC category	2019		2020		2021	
	no	yes	no	yes	no	yes
Negative	0	0	0	0	0	0
Low (<100 epg)	3	9	3	6	3	1
High (>100 epg)	4	14	14	16	14	10

FEC: faecal egg count, LW: liveweight, epg: eggs per gram

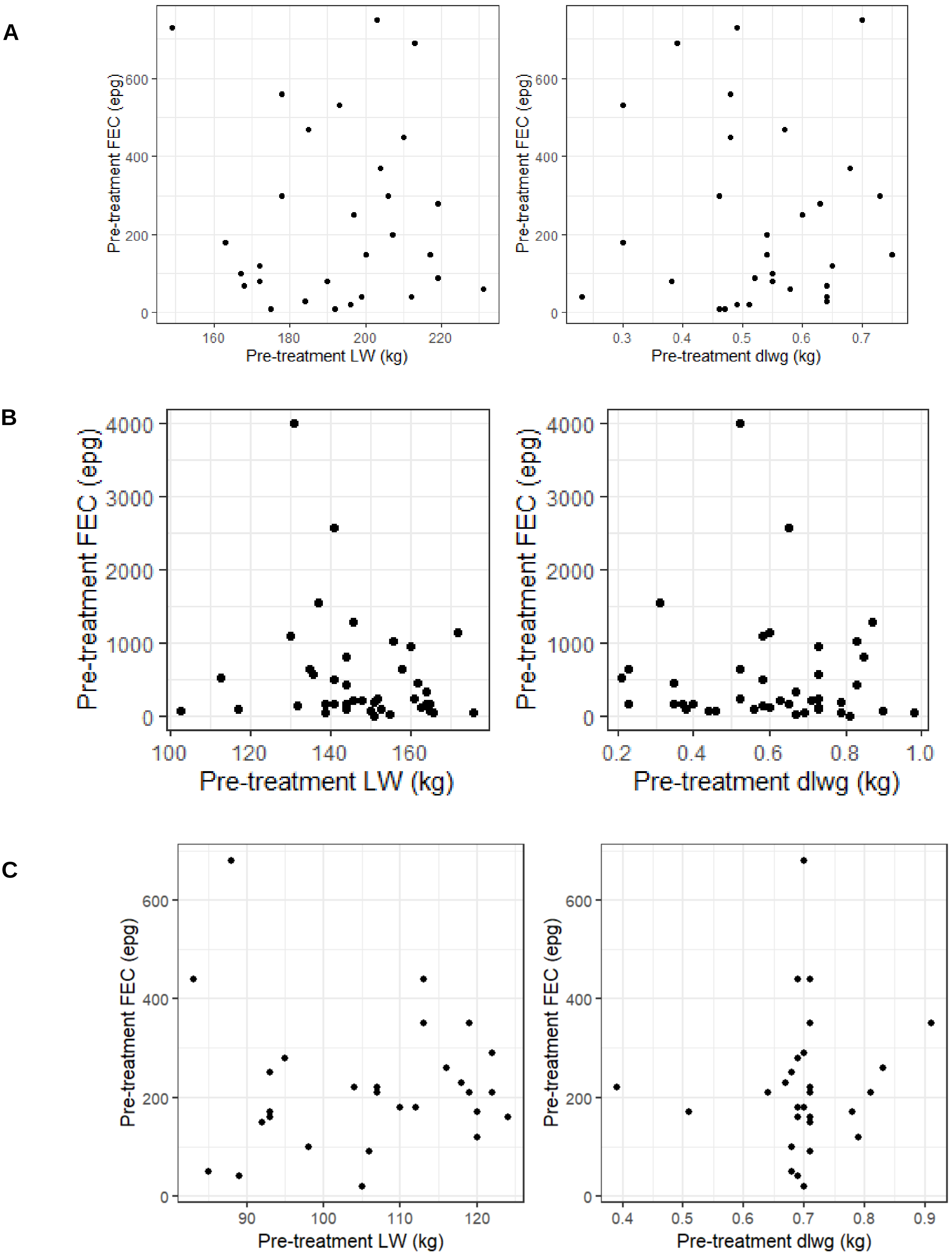


Figure 6: Farm J: There was no clear correlation between R1 (first season) cattle faecal egg counts (epg) and liveweight (kg) in (A) 2019, (B) 2020 and (C) 2021. FEC - faecal egg count; LW – liveweight; epg – egg per gram.

In 2019, 30 6-8 month-old calves were divided into two treatment groups. Day 0 (treatment and sampling date, and pre-treatment weights) was 25/09/19. Day 13 (sampling date) was 8/10/19. Post treatment weights were measured on 25/10/19. Pre-treatment FECs were significantly lower for the ML-inj group, but LWs were similar (Table 6, Figure 7)

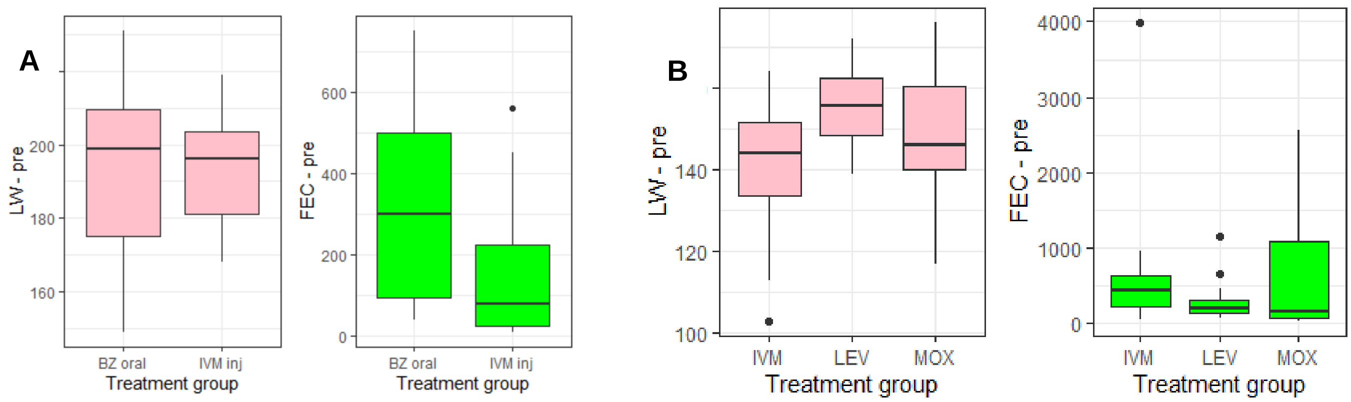
In 2020, 45 7-month-old claves were divided into 3 treatment groups. The LEV group were bigger calves when separated out (approx. 2 months prior to the study), and were in a different management group from the MOX and IVM-inj groups. The LEV group had treatment and FEC on different dates, and post treatment FEC was carried out on d7. The average DLWG of the LEV group was significantly lower than the other two groups (p=0.001), although the LW were slightly higher.

In 2021, 30 approximately 8-month-old calves were divided equally between two treatment groups. Two calves in the ML group had incomplete weight measurements recorded so were excluded from the analysis. Pre-treatment weights were taken on 15/06/21. Day 0 (treatment and sample date) was 17/06/21. Day 14 (sample collection date) was 30/06/21. Post treatment weights were measured on 23/7/21. A t-test indicated that there were no significant differences between the two treatment groups for FEC, LW or DLWG.

Table 6: Summary of Farm J pre-treatment FECs, mean LW and DLWG in R1 cattle, from 2019-2021.

	2019		2020			2021	
Treatment group	BZ	ML-inj	LV	ML-inj	MOX	BZ	ML-inj
Number of calves	15	15	15	12	15	15	13
Median FEC (epg)	300	80	430	180	140	170	210
Mean LW (kg)	193.3	193.1	140.5	154.8	148.3	102	111
Mean DLWG (kg)	0.52	0.54	0.63	0.41	0.72	0.69	0.71

BZ: Benzimidazole, ML-inj: Macrocyclic lactone injection, ML-pour: Macrocyclic lactone pour-on, MOX: Moxidectin. FEC: faecal egg count, LW: liveweight, DLWG: daily liveweight gain, epg: eggs per gram



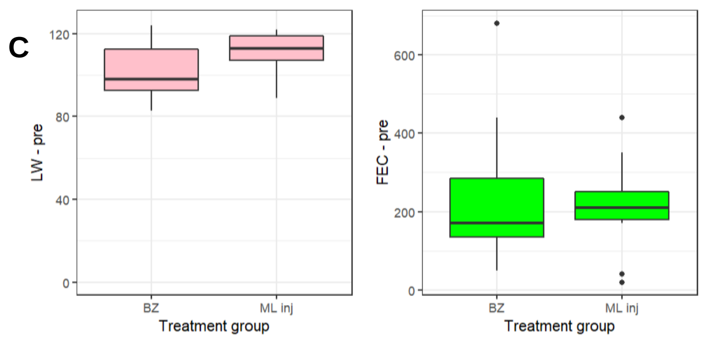


Figure 7: Farm J: Boxplots of pre-treatment LW (pink) and pre-treatment FECs (green) of R1 (first season) cattle, per anthelmintic treatment group from (A) 2019, (B) 2020 and (C) 2021. LW-pre: pre-treatment liveweight, FEC – pre: pre-treatment faecal egg count, BZ: Benzimidazole, IVM-inj: Ivermectin injection, ML-inj: Macrocyclic lactone injection, LEV: Levamisole, MOX: Moxidectin.

Farm J post treatment summary

On Farm J in 2019 BZ was more efficacious (100%) than IVM (81%) although no significant differences in DLWG and LW compared to target were seen the pre-treatment, nor for DLWG and LW compared to target post treatment. (Figure 8A).

In 2020 IVM was the least efficacious (62%, Table 2) followed by MOX (80%) while the LEV group showed a 100% efficacy The LEV groups DLWG was significantly lower than the other two groups, both before and after treatment (*p* < 0.001, Figure 8B) but were managed differently and treated at different times so a direct comparison is mis-leading. The liveweights compared to target of all three groups were similar before treatment. After treatment, the LEV group had a lower liveweight gain compared to target, but this difference was not significant (*p* = 0.07).

In 2021, both wormers saw a reduction in epg of > 95%, and there were no significant differences reported in DLWG or LW between treatment groups, either before or after treatment (Figure 8C).

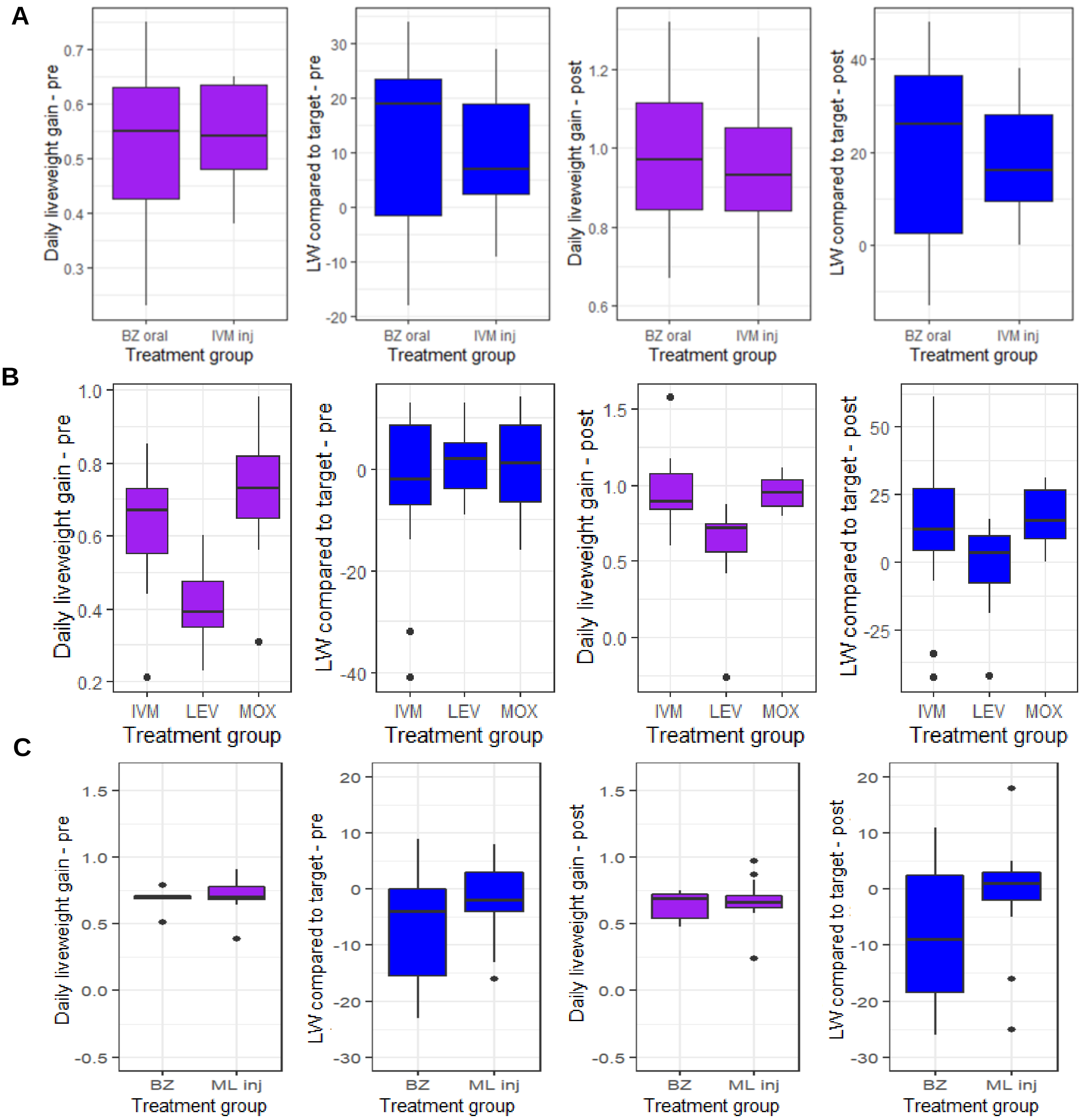


Figure 8: Farm J: Boxplots showing the pre- and post- treatment daily liveweight gain (DLWG, purple) and liveweight (LW) compared to target (blue) for the different treatment groups in (A) 2019, (B) 2020 and (C) 2021. LW liveweight, BZ: Benzimidazole, IVM-inj: Ivermectin injection, ML-inj: Macrocyclic lactone injection, LEV: Levamisole, MOX: Moxidectin.

Farm J pre and post treatment FECs and liveweight summary when considering High FEC Cattle only

Normally, it is expected to observe a difference in post treatment performance in calves that had high worm burdens. But here, no difference in post treatment performance was observed between treatment groups when they had (in some cases) significantly different treatment efficacies. The individual R1 calves had different FEC levels pre-treatment and therefore, it was decided to remove the low FECs results and re-analyse the data. The only time a difference was detected was in the 2019 test where the IVM-inj group had poor efficacy (81% efficacy) and a significantly lower DLWG than the BZ group (100% efficacy) after treatment when calves with low burden pre-treatment were removed from the dataset (Figure 9). The BZ Oral groups DLWG increased from 0.55 kg / day pre to 1.05 kg / day post treatment while the IVM-inj increased from 0.55 kg / day to 0.85 kg day. This a difference of 200g a day in growth which would have significantly affected the performance and production efficiency of those calves.

Care needs to be taken with interpretation as eliminating low FEC calves results in smaller data sets of 11 and 7 animals per group respectively.

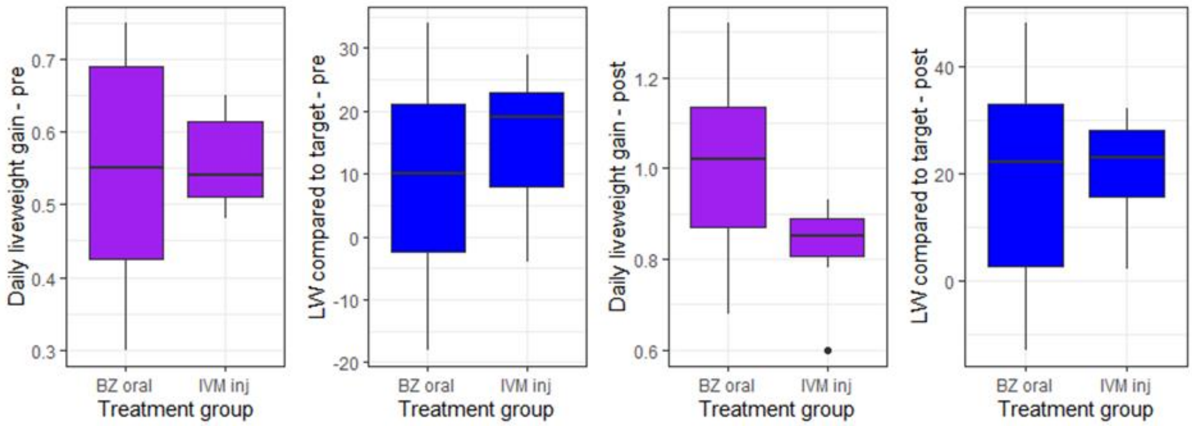


Figure 9: Farm J comparison of the pre- and post- treatment daily liveweight gain (DLWG, purple) and liveweight (LW, blue) compared to target for the different treatment groups in 2019 – High FEC Cattle only. LW liveweight, BZ: Benzimidazole, IVM-inj: Ivermectin injection.

3.6 Nematode speciation

3.6.1 Species of GINs infecting calves grazing pasture over summer of 2020 and 2021

Six pooled faecal samples were analysed from three farms between 9/7/2020 and 5/11/2020. Faecal samples were cultured and third stage larvae (L3) extracted (Avramenko et al., 2015). Larvae were speciated using a nemabiome approach at the University of Calgary (Avramenko et al., 2015). Nemabiome is a deep amplicon sequencing method used to provide a detailed picture of GIN species composition in a sample.

In all six samples, the majority of eggs were identified as *Cooperia oncophora*, the remaining eggs were predominantly *Ostertagia ostertagi*. The proportion of *O. ostertagi* eggs present ranged from 18% to 55% of the total number identified. A small number of *Trichostrongyle axei* eggs were identified on both farms at all time points. A small percentage of *Nematodirus helvetianus* was found on Farm J in the July sample. There did not appear to be any significant trends in terms of differences between farms or between sampling points (Figure10). A similar result was obtained when eggs from calves grazed in 2021 were speciated (Figure 11). In 2021 a small number of *Dictyocaulus viviparus* larvae were identified on Farms E and M.

Figure 10: Graph showing mix of nematode species on three farms at different timepoints between July and November 2020

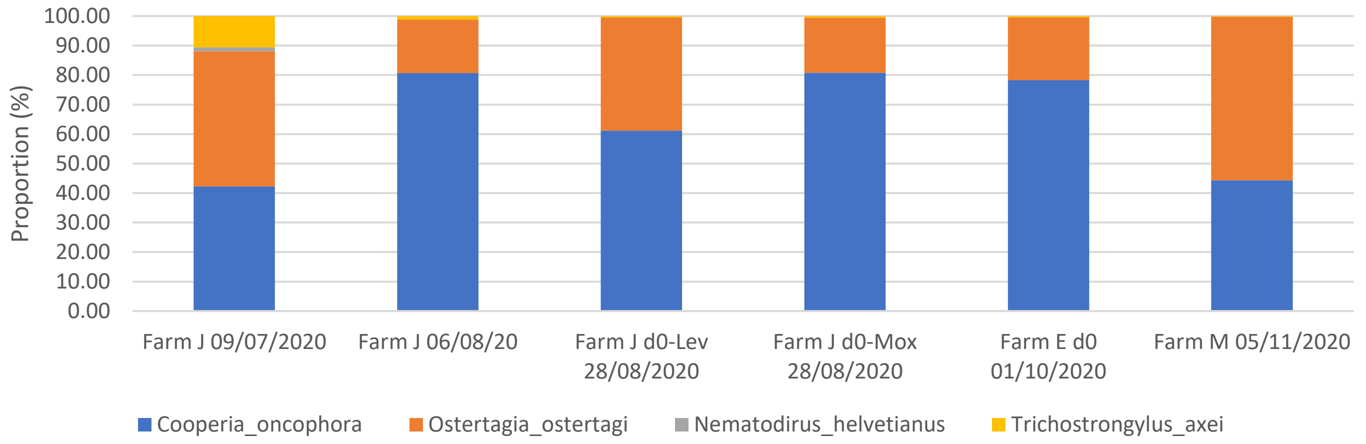
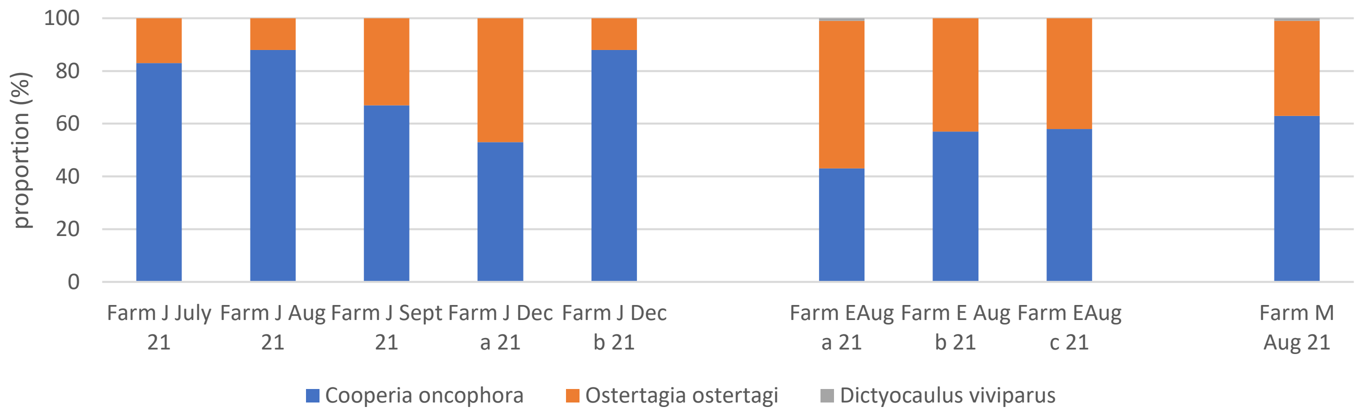


Figure 11: Larval identification from faecal samples collected from calves from three farms over summer 2021



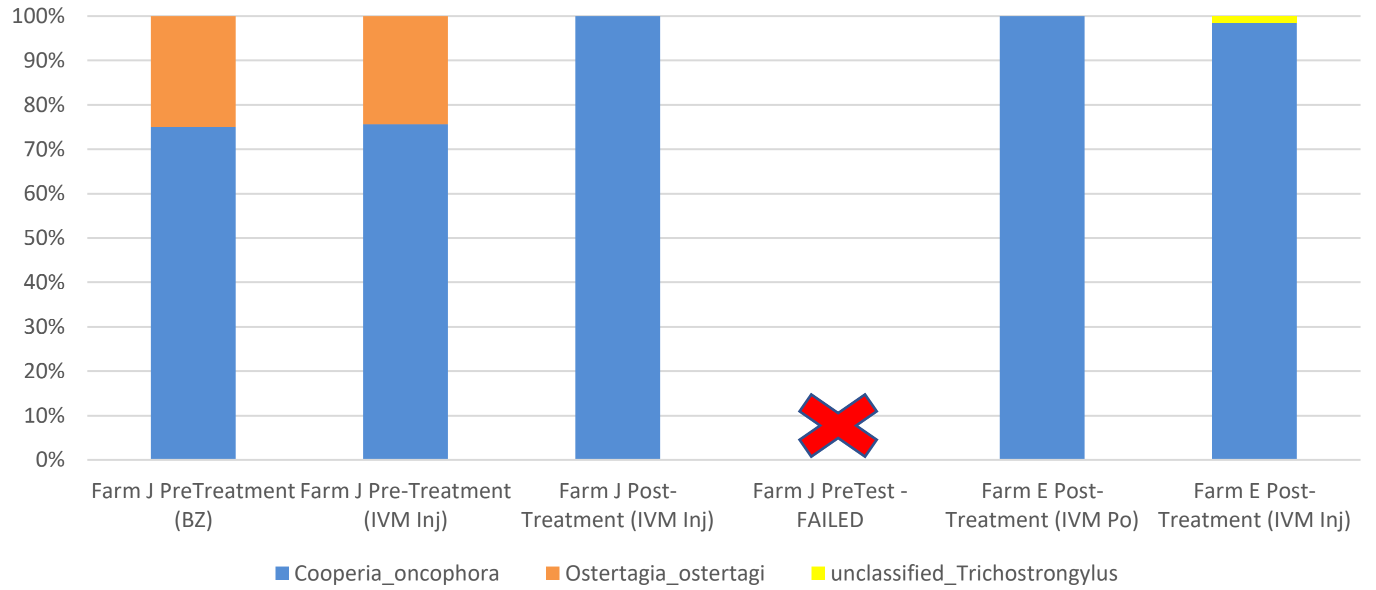
3.6.2 Comparing species of nematodes in pre and post treatment samples

On two farms (J and E), FECRTs were carried out in 2019, and as reported previously failures were recorded following either injectable ivermectin (IVM-inj) or pour-on ivermectin (IVM pour) treatment. The results of the speciation tests are illustrated in Figure 12.

On Farm J both the pre-treatment tests identified approximately 25% of the species identified were *O. ostertagi*, the remainder being *C. oncophora*. Following IVM-inj and treatment on farm J, there was an 81% reduction in FEC and 100% of the worms surviving treatment were *C. oncophora*.

On farm E there was a 44% and an 8% reduction in FEC following IVM-inj and IVM-pour treatment respectively. On this farm the pre-treatment test failed to yield any results, so the species mix is unknown, however 98-100% of eggs recovered post treatment in both group were *C. oncophora* (Figure 12). It is not possible to know from Farm E whether *O. Ostertagi* is susceptible to the 3ML wormers tested.

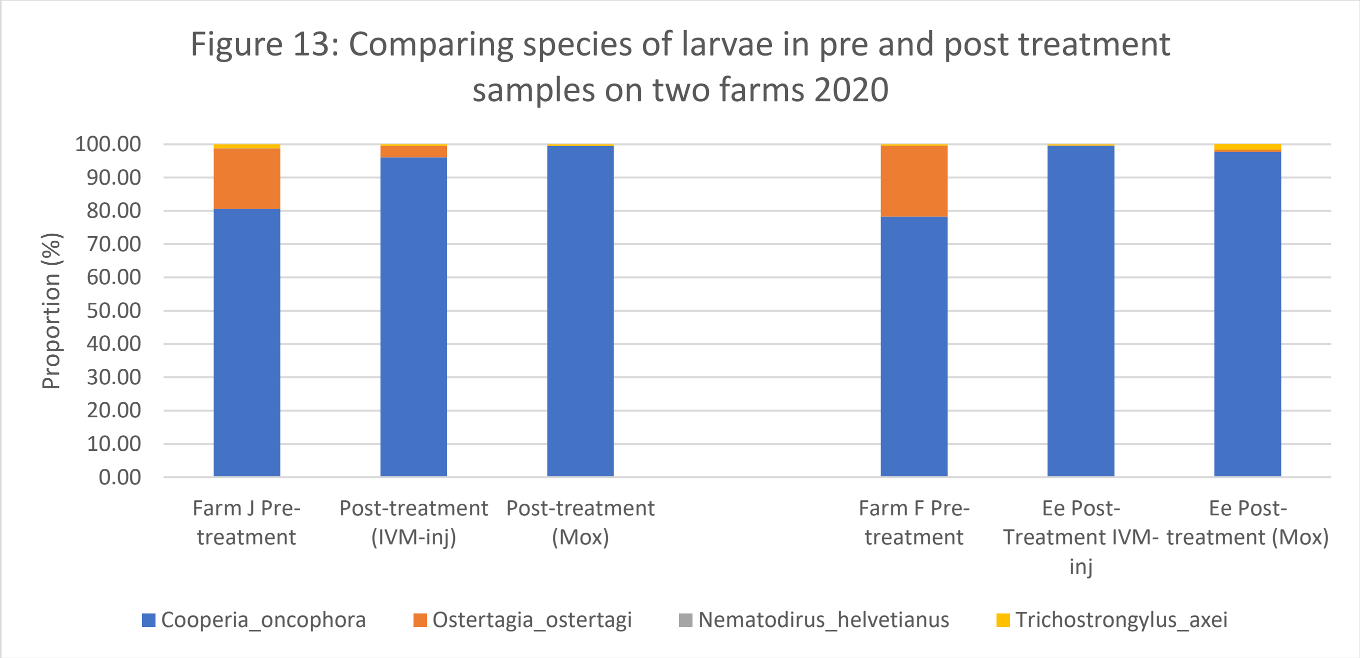
Figure 12: Comparing species of larvae in pre and post treatment samples on two farms 2019



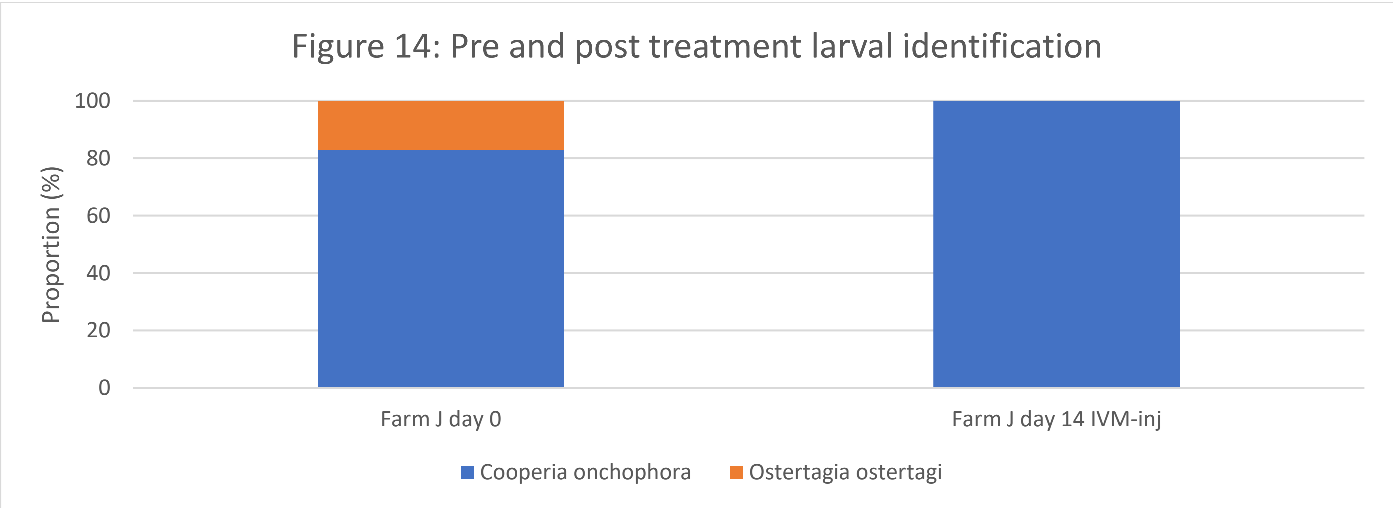
The FECRT tests were repeated on the same two farms (J and E), in 2020, and failures were recorded following either injectable ivermectin (IVM-inj) or pour-on moxidectin (MOX) treatment.

Before treatment, about 20% of the eggs recovered from the faecal samples were *O. ostertagi*, the remainder were mostly *C. oncophora*. Following IVM-inj and MOX treatment on farm J, there was a 62% and 80% reduction in FEC respectively. 96-99.5% of the worms surviving treatment with either IVM-inj or MOX were *C. oncophora*. About 3% of eggs were identified as *O. ostertagi* in the IVM-inj post treatment sample.

On farm E there was a 57% and a 20% reduction in FEC following IVM-inj and MOX treatment respectively. On this farm, 79% of eggs before treatment were *C. oncophora* whilst post treatment, 98-100% of eggs were *C. oncophora* (Figure 13).



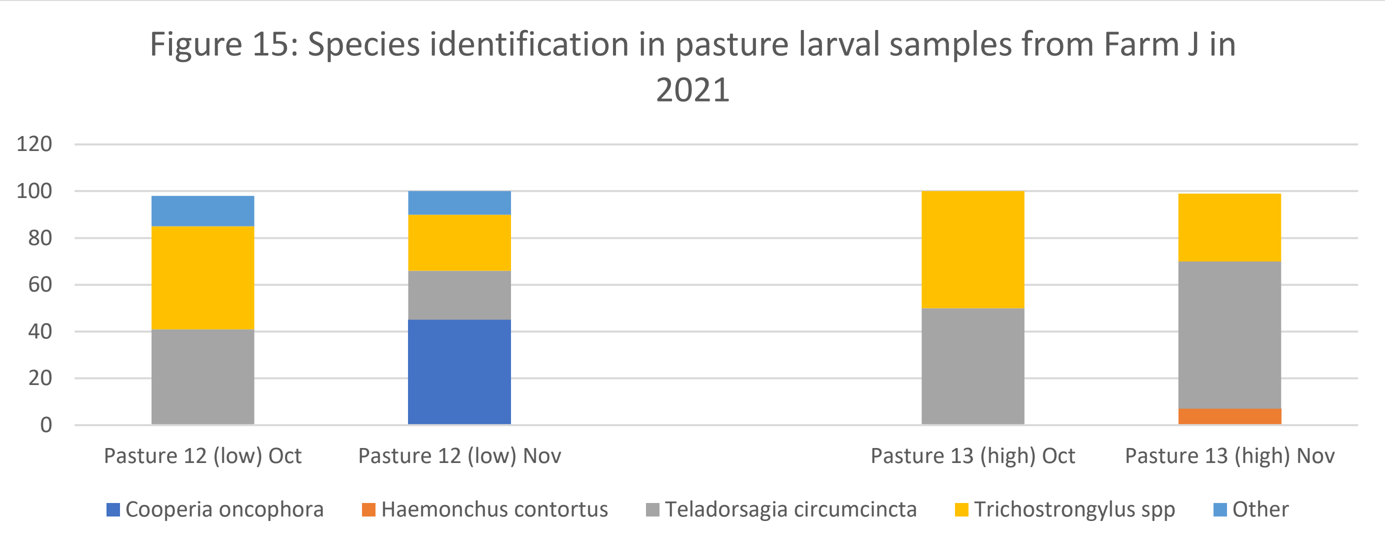
A similar result was obtained when the FECRT was repeated on Farm J in 2021, the calves were treated with IVM-inj, the FECR was 96%. Speciation showed that the small number of worms surviving treatment were all *C. oncophora* (Figure 14).



In conclusion, there was evidence from the FECRTs that both IVM-inj and pour-on MOX had reduced efficacy against *C. oncophora* but both products showed efficacy against *O. ostertagi*.

3.6.3 Pasture larval counts (PLCs)

Pasture larval counts were completed on 2 paddocks in Farm J as part of validation of the L3 pasture contamination modelling as described in section 3.7 below. L3 recovered from the pasture samples were speciated (Figure 15). L3 detected on pasture included *Cooperia oncophora, Teladorsagia circumcincta, Trichostrongylus* spp. and *Haemonchus contortus*. Other species detected in low numbers included *Chabertia ovina* and *Oesophagostomum venulosum.* No *O. ostertagi* or *D. viviparus* were detected on either pasture over the Autumn of 2021.



Speciation of eggs passed by grazing calves on three farms showed that the majority of worms parasitizing the calves were either *Ostertagia ostertagi* or *Cooperia oncophora*. *C. oncophora* is considered to be less pathogenic compared to *O. ostertagi* and less susceptible to macrocyclic lactones. Results from the faecal egg count reduction tests on two farms showed that *O. ostertagi* was susceptible to injectable ivermectin and pour-on moxidectin, but *C. oncophora* survived treatment. It is not known if these surviving populations of *C. oncophora* have developed heritable resistance to the macrocyclic lactones or their survival is linked to a lack of susceptibility. Avramenko *et al.,* (2017) found similar results in Canadian beef herds following macrocyclic lactone treatment. There was a significant increase in *C. oncophora* and a decrease in *O. ostertagi* following ivermectin treatment (Avramenko et al., 2017).

Small numbers of *Trichostrongylus axei* were detected on farms J and E in 2020 and small numbers of *Dictyocaulus viviparus* were found on two farms E and M in 2021. *T. axei* are rarely associated with disease or production losses in cattle in UK. In contrast, *D. viviparus* is a serious pathogen of calves and measures are needed to ensure control of this parasite.

Pasture larval counts were conducted on farm J. A similar but relatively low burden of larvae were detected on both pastures in October and November of 2021. A mixture of species of nematode were detected. In September, insufficient numbers of larvae were recovered to analyse. Samples collected in October and November showed a mixture of species present. The predominant species were *Teladorsagia circumcincta* and *Trichostrongylus vitrinus* and *Trichostrongylus colubriformis*. These genera are parasites of sheep. These are surprising results as this grazing block is not grazed by sheep other than the odd time when neighbouring sheep break in. *Cooperia oncophora* L3 were detected on one pasture in October and a small proportion of *H. contortus* L3 were detected in November on one pasture. This is consistent with the epidemiology of *H. contortus* in the UK (Rose et al., 2014).

The species of nematode detected on pasture did not reflect the species of nematode detected in the calves, but there was a difference in the time point when the pasture samples collected relative to the analysis of species present in the calves grazing those pastures.

3.8 Predicting future worm burdens

The early-stage investigation for this part focussed on Farm J only. A digital farm map of the R1 grazing block (as seen in Figure 16) was shared with Dr. Christopher McFarland from QUB along with the following data during 2019, 2020 and 2021 which was updated on a regular basis:-

- Grazing movement records– the number of stock and the date moved on and off each block
- Management of pastures (reseeding / harvesting etc.)
- Liveweight records
- FEC results (from FECPAK^{G2})

Figure 16: Digital map of Llechwedd Dderi which is the grazing block for Farm J's R1 calves.

Meteorological data was also collated, initially this data was sourced from available local weather stations. In 2021 a digital weather station was used, which was placed on the block and provided regular localised weather information on a regular basis.

This data was then fed into GLOWORM-FL which is a mathematical model that combines meteorological data, animal movement records and data generated by the FECPAK^{G2} system to predict future parasite contamination on pasture at a farm-level or individual field basis. It was developed under the EU funded GLOWORM and BBSRC funded BUG projects.

An example of the model output is shown in Figure 17 and this shows the predicted variation on pasture burden (measures as L3 on herbage per ha). This model is predicting that Paddock 1.1 will carry nearly twice as much parasite burden as Paddock 1.2.

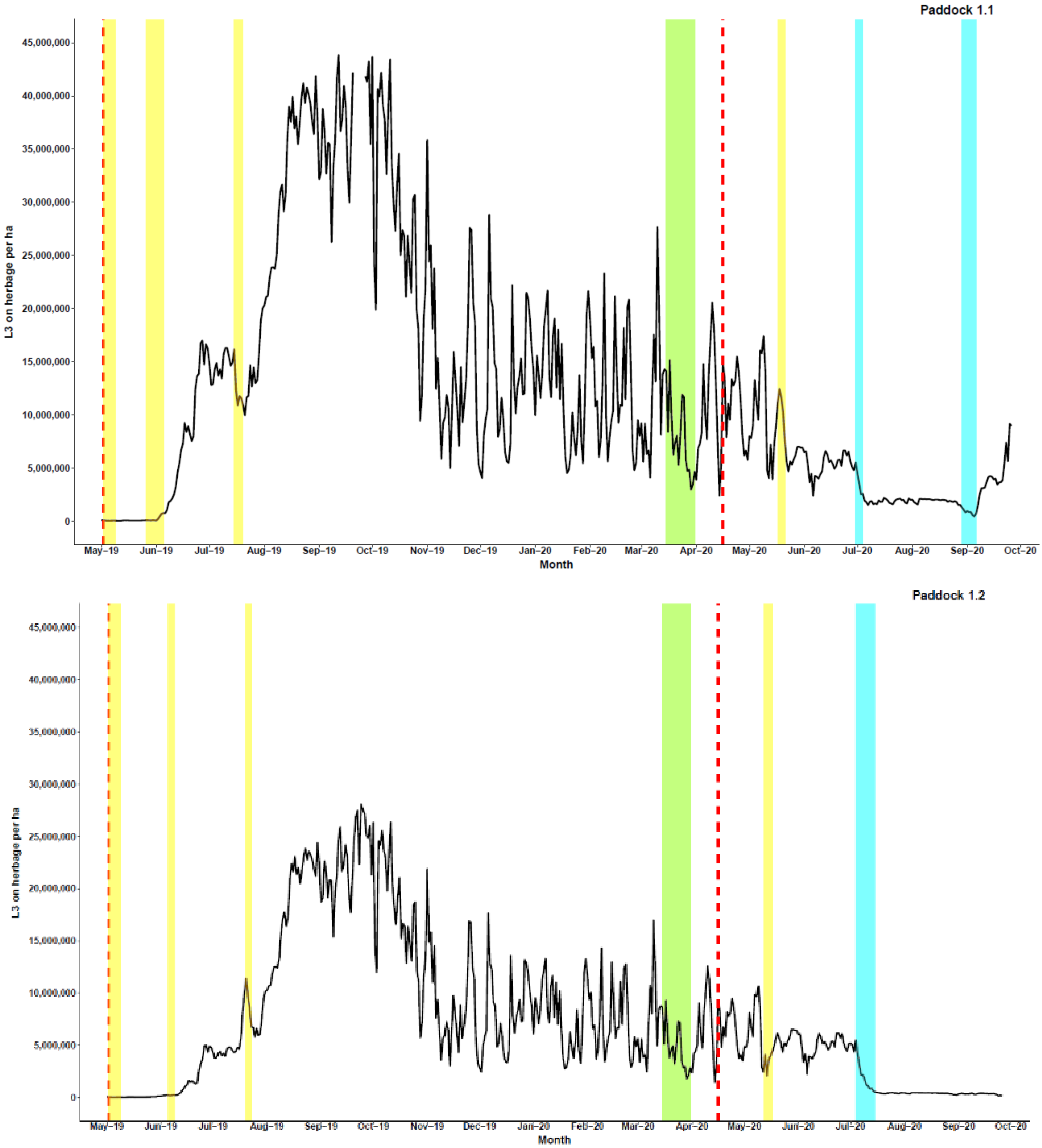


Figure 17: Example of outputs from the GLOWROM-FL model showing predicted L3 on herbage per ha on 2 different paddocks at Llechwedd Dderi (paddocks 1.1 and 1.2).

To test the accuracy of these predictions, PLCs were done on separate occasions in the autumn of 2021.Two pastures were identified, paddock 12 (identified on the map in Figure 16) was predicted to have a low L3 burden, based on previous grazing history and pasture 13 (Figure 16) was predicted to have a high L3 larval burden. Each pasture was sampled using standard ‘W’ transects done induplicate. L3 were isolated using Baermann methodology and the number of L3 per kg dry matter calculated (Griggs, 2005). Parasitic larvae were separated from free living larvae and speciated using a nemabiome approach (Avramenko et al., 2015). Data are shown as mean burden per pasture (Table 7).

Table 7: Summary of the average number of infective L3 recovered per kg of dry matter samples, per paddock in September, October and November 2021.

Pasture	September (L3 per kgDM)	October (L3 per kgDM)	November (L3 per kgDM)
12-Low	7.5	2850	3041
13-High	8	4832.5	1436

The number of L3s recovered in September was low, possibly as a result of a dry spell immediately prior to sampling. Numbers of L3 increased on both pastures in October and the pasture predicted to be highly contaminated had higher numbers of L3. However, by November numbers of L3 had declined on the paddock 13 (Figure 16), the high pasture whilst numbers of L3 were maintained on the low pasture, paddock 12 (Figure 16). The reasons for this are unknown. Further speciation work (as described in section 3.6.3) indicated the population of L3 was dominated by sheep nematodes with *Cooperia spp.* only detected in one of the 4 samples and *Ostertagia spp.* not detected at all. These are surprising results as no sheep graze this grazing block at all other than the odd time when neighbouring sheep break in.

The current variables used in GLOWORM-FL was based on parameters for *O. ostertagi*. A low prevalence of *O. ostertagi* was detected on Farm J, in comparison to *Cooperia spp.* and this would likely affect the outcome of the models estimates. An improved model has just been published (Wang et al., 2022) which now incorporates *C. oncophora*. and it would be interesting to rerun the data through the new model using *Cooperia* spp. parameters.

The most recent data is still being analysed and validated as QUB’s ongoing research work and more results may become available in the future.

4. General Discussion

The impact of wormer resistance on cattle production

The project has unearthed a significant lack of efficacy of the 3ML (macrocyclic lactones) group of anthelmintics on two of the farms where several FECRTs were carried out. This was found in wormers where both ivermectin, and the longer acting moxidectin, were the active ingredient. The mode of application did not appear to make a difference either as both injectable and pour-on applications showed reduced efficacies, although on Fam E the pour-on method did appear to have a lower efficacy at between 8% and 20% reductions only. The speciation tests infer that lack of efficacies, in most cases, was down to *Cooperia oncophora* surviving treatment.

A lack of treatment efficacy was confirmed on both farms on each occasion in 2019 and 2020. However, in 2021 on Farm Js FECRT the injectable ivermectin gave a 96% reduction. This FECRT was done much earlier in the season (early summer) than in previous years. It was suggested that *Ostertagia* might be the more dominant species at this time of year and this might account for the difference in FECRT result. However, the speciation results showed *Cooperia* was still the dominant species making up 83% of eggs present in the faecal sample before treatment, which raises interesting questions as to why the 3ML wormer showed better results in 2021 compared to previous years. It remains unanswered as to whether the lack of efficacy of the 3ML treatments is due to heritable anthelmintic resistance, or if *Cooperia* sppis is less susceptible to the 3ML wormers.

In the 2020 FECRT done on Farm J, there was some evidence that a small proportion of O. ostertagi survived the ivermectin injectable treatment; 18% of eggs were identified as *O. ostertagi* before treatment compared to 3% after treatment. It is possible that this may suggest evidence of ivermectin resistance developing in *O. ostertagi* on Farm J, but this requires confirmation especially considering ivermectin was shown to be fully effective in the FECRT carried out on Farm J in 2021. There are reports of ivermectin resistance emerging *O. ostertagi* in UK (Geurden et al., 2015).

It would be expected that the significant failures recorded here against the *Cooperia* spp. would negatively impact performance to some extent despite being the least pathogenic of the two species. However, no evidence was found that positive FECs or treatment with any of the classes of anthelmintic affected LW or DLWG. The only exception to this was when high FEC R1s alone were considered and a significantly lower DLWG was observed in the ivermectin injectable treated R1s compared to the benzimidazole treated group. The IVM-inj had an efficacy of 81% compared to the BZ treated group which was 100%. It must be remembered that this project was run under commercial conditions and other factors would have influenced growth rates such as where calves were grazed, when they were housed etc. One of the challenges encountered was the timing of post treatment weight recording, as this was often done several weeks after treatment, so initial differences in DLWG may have been missed.

Macrocyclic lactone wormers have residual effects that may have a positive impact on performance versus the other wormer classes. Both ivermectin (14 to 21 days) and moxidectin (35 to 120 days) have a persistent effect which prevents reinfection by certain worm species. Reinfection could mask differences in performance post treatment. Farm J in particular had high challenge pastures. Due to the lack of persistence for 1BZ and 2LEV wormers, if calves were turned back on to highly infected pasture, they would have been immediately reinfected whereas the 3ML treated animals would be protected due to their persistency. The 3ML wormers also control several ectoparasites such as mites, lice and biting flies and their removal may have effected LW gains.

The other important consideration with reduced efficacy is the impact on future pasture contamination and subsequent GIN infections. By not controlling the parasite burden, the calves will continue to seed the pasture with GIN eggs. The effects of this will be cumulative.

The role of FEC and FECRTs in cattle parasite control

Many have questioned the usefulness of FEC and FECRT's for cattle in the past with even an early version of the COWS manual (www.cattleparasites.org.uk) stating 'Faecal egg counts are of limited use'. The authors are well aware of the limitations of FEC testing; however, it is important to note they have proved extremely useful as a monitoring tool during the course of this project. One important point affecting the usefulness of FEC in cattle, is the dilution effect of the large quantities of faeces produced by cattle. For this reason, a test with a lower egg detection threshold compared to the standard McMaster test is essential. The McMaster test has an egg detection sensitivity (detection limit) of 50 eggs per gram (epg). The FEC method used for this project has an egg detection limit of 20epg, and in many cases 2 cassettes were analysed (always within an FECRT).

Another factor to improve the value of FECs is to test on a more regular basis so that trends can be observed. This would mean if a decision was made not to treat based on low FEC, then a follow up test could be done about three weeks later to ensure increasing burdens are detected. Regular monitoring helped identify significant differences in GIN infection patterns between farms, between different years and even between management groups on the same farm. Regularly monitoring R1 and R2 FECs has helped each farm better target the timing of treatment, reduce reliance on anthelmintics without any detriment to performance. It is important to take into account other factors such as growth rate, condition, age and history in conjunction with FEC data when making these decisions. FECs are a valuable tool that provide more data to enable farmers and their vet / animal health advisors to make better informed decisions. FECs are particularly useful in assessing contamination of pastures which is an important factor in parasite control management.

Previous studies have suggested that FECRTs in cattle are not sensitive enough to be useful. This may well be true when considering early stages of resistance and detecting low levels of treatment failure. However, in this project we detected different levels of treatment failure on several occasions following ML treatment. In the project, we ensured that the initial FEC in the pooled samples were high enough to conduct a FECRT, we also followed a rigorous protocol to ensure the accuracy of the result. The results obtained persuaded the farmers to move away from complete reliance on MLs and to rotate their anthelmintic classes each year. The newly published COMBAR and WAAVP guidelines for carrying FECRTs address previous limitations of FECRT in cattle. In conclusion, FECs and FECRTs are useful tools to include in parasite control strategies and herd health programmes for dairy farms.

Reducing reliance on anthelmintics

In this project, a monitoring led approach and better targeting of treatments reduced reliance on anthelmintics for all three farms involved. The biggest impact on all farms has been with the R2 cattle as regular FECs showed they rarely needed treatment and most of the routine treatments used previously were not administered.

For the R1 cattle, some treatments were given on farms E and J but fewer compared to previous years. Farm M had a much lower parasite burden compared to the other two farms, there was a substantial reduction in anthelmintic treatments, with no detriment to DLWGs. Some treatments were still administered regardless of the FEC due to the risk of lungworm infection (as detailed below) and in late autumn / winter to prevent the risk of Type II ostertagiosis. Even though R1 calves at Farms E and J received multiple treatments during the seasons they were much more targeted.

A second, important change in usual practice, was that all farmers stopped relying solely on group 3ML wormers, based largely on the FECRT data. This has meant going back to oral administration of BZ and LEV wormers, but with suitable handling facilities and restraining crush / crate this was not an issue for any of the farms.

Parasite speciation in monitoring

The differences between the two principal species of GIN have been discussed earlier, *O. ostertagi* and *C. oncophora* are thought to have different levels of pathogenicity and susceptibility to certain anthelmintic classes. The lack of efficacy of the ML group of anthelmintics observed during the project appears to be due to *C. oncophora*. Similar findings have been reported in the UK and in Canada (Geurden et al., 2015; Avramenko et al., 2017). Considering these findings, being able to readily detect what species are present during routine monitoring would be of great value for cattle GIN control. There are a few potential future developments that may address this, such as, the development of affordable molecular diagnostics, or training computer models to detect the differences in egg morphology from sample images. Tests need to be quick and affordable if they are to be used widely in the industry. Having immediate data about the species of GIN present in a faecal sample may aid treatment decisions, given the differences in pathogenicity and treatment efficacy against specific species.

Lungworm complications

One of the challenges encountered during this project was the perceived lungworm challenge which dominated decisions about when to treat. None of the farmers vaccinated for lungworm and it does not seem to be a common practice on many farms in the area. Vaccinating against lungworm is costly and laborious; two oral doses need to be administered four weeks apart and before they are exposed to any lungworm infection. Also, in recent years there has been a shortage of lungworm vaccines. There were several occasions when FECs were low but an anthelmintic treatment was still administered due to nervousness around lungworm. This hampered one of the project objectives of reducing reliance on anthelmintics, especially in the R1 group of cattle. Regular treatments for lungworm (especially persistent wormers) decrease exposure to the parasite, which could in turn prevent the development of natural immunity against lungworm. But the dangers from even low lungworm burdens are considerable in naïve calves (R1s), so some protection is necessary.

One of the messages to the industry would be to encourage lungworm vaccination. A positive outcome of engagement with this project is that Farm M has now started a vaccination program for their calves.

Ecological impact

An interesting positive side effect of the actions taken during this project is the impact on the immediate environment. It has been well documented that frequent exposure to certain wormers (especially 3ML wormers) can have a detrimental effect on important soil and dung dwelling invertebrates such as dung beetles and other insects, that are important food sources for many wildlife species. Dung beetles have gained considerable attention recently, as they are important insects that perform beneficial tasks for farmers. Not only do they clear dung pats and help distribute the nutrients into the soil, but they have an antiparasitic role as the aid in the drying out of faecal matter, making it a less habitable environment for GIN eggs and larvae to develop.

Although we did not evaluate this as part of the project, by reducing the reliance on anthelmintics and changing to different classes of wormers, these farms are helping protect these important organisms. Indeed, this gained a lot of positive interest from the three project farmers.

For more detailed information on this aspect please visit www.dungbeetlesforfarmers.co.uk or www.dungbeetletrust.co.uk.

Changing farmer behaviour

Changing farmer behaviour, in terms of administering regular treatments has proved difficult, despite providing data to support decision making. This has partly been due to concerns around lungworm and the risk of Type II ostertagiosis. Although significant progress was made, there could have been further improvements. However, change takes time and needs considerable support. The role of veterinarians, RAMAs and animal health advisors is key to provide this support and it is imperative they have the resources and correct training to do so.

Anyone interested in getting best advice and latest recommendations should use resources such as those found on the COWS website - www.cattleparasites.org.uk.

5. Knowledge exchange

This project has generated a substantial amount of interest, and considerable effort has gone into communicating the project and its findings.

4.1 Preparation of knowledge exchange materials

Two technical articles / posters were prepared in 2020 and 2021, respectively, and disseminated through EIP Wales and Farming Connect websites.

Following the completion of this report, a shortened summary in the form of a technical article will be produced for further dissemination. Communications will also be prepared to share with various industry bodies including COWS, BCVA, AHDA and VMD.

4.2 Press (broadcast and print)

An article highlighting the project was published in the farmer weekly on 28th October 2020 featuring Irfon Jenkins, one of the project farmers. An image of article is shown below. A full copy can be found here: https://www.fwi.co.uk/livestock/health-welfare/parasite-project-helps-dairy-farm-to-improve-worm-control

FARMERS WEEKLY

PPA SPECIALIST BRAND OF THE YEAR 2021

Parasite project helps dairy farm to improve worm control

Debbie James 28 October 2020

Irfon Jenkins is now more selective in his use of the clear group of wormers © Debbie James

A Ceredigion dairy farm is revising worming protocols for its replacement heifers after a parasite control research project detected early stage resistance to clear wormers.

The Jenkins family, who milk 400 New Zealand Friesians at Pentrefelin Farm, Talsarn, outwinter 210 R1 and R2 heifers on deferred grazing. This system increases vulnerability to roundworms because it limits opportunities for clean grazing in the spring.

See also: Tips on evaluating your dairy vet spend

The research project will run for three years and involves two other dairy farms in the region.

About the project

At Pentrefelin, two wormers – benzimidazole oral, a white wormer, and ivermectin injectable, a clear wormer – were tested on 15 calves. Faecal egg counts (FECs) were recorded before and after treatment.

- A press release is intended to coincide with publication of this report.

4.3 Presentations at conferences and events

- In July 2021 Eurion Thomas presented at the WAAVP (World Association for the Advancement of Veterinary Parasitology) International Conference (Held online due to Covid 19)
- Eurion Thomas presented the most recent finding of the project to COWS steering group on 2 separate occasions (May 2021 and May 2022).
- Eurion Thomas presented findings in a Webinar hosted by RM Jones (animal health distributor) in conjunction with Norbrook.
- Results have been further disseminated and discussed when Techion have attended events with an exhibition stand and these include –
    - COMBAR Conference, Greece May 2022
    - RAFT Precision Livestock Conference, York, November 2021
    - Royal Welsh Show, July 2022
    - Beef Expo, Darlington June 2022
    - Groundswell Event, June 2022
    - Morrisons Farming Event, June 2022
- Results will be further disseminated at 2 key events planned for late Autumn 2022 where Techion are attending –
    - AHDA Conference – September 2022 (Animal Health Distributors Association)
    - BCVA Congress – October 2022, Birmingham (British Cattle Veterinary Association)

6. Lessons learned

The Cattle EIP Project has been a large success as evidenced by the large volume of quality data presented in this final report. However, there have been some practical difficulties experienced along the way.

- COVID 19 restrictions presented some challenges as it was not possible to have face to face meetings, but online meetings worked well as an alternative.
- Veterinary practice involvement was challenging on occasions due to Covid 19 impairing the operational running of vet practices and staff absences. The implication of BREXIT also played its part as it has resulted in increased workloads (import and export paperwork) and a shortage of staff within the industry. This was felt by both practices involved in the project.
- The protocols for collecting performance records around the time of efficacy testing could have been more robust. Recording liveweight gains at 2 and 4 weeks post treatment on each occasion would have made performance data more comparable. In hindsight, we could have budgeted for some technicians to help the farmers and vets with these tasks, as it is understandably difficult to fit in these additional tasks within the normal routine of busy commercial farms.

7. Acknowledgements

The project is indebted to the co-operation and dedication of:

- The three participating farms and their vets
- University of Liverpool– Prof. Diana Williams, Dr. Alison Howell
- University of Calgary, Prof. John Gilleard, Dr. Elizabeth Redman (*Nemabiome Sequencing for speciation*)
- Queens University Belfast – Dr. Chris McFarland, Prof. Eric Morgan - (Pasture Larval Counts – disease prediction models)
- Tony Little - Sustainable Farming Consultancy / ADAS (project Innovation Broker)
- EIP Wales

8. References

Avramenko RW, Redman EM, Lewis R, Bichuette MA, Palmeira BM, Yazwinski TA and Gilleard JS 2017. The use of nemabiome metabarcoding to explore gastro-intestinal nematode species diversity and anthelmintic treatment effectiveness in beef calves. International Journal for Parasitology 47, 893–902.

Avramenko RW, Redman EM, Lewis R, Yazwinski TA, Wasmuth JD and Gilleard JS 2015. Exploring the Gastrointestinal “Nemabiome”: Deep Amplicon Sequencing to Quantify the Species Composition of Parasitic Nematode Communities. PLOS ONE 10, e0143559.

Brown LD, Cai TT and DasGupta A 2001. Interval Estimation for a Binomial Proportion. Statistical Science 16, 101–133.

COWS 2020. Control of roundworms in cattle (part of the COWS technical Manual).

Dobson RJ, Hosking BC, Jacobson CL, Cotter JL, Besier RB, Stein PA and Reid SA 2012. Preserving new anthelmintics: a simple method for estimating faecal egg count reduction test (FECRT) confidence limits when efficacy and/or nematode aggregation is high. Veterinary Parasitology 186, 79–92.

Geurden T, Chartier C, Fanke J, di Regalbono AF, Traversa D, von Samson-Himmelstjerna G, Demeler J, Vanimisetti HB, Bartram DJ and Denwood MJ 2015. Anthelmintic resistance to ivermectin and moxidectin in gastrointestinal nematodes of cattle in Europe. International Journal for Parasitology: Drugs and Drug Resistance 5, 163–171.

Griggs TC 2005. Determining Forage Dry Matter Concentration with a Microwave Oven. from *chrome-extension://efaidnbmnnnibpcajpcglclefindmkaj/https://core.ac.uk/download/pdf/84290306.pdf*.

Höglund J, Dahlström F, Sollenberg S and Hessle A 2013. Weight gain-based targeted selective treatments (TST) of gastrointestinal nematodes in first-season grazing cattle. Veterinary Parasitology 196, 358–365.

Kaplan RM 2020. Biology, Epidemiology, Diagnosis, and Management of Anthelmintic Resistance in Gastrointestinal Nematodes of Livestock. Veterinary Clinics of North America: Food Animal Practice 36, 17–30.

Rose H, Hoar B, Kutz SJ and Morgan ER 2014. Exploiting parallels between livestock and wildlife: Predicting the impact of climate change on gastrointestinal nematodes in ruminants. International Journal for Parasitology: Parasites and Wildlife 3, 209–219.

Taylor MA, Coop RL and Wall R 2007. Parasites of cattle. In Veterinary parasitology, pp. 60–62. Blackwell Pub., Oxford; Ames, Iowa.

Wang T, Vineer HR, Redman E, Morosetti A, Chen R, McFarland C, Colwell DD, Morgan ER and Gilleard JS 2022. An improved model for the population dynamics of cattle gastrointestinal nematodes on pasture: parameterisation and field validation for Ostertagia ostertagi and Cooperia oncophora in northern temperate zones. Veterinary Parasitology 310, 109777.

Appendix I: KE Materials and activities

- Article in the 'European Innovation Partnership (EIP) Wales: Collaborating for rural success' Booklet (2021)
- Project update, summarising findings to (February 2021)
- Article on ''Tackling wormer resistance in dairy youngstock' (October 2020)
- Article in Farmers' Weekly 'Project helps dairy farm to improve worm control' (28 October 2020)
- Project update (May 2020)