EBLEX Farm Innovation Grant ‘Improving detection and uptake of genetic resistance to roundworms in the UK national sheep flock’

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Project Background

Infection with gastro-intestinal roundworms is a significant limiting factor for lamb production in UK farming systems. Anthelmintic resistance is increasingly common with some flocks now subjected to drench failure in three of the five chemical families available. Roundworm infections can sometimes be successfully managed through good animal nutrition and management strategies such as the use of 'safe' grazing. However, lamb production systems often rely on unimproved grazing and therefore pasture improvement and use of mixed-species grazing is limited. Selecting individual animals for improved genetic resistance to roundworms is an important tool to combat worm challenge. Selection of breeding animals on the basis of their low faecal egg counts (FEC) has led to reductions in FEC in future generations and to reduced pasture contamination.

Breeders in the UK have been able to undertake selection for reduced FEC for a number of years but uptake has been limited in part due a lack of awareness amongst commercial ram buyers. The practical implications of having to develop sufficient levels of exposure within a group of lambs has also been cited as a reason for lack of uptake although in practice low levels of infection are actually required. Breeding for improved resistance is particularly applicable to the maternal breeds with research work having demonstrated that selection for low FEC in lambs reduces the magnitude of the peri-parturient rise in the same animals as breeding ewes.

A newly formed group, Performance Recorded Lleyn Breeders has been developed in order for like-minded breeders to develop their breeding goals and to increase the uptake of performance recorded stock within the commercial sector. Breeding for parasite resistance has been identified as a key requirement for the group. The project therefore aims to tackle both the need for increased uptake of the technology and to address potential improvements in the phenotype selected for.

Research carried out by Professor Mike Stear at Glasgow Veterinary School has shown that antibody responses against the larval stage of *Teladorsagia circumcincta* are an important marker of host response to infection.

Mucosal IgA levels have been shown to regulate worm growth and fecundity. Using regression analysis, parasite specific plasma IgA has been compared with FEC as an indicator of resistance to infection. Following simulation of the impact of 10 generations of selection, selection on plasma IgA gave a much faster reduction in FEC than selection based on FEC alone (Figure 1). This was because high levels of IgA reduced worm growth and fecundity and therefore decreased egg output. The research concluded that plasma IgA was a better indicator of intensity of infection than FEC and has a number of practical advantages. Plasma IgA was suggested to be easier to sample and to result in more precise measurements. It should also reduce pasture contamination (through a correlated reduction in faecal egg output) and has applications for both diagnosis and selective breeding.

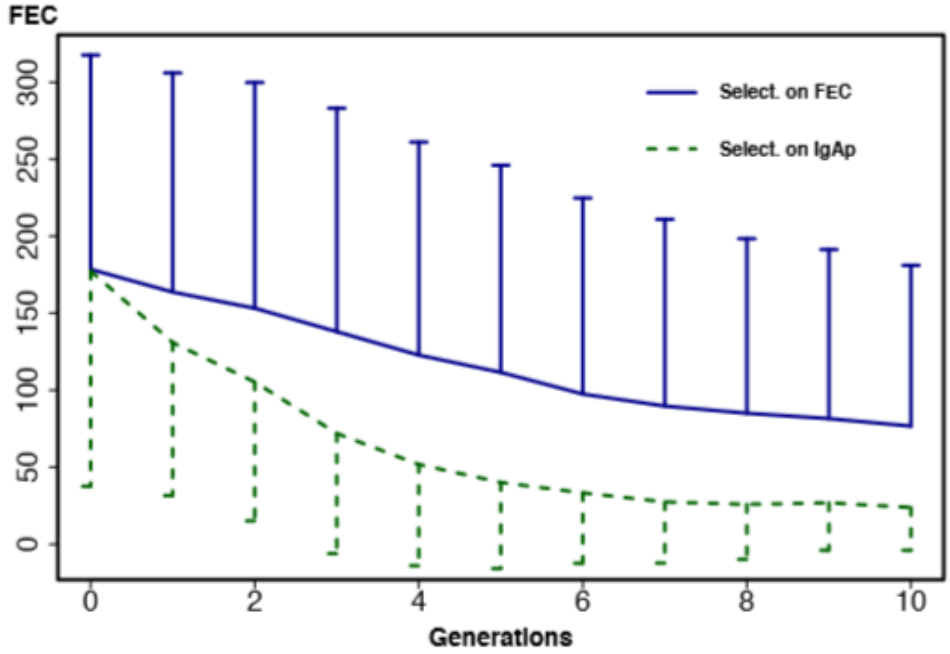

Figure 1. Graphical representation of reduction in faecal egg counts over 10 generations

The research concluded that plasma IgA was a better indicator of intensity of infection than FEC and also has a number of practical advantages. Like faecal egg counts, detection of IgA in the saliva requires animals to have been exposed to infection. However, unlike FEC, it is not affected by anthelmintic treatment and therefore is easier to integrate this marker for resistance into flock management procedures. Following discussions with individual breeders it is clear that the low uptake of faecal egg counts in the pedigree sector is in part due to the potential loss of performance associated with allowing sufficient levels of exposure to develop. It is therefore expected that the move to the saliva IgA test will increase uptake of breeding for resistance to roundworms within the UK national sheep flock.

The project therefore proposes to apply the technology to on-farm applications with the aim of developing improved ranking of individuals animals based on their genetic resistance to infection with *Teladorsagia circumcincta*. The project also aims to use this new development to highlight to commercial lamb producers the importance of selecting maternal rams bred for improved resistance to roundworms.

Project Objectives

The IgA assay has been validated in large field trials conducted by researchers at Glasgow Vet School and the University is now looking to work with commercial producers and ram breeders to develop a service with potential wide-scale uptake. The project therefore proposes to apply the technology on-farm with the aim of developing improved ranking of individuals animals based on their genetic resistance to infection with *Teladorsagia circumcincta*. The project also aims to use this new development to highlight to commercial lamb producers the importance of selecting maternal rams bred for improved resistance to roundworms. The project objectives therefore include:

1. Development of 'user-friendly' saliva collection procedures and protocols.
2. Correlation of saliva IgA levels with associated faecal egg counts.
3. Production of IgA estimated breeding values for participating flocks.
4. Correlation of saliva IgA levels with growth rate, ultrasonic muscle and fat depth measures and dag scores.
5. Production of literature and press coverage to highlight the importance of performance recording and breeding for improved resistance to roundworms.
6. Feedback on market potential to academic partner and development of routes to full commercialization.
7. Identification of requirements for reporting tools associated with sample results.

Key Findings

- In total circa 2,800 saliva samples were submitted for analysis with the collection of circa 2,700 FEC samples. 12 flocks participated in the project
- Feedback from the sample collection procedure was that collection of saliva was a practical on-farm procedure. Furthermore, all breeders would be happy to carry out the saliva collection procedure themselves
- Sampling began in early August and finished on the 20th of November. The range in dates reflected both a range in lambing dates and the time required for the average FEC within the group to reach 250 eggs per gram
- Significant variation in FEC results was observed within the individual flocks sampled. Average FEC at sampling ranged from 250 eggs per gram to over 1,000 eggs per gram
- The project confirmed that saliva was successfully extracted from the swabs and the ELISA tests was successful in detecting *T. circumcinta* antigens
- The association of IgA activity with faecal nematode egg count was estimated by fitting IgA activity as a covariate. In this analysis, IgA activity, farm and the interaction between farm and IgA activity were all highly significant ($p<0.001$).
- The association between FEC and IgA was negative; high IgA activity was associated with reduced egg count. The significant interaction indicated that the strength of this association differed among farms
- There was a significant association between increased IgA activity and increased muscle depth and index2 values. Other associations with traits of interest were not significant
- The project aims and objectives have been shared with a number of breeders and within the scientific community with further activities planned for 2015.

Project achievements

All objectives were met.

Objective 1. Approximately 3,000 lambs were sampled for saliva and faeces from 12 farms in the Performance recorded Lleyn breeders group. The collection of samples went well. There were no problems and the recovery of saliva was sufficient to allow each animal to be tested in triplicate in the ELISA reaction.

Both faecal and saliva samples were collected in tandem at each participating farm. Upon sample collection, faecal samples were analysed by KN Consulting with saliva samples posted to Glasgow Veterinary School for the completion of the ELISA test to estimate IgA levels to whole worm antigen of the Teladorsagia species. On arrival at Glasgow Veterinary School, saliva was removed from the collection swabs through centrifugation. Following this samples were frozen before further analysis took place. Of the twelve participating flocks, eleven took up the option of sample collection by KN Consulting with one breeder taking their own faecal and saliva samples following the postage of the required sampling equipment (Appendix 1).

Where sampling took place by a representative from KN Consulting the breeder also took at least a proportion of the saliva samples for feedback on ease of the process. The collection of faecal samples were marginally quicker compared to the saliva samples but did suffer the issue of some lambs being 'empty' whereas a saliva sample could be collected from all animals. Conversely, when a faecal sample was collected and provided it weighed over 2grams (minimum) then it was always possible to carry out a faecal egg count. There is the risk with the collection of a saliva sample that for wide-spread commercial use sufficient saliva might not be present. This was however not observed in the circa 3,000 samples collected as part of this project. Where lambs were 'empty' it was possible to re-sample however guidance from KN Consulting is that this should only be attempted twice in order to minimise any potential stress to the animal.

With the option of either collecting faecal or saliva samples, breeders always took the option of collecting the saliva samples therefore suggesting that this is a more acceptable form of the collection of phenotypic data compared to the collection of a faecal sample.

The speed of sample collection varied in part with the handling facilities available but it was possible to take up to 100 faecal samples per hour and 70 saliva samples when another member of the team ensured samples were labelled. Where a faecal sample was available then this could be collected immediately whereas it was important to keep the swab in the mouth of the animal for at least 10 seconds to allow for sufficient absorption of saliva. This was the main reason for the slightly longer time required to collect the saliva sample. It is however important to note that feedback from the breeder who took both FEC and saliva samples indicated that without prior experience of the collection of faecal samples, it was the saliva collection which they found quicker to carry out.

Saliva sample collection	Saliva sample swabs in test tubes	Faecal samples



The sampling requirements for both faecal and saliva samples can be easily purchased and packaged for commercial use.

For the benefit of the project the following costs associated with the consumables for sample collection were incurred:

Faecal samples
Glove – £0.03
Nytraguard Bluple Nitrile Gloves
Bag – £0.03
Niceday Grip Seal Bags With Write On Strip Clear 152 x 229 mm 1000 Per Pack
Total cost of sample collection equipment: £0.06/sample

Saliva samples
Swab – £0.01
Robinson Dental Rolls Size 3
Sampling tube - £0.3
Karter Scientific 208O2 Centrifuge Tubes, 15ml, 17x120mm
Total cost of sample collection equipment: £0.31/sample

Also required for sample collection was a pair of 10" straight forceps for the handling of the swabs. At least one forcep would be required per flock as part of the sampling kit. Where multiple flocks were sampled by KN Consulting, three forceps were available with appropriate disinfection between farms. The cost of each forcep is circa £3.99.

All costs above are excluding VAT.

Objective 2. To estimate the correlation, we transformed the faecal nematode egg counts by taking the natural logarithm of the estimated eggs per gram pplus 15. The sensitivity of the technique is 30 eggs per gram and 15 is half this value. This is a widely recommended procedure in parasitology. The ELISA produces an optical density for each sample. The mean of the three values for each animal was transformed into an optical density index by comparison with the mean of the three high and low standards on each plate. The formula was odin = (igaod-low) / (high-low)where odin is the optical density index, igaod is the mean value for each animal and high and low are the mean values on each plate for the high and low standards. This equation is standard in ELISA tests and is used to minimise variation between plates.

The overall correlation across the 12 farms was positive and weak at 0.14. However, the intensity of infection varied across farms. At the time of sampling the mean egg counts ranged from 140 to 906 eggs per gram. The immune response is dose dependent and high levels of infection lead to higher responses creating a spurious positive association when data are pooled across farms. The correlations on each farm ranged from -.35 to 0.06. A more appropriate procedure is to use a general linear model that removes the effect of the differences in exposure on different farms by fitting farm as a fixed effect and estimates the association of IgA activity with faecal nematode egg count by fitting IgA activity as a covariate. In this analysis, IgA activity, farm and the interaction between farm and IgA activity were all highly significant ($p<0.001$). The association was negative; high IgA activity was associated with reduced egg count and the significant interaction indicated that the strength of this association differed among farms. Refitting the model without the interaction indicated that the estimated effect of transformed IgA activity on transformed egg count was -0.81 $\pm$ 0.13.

Objective 3. The distribution of IgA activity was overdispersed and fell between a normal and a gamma distribution. The log transformation was not entirely satisfactory and a Box-Cox procedure was applied to determine the most suitable transformation to normalise the data. The Box-Cox transformation was odinbc = ((odin+**1**)**-**0.25**)/(-**0.25).**
A general linear mixed model was then used to determine the breeding values. Odinbc was the response variable. Farm and sex were fitted as fixed effects and sheep identity was fitted as a random effect. The numerator relationship matrix included only the parents. More detailed pedigrees going back over 50 years were supplied by Sam Boon of EBLEX but we have not yet extracted the relevant relationships and or computers will not readily invert matrices of this size.
Figure 1 shows the distribution of breeding values for nematode-specific salivary IgA. The distribution is a good visual fit to the normal distribution as shown by the superimposed normal curve.

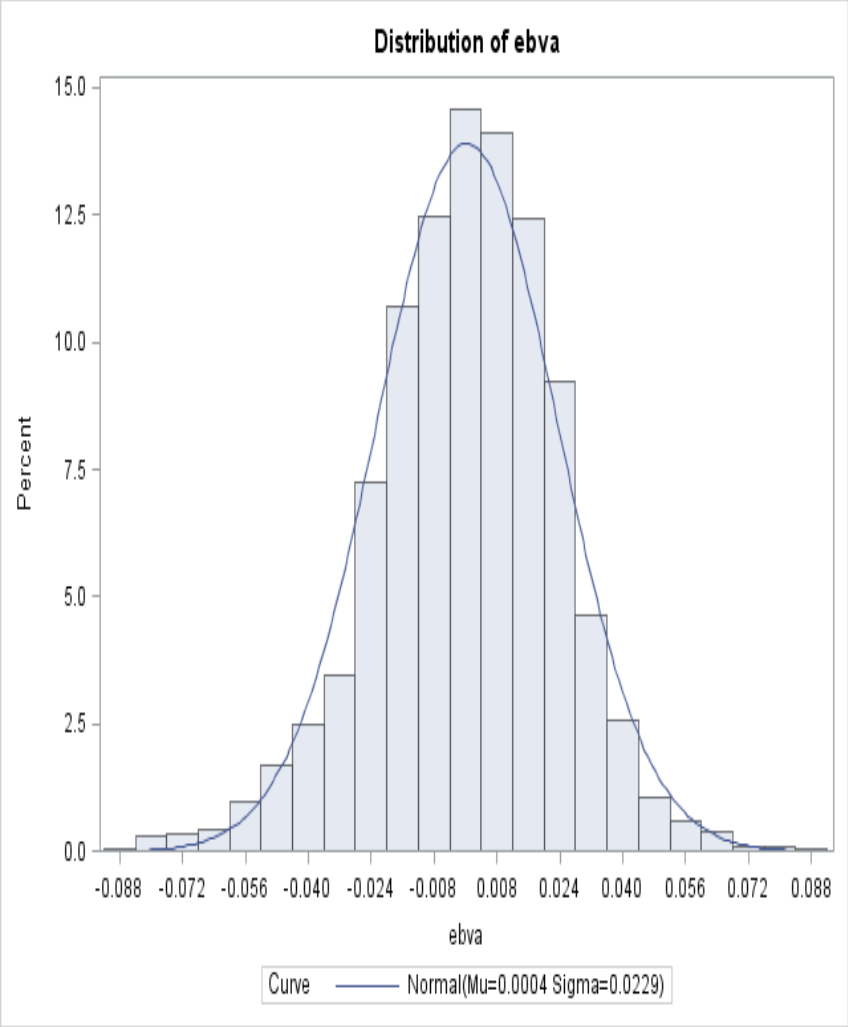

Figure 2. The estimated breeding values for nematode-specific salivary IgA.

Objective 4. EBV for Birthweight, week 8 weight, mature size, litter size, maternal ability, scan weight, muscle depth, fat depth index and index2 were supplied by Sam Boon. A general linear model was used that fitted logodin and farm. The interaction between logodin and farm was not significant. There was a significant association between increased IgA activity and increased muscle depth. The estimated effect was +0.36 $\pm$ 0.08 ($p<0.0001$); there was also a significant association with increased index 2 values. The estimate was +10.88 $\pm$ 4.56 ($p=0.0173$). Other associations were not significant. Dag scores were not collected as part of the project as some farms had shorn lambs or 'dagged' prior to sampling. Observations at sample collection suggested that most lambs had a clean breech. Should further work be required in this area, the required protocols can be developed to ensure that lambs are presented at sampling with a full fleece so that the scoring can take place.

Objective 5. The information generated by these data has been made into a power point presentation. This was delivered to the maternal sheep group and a copy of the slides will be posted on their website www.maternalsheep.co.uk. The results from the project were also included in a presentation to the Animal Health Research group. The project has been very successful and we are preparing the results for publication in a scientific journal. Once this has been published, we will organise a press release through Glasgow University. We also plan to present these results to the sheep breeders Round Table. Glasgow Science Centre will also highlight this research as a poster available to the general public. KN Consulting has offered to attend an open-day at one of the participating breeders farms in order to promote both the project and breeding for worm resistance. Given the seasonality of lamb production this event will likely be held in June or July to coincide with ram selling and also to promote the science to an audience of potential users. KN Consulting has also received interest in the technology from other ram producers of different breeds. Following discussions with the participating breeders in this project it would therefore be possible to further promote the technology to a targeted audience.

Objective 6. KN consulting has in collaboration with Glasgow University prepared an Agritech Catalyst grant proposal that is under review by Innovate UK. We have also submitted a proposal to the Marie Curie program in collaboration with EBLEX to further develop this work. We have also had useful discussions with the Research Strategy and Innovation Office of Glasgow University. The main obstacles to commercialization have now been overcome.

The group interest in the use of this technology exceeded initial expectations. At the project start a minimum of 2,000 faecal and saliva samples were set for analysis from four breeders. However by the end of the project nearly 3,000 samples were collected from twelve breeders. Expression has also been expressed in the use of the technology by a number of breeders outside of the group involved in this project. The interest expressed has come from a number of maternal breeds and could represent a further 3,000 samples processed in 2015 before any widespread promotion of the test. Group expansion over the last 12 months is also likely to increase uptake of the technology. Notwithstanding any funding requirements and with successful implementation into the Signet package, it could be expected that up to 8,000 lambs undergo phenotyping for worm resistance. Previous to the start of the project, approximately 1,200 lambs underwent faecal egg counting (2013).

Informal discussions amongst both pedigree ram breeders and commercial lamb producers has also shown an increased interest in the identification, selection and purchase of individual animals which have improved genetic resistance to roundworms.

The value of using such genetics will vary from flock to flock depending on a number of factors including:

- Levels of worm challenge on the farm
- Effectiveness of anthelmintic groups
- Stocking rate
- Breeding and replacement policy

The actual cost of wormer use in lambs is relatively cheap ranging from £0.05 to £0.35 per lamb per treatment. For the two new classes of wormers (Zolvix and Startect) the cost is increased to between £0.70 and £1.00 but treatment is normally limited to a single treatment unless anthelmintic resistance to more than one class of wormer is detected.

There is however a hidden cost of high levels of worm infections in lambs and this is the reduction in performance. In its early stages this is detected through a reduction in growth rates but if left untreated morbidity and mortality can occur. Furthermore labour associated with increased frequency of treatments can also increase production costs as well as the loss of production resulting from the handling of growing lambs on multiple occasions.

Lambs growing at 200g per day take 6 months to finish

Lambs growing at 100g per day take 12 months to finish

Research and demonstration projects are finding increasing levels of resistance throughout the UK. In Wales an ongoing study being carried out by Hybu Cig Cymru (Appendix 2) has shown resistance to the three main wormer groups as well as resistance to Moxidectin which is part of the Group 3 Macrocyclic Lactones. It could be expected that similar results would be seen in the English national flock.

Modelling the financial benefits of selecting for worm resistance is outside of the scope of the project since as part of a planned breeding programme this requires a number of calculations.

However, initial figures suggest that the following assumptions can be used in calculations:

- Annual saving of a single drench treatment with associated treatment and labour costs equates to £0.50 per lamb
- Increased growth rates associated with reduced pasture challenge of 10g/day or 1.5kg over a season equates to £2.50 per lamb (valued at £1.70 per kg live-weight)
- 100 lambs reared per ram per year.

The above assumptions lead to an annual financial benefit of £300 per ram per year or nearly £1,000 over the lifetime of each ram purchased. Further modelling would be required to fully cost the cumulative genetic benefits over a number of generations of selection including reduced faecal egg counts over the peri-parturient period. The above calculation is however a useful exercise to demonstrate the associated costs of worm infections in lambs.

Objective 7. Dealing with a large amount of phenotypic data without an associated database led to challenges in the provision of data to the academic partner for further analysis. For commercial application this would have to be addressed to allow for the timely reporting of results although with the experience gained by all project partners this is not expected to compromise the delivery of a commercial service in the future.

It is also likely that a move to a 'saliva only' test would increase the ease of testing, analysis and reporting. Due to the need to allow for sufficient parasite exposure to generate variation in faecal egg count results the sampling process ran over 4 months. The move to a saliva only test would help reduce this issue and associated timescale of testing although exposure would still be required as an active patent infection would not be needed.

Of the twelve flocks sampled there was significant variation in egg counts. Some lambs may have suffered associated production losses although this was not as a direct result of participation in the project and more a reflection of parasite levels on an individual farm. Table 1 shows the typical variation in faecal egg counts results across the farms sampled.

Table 1. Typical variation in faecal egg counts results across the farms sampled.

Farm/group	Average	Minimum	Maximum	% difference between average and maximum counts
A	284	0	1,440	+ 507%
B	152	0	810	+ 533%
C	62	0	690	+ 1113%
D	346	0	2,130	+ 616%
E	610	0	2,400	+ 393%
F	908	30	3,900	+ 430%
G	547	0	8,100	+ 1481%
H	700	0	7,050	+ 1007%
I	1,251	0	5,580	+ 446%

From a flock management perspective and when undertaking group FECs for the purpose of drenching decisions the following thresholds are quoted:

	Faecal egg count		
	Low	Medium	High
Mixed (H. contortus absent)	<250	250 - 750	>750
Mixed (H. contortus present)	<500	500 - 1500	>1500

Source: *SCOPS manual 4th Edition*

The results obtained from the 12 flocks participating in the project show that:

1. There is sufficient variation in individual FEC results at average levels which are classed as 'low'
2. It should be possible to both expose animals to sufficient parasite exposure without compromising animal performance.

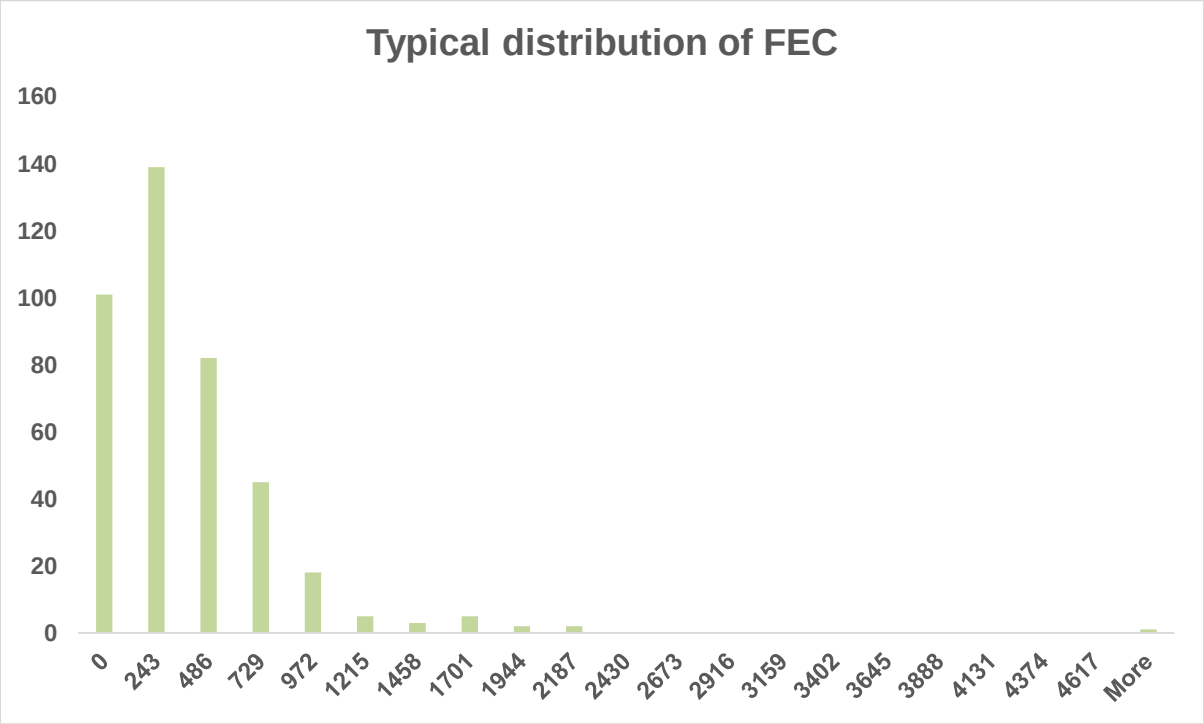

Figure 3. Typical distribution of faecal egg counts in a contemporary group of lambs. The average FEC for the group was 295 eggs per gram.

Additional results. The project has been scientifically very informative and we anticipate at least two scientific papers will result from this project. Additional results include heritabilities and genetic correlations between parasite and production traits. In addition these associations will be used to develop a genetic model to predict the response to joint selection on parasite resistance and improved performance.

In conclusion, this project has provided a great deal of information and met its objectives.

Report compiled by Professor Mike Stear and Dr Catherine Nakielny. March 2015

Appendix 1

FEC and Saliva Testing Instructions

FEC:

1. Firstly make sure the lamb is restrained, either by hand, in a race or combi-clamp.
2. Put a glove on the hand you are going to use.
3. Insert 1 or 2 fingers into the rectum and with gentle movements manipulate some faeces towards you and out.
4. Try to get at least 4 grams of faeces although this is not always possible and occasionally the lamb is empty*
5. Place the faeces in a bag and try to wipe all faeces from the glove into the bag
6. Use a fresh glove for each lamb.
7. Label the bag with the tag number of the lamb.
8. Please package and send the FEC samples back to KN Consulting using the packaging provided.

*Even when a lamb is empty it can still be worth coming back to it at the end of the session as 'refilling' normally takes place within 30 minutes or so. The process should however only be carried out twice in total so if the lamb is still empty then discard from the sampling process.

*Aim for a minimum sample size equivalent of a tablespoon of faeces per animal

Saliva Testing:

1. Put the dental swabs in a clean and dry container next to you to be easily picked up by the forceps as you should try not to touch them.
2. Make sure the lamb is restrained, either by hand, in a race or combi-clamp.
3. Use the forceps to clamp a swab at one end and gently insert it into the side of the lamb's mouth.
4. Turn and twist the swab around the mouth and over the tongue collecting saliva for around 10 seconds.
5. Using the forceps place the swab into a tube, and label the tube with the lamb's tag number.
6. Please package and send the saliva samples to Karen Fairlie-Clarke, address provided.

Appendix 2

Feb 11 2015
Resistance to wormers has increased in Wales since the previous study in 2006, including those products that were previously considered effective at killing the parasites in sheep.

These are the preliminary results from a project into anthelmintic resistance overseen by Hybu Cig Cymru – Meat Promotion Wales and funded by the Rural Development Plan for Wales.

The initial results indicate that the extent of anthelmintic resistance has increased. Anthelmintic resistance means that the wormer loses effectiveness because a proportion of the worms are resistant and survive treatment.

Generally this process occurs over several years but if detected at an early stage, famers can adopt practices to maintain the wormer's useful life for longer.

The Wales Against Anthelmintic Resistance Development (WAARD) project is half way through its surveillance on 45 farms.

The three older wormer groups (1-BZ, 2-LV and 3-ML) have been tested as well as a separate test on Moxidectin (a member of the 3-ML group with persistent action), to see how effective they are.

The test involves looking at Faecal Egg Counts (FEC), before and after treatment and calculating the percentage reduction. To be fully effective the count must drop by at least 95 per cent. A reduction of less than 95 per cent suggests that there are resistant worms present.

Of the 24 farms tested so far, 83 per cent have evidence of resistance to White (1- BZ) and 67 per cent to Yellow (2-LV) drenches. While this represents an increase compared to previous surveys, it is no real surprise.

Resistance has also been detected on 29 per cent of farms to the Clear (3-ML) wormer group and on 21 per cent of farms tested so far there is evidence of resistance to Moxidectin.

Moxidectin is tested separately because although it is a member of the 3-ML group only rare instances of resistance has previously been recorded, with Moxidectin remaining effective even where other 3-ML products such as ivermectin are failing.

"These results are therefore, of concern," said Eirion Thomas of Techion UK, who is carrying out the research on behalf of HCC. "Additionally four of these farms have evidence of worms that are resistant to all four of the wormers tested.

"The challenge will be to help them maintain their worm control in the future.

Drug group	**1-BZ**	**2-LV**	**3-ML**	
			IVM	**Moxidectin**
No. of farms with resistance detected	20	16	7	5
% with resistance detected	83%	67%	29%	21%

Lynfa Davies, HCC's Technical Development Executive, said: "This is an important project providing sheep farmers with valuable information about the level of resistance to these drugs."

"While the preliminary results are worrying, HCC together with SCOPS, will work to provide clear messages to farmers to help them understand and react to these findings."

Lesley Stubbings sheep consultant representing SCOPS, said "We shouldn't be surprised by these results because we know that the incidence of anthelmintic resistance has continued to increase in recent years.

"The detection of Moxidectin resistance on one in five of the farms, however, is a real concern and a very timely reminder that we must act fast if we are to maintain good worm control on sheep farms.

"The over-riding message coming out of the WAARD project is that it is vital that sheep farmers know which drugs work effectively on their farm because so many are continuing to use products with reducing efficacy.

"As a result they are losing lamb performance as well as heading down the slippery slope to a fully resistant worm population, from where there is no return," she said. "A simple drench test after treatment will give an indication of the resistance status and should be a routine on all sheep farms."

Mr Thomas said: "An important element of the project is advising the farmers involved on what steps they should take in response to their results.

"Although the results so far are concerning there are steps they can take to maintain worm control, which means adopting SCOPS principles rather than just reaching for the drench gun."

The project runs until June 2015 and the remaining farms will be tested in the spring and early summer. Further analysis is being carried out to see what species of roundworms have survived treatment to complete the picture of what is happening on each farm.

Source: http://hccmpw.org.uk/news_and_events/news/story/resistance_to_wormers