Harper Adams University and the Performance Recorded Lleyn Breeders

Sheep Worm Resistance

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Summary

Anthelmintic resistance is a growing problem worldwide, threatening the long term viability of sheep production. In the UK there are currently five chemical groups of wormer (anthelmintic) available and resistance in some of the target parasites has been reported to four of the five groups. Resistance is defined as the ability of parasites to survive doses of drugs that would normally kill parasites of the same species and stage (Geary et al., 2012). Resistance is inherited and selected for during treatment, as resistant parasites escape the effect of treatment and pass resistance to the next generation. The resistance genes that occur through mutation are initially rare in the population but, as selection continues, their relative proportion in the population increases and consequently the proportion of resistant parasites increases too.

This study has looked at an alternative approach to dealing with internal parasites – trying to identify sheep that are themselves more resistant to parasites, hence needing fewer therapeutic treatments in order to grow efficiently.

Selective breeding based on faecal egg count (FEC) has been adopted by some sheep breeders but FEC is time consuming, costly and potentially unreliable and the use of immune markers such as immunoglobulin A (IgA) have been investigated. IgA is produced in the gastro-intestinal tract in response to challenge from gastro-intestinal nematodes (GINs) and a specific IgA produced in response to the parasite *Teladorsagia circumcincta,* (a round worm commonly found in sheep) has been identified(Strain, 2001) This IgA has been detected in, gastric mucous, blood and saliva. This study aimed to further assess the reliability of FEC and saliva IgA as indicators of host resistance to parasites.

Operational group members of the Performance Recorded Lleyn Breeders (PRLB) took faecal and saliva samples (a total of 4697 and 5281 respectively) from their own sheep in the autumn of 2017 and 2018 to add to the already large dataset being used by Signet Breeding Services to create the Estimated Breeding Values for FEC and saliva IgA.

At Harper Adams University FEC and saliva samples were collected from 200 ewe lambs in 2017 – taken on two consecutive days. In 2017 a breeding plan was designed to test the heritability of FEC and IgA with 180 ewes mated to six high or low FEC and IgA EBV rams. In 2018 samples of faeces and saliva were taken from 235 lambs from the planned matings and further faeces, saliva and serum samples taken from 84 of the same lambs over a period of 38 days.

Results from both years showed how variable both FEC and saliva IgA can be from one day to the next and even between duplicate samples taken on the same day. Ranking of individuals over the course of the trial was also variable for FEC and saliva IgA but was much more consistent for serum IgA. This suggests that relying on a single sample for FEC or IgA at one point in time is likely to be inadequate as an indicator of worm resistance.

The findings from this project have highlighted inconsistencies in both FEC and saliva IgA testing and questioned their usefulness in selective breeding. It proposes further research into the use of saliva IgA for selecting genetically superior animals and that serum IgA may be a more reliable indicator of host immunity.

1.0 Aim of the project

This project investigates the use of saliva IgA and faecal egg output to improve selection of animals for resistance to round worms.

The project also aims to use the information to highlight to commercial lamb producers the importance of selecting rams for improved resistance to roundworms, in the light of increasing worm resistance to the available anthelmintics.

Research at Glasgow University had indicated that saliva IgA could be used as a new phenotype for worm resistance. The project therefore proposed to further apply the technology on-farm with the aim of developing improved ranking of individual animals based on their genetic resistance to natural roundworms (predominantly infection with *Teladorsagia circumcincta)*.

2.0 The Operational Group

The group was set up in 2013 to bring together like-minded Lleyn breeders who are performance recording their flocks with Signet Breeding Services, and to promote breed improvement. In terms of this RDPE EIP project application the group agreed to collect faecal samples for worm egg counts and to take saliva samples for saliva IgA analysis from their own lambs.

The group is chaired by Richard Evans and the current secretary is Chere Border. Meetings are held twice a year for the whole group or more frequently if the need arises. Farm walks are organised on member's farms and conference calls are arranged as and when needed. George Cullimore is responsible for technical issues and for submitting claims to AHDB for analytical testing for FEC and IgA. He is also responsible for liaison with Signet Breeding Services, Glasgow University, Harper Adams University and The Moredun Institute. A full list of members is shown in appendix 1 and the terms of reference for the group are shown in appendix 2.

3.0 Literature Review

3.1 Introduction

Gastrointestinal nematodes (GIN) pose a significant threat to the health and welfare of sheep (Learmount *et al.,* 2018a and b). In 2005 it was estimated that nematodes cost the UK sheep industry £84 million annually due to cost of treatment and prevention and production losses associated with reduced live weight gain and poor quality of meat and wool (Nieuwhof and Bishop, 2005). Most farmers rely on anthelmintics to control GINs in sheep; however there has been widespread development of anthelmintic resistance (AR) in parasite populations (Shaw *et al.,* 2012). AR is the worm's ability to survive a normally effective doses of an anthelmintic. It has increased as a result of selection from overuse of products and from incorrect and unnecessary dosing (SCOPS, 2013).

3.2 Faecal Egg Counting

Faecal egg counts (FEC) are the most commonly used way of assessing the intensity of gastrointestinal nematode infections (Kenyon, *et al.,* 2016). However, worm egg output does not necessarily correlate with worm burden for a number of reasons. FECs do not take into account the developmental stages of the parasite that are not yet producing eggs (Storey, 2015). GIN species vary in the number of eggs they produce but as most species cannot be differentiated in a FEC, this can often not be accounted for.

FEC is recognised to lack sensitivity, specificity, reliability and repeatability (Roeber *et al.,* 2012). Variability in results can be due both to factors associated with the animal (e.g. fluctuation in egg output over time and aggregation of eggs within faeces) and to factors associated with the collecting, handling and testing of samples.

Currently there are three commonly used methods of FEC: the traditional McMaster technique, use of FECPAK and the Flotac method (Bosco *et al.,* 2014).

It should be noted that research conducted by Bosco *et al.* (2014) was in cattle, rather than sheep, and as worm egg counts in cattle are generally much lower than in sheep it may not be reasonable to apply these findings to FECs from sheep. Additionally, the sensitivity results for FECPAK are from the first generation of technology; the second generation (FECPAK^{G2}) is now the method used but there is no available literature that has reviewed this.

The Flotac method is the most sensitive, however, Kenyon *et al.* (2016) found that a McMaster test at epg sensitivity of 15 produced the same results as the Flotac. It is important to maximise the sensitivity of the tests to avoid false negatives (Kenyon *et al.,* 2016); Levecke *et al.* (2011) found that a McMaster epg sensitivity of 50 produced 36.7% false negatives.

Repeatability ranges from 0.25-0.57 (Stear *et al.,* 1995a; Stear *et al.,* 1995b; Bouix *et al.,* 1998) and is highest when samples are taken at short intervals. For group sampling Cabaret and Berrag, (2004)

advise that a minimum of ten samples are used and that results are most reliable when over 300 epg.

The literature reveals variation in results; Stear *et al., (*2009), estimate that 22% of variation can be attributed to measurement technique including, counting technique (Storey, 2015), variation in flotation solution, sample dilution, time, choice of slide area (Cringoli *et al.,* 2004) and the amount of mixing (Morgan *et al.,* 2005). Taylor *et al.* (2002) found that there is better correlation between FEC and actual worm burden for some species including Haemonchus species but not others including *Trichostrongylus colubriformis* or *Teladorsagia circumcinta*. Storey, (2015) also found different correlations between species, however, good correlation was found for *Trichostrongylus colubriformis*.

Small sample size reduces test precision (Cringoli, *et al.*, 2004; Bosco *et al.,* 2014). The interval between sampling and egg counting, the temperature at which the sample is maintained during this time and the amount of exposure to air will all also affect the count.

The consistency of faeces is a further variable that may have a significant effect on FEC. Diarrhoea increases faecal moisture and may dilute the number of worm eggs observed. Le Jambre et al. (2007) suggest that calculating the dry matter of samples and adjusting the sample size according to faecal moisture may provide an improved estimate of FEC. However, calculating FEC per unit dry matter may not be practical for industry application.

Genetic factors, for example, host level immunity, will impact on FEC and may account for approximately 30% of the variation (Stear *et al.,* 2009).

FEC tests have the advantage that they are simple to do, user friendly (Levecke *et al.,* 2011) and, now with the invention of FECPAK^{G2}, can be done on farm with rapid results (Techion UK Ltd).

3.3 Immunoglobulin A (IgA)

The use of immunological markers has been proposed as a more reliable alternative to FEC to indicate worm burden and resistance in sheep (Shaw and Sutherland, 2012). Studies have demonstrated that an increase in immunoglobulins A (IgA), E (IgE) and G (IgG) are associated with a worm challenge and could therefore be used as potential markers (Murphy *et al.,* 2010; Shaw and Sutherland, 2012; Arsenopoulos *et al.,* 2017). However, selection for IgE has been associated with reduced live-weight gain and is less heritable than IgA (Murphy *et al.,* 2010; Shaw *et al.,* 2012). IgA has been identified as the most abundant (Watt *et al.,* 2015) and useful marker (Stear *et al.,* 1999a) that is the most important mechanism in controlling worms (Strain, 2001).

3.3.1 The effect of IgA on worms

A number of studies have identified that IgA regulates worm growth, rather than worm number, by acting on third (L3) and fourth (L4) stage larvae (Stear *et al.,* 1997; Stear *et al.,* 1999a, b; Strain *et al.,* 2002; Henderson and Stear, 2006; Stear *et al.,* 2009; Venturina *et al.,* 2013; Arsenpoulos *et al.,* 2017).

Harrison *et al.* (2003a,b and 2008) explain that sheep produce an IgA parasite specific antibody against a carbohydrate larval surface antigen known as CarLA which is present on the (L3) of *Trichostrongylid* species. CarLA IgA can be identified using an ELISA test, therefore ensuring that

elevated levels of IgA are indeed a result of parasitic infection not an alternative cause (Shaw *et al.,* 2012). The exact response to fourth stage larvae is unknown, but studies demonstrate that the strongest response in IgA was to L4 (Stear *et al.,* 1995; Strain, 2001). However, the exact immune defence mechanisms differ between worm species (Henderson and Stear, 2006).

IgA reduces worm length by inactivating metabolic enzymes and suppressing the feeding of the parasite (Shaw *et al.,* 2012). Worm growth is correlated with worm fecundity, as figure 1 shows, therefore causing the worms to produce less eggs (Stear *et al.,* 1999; Strain *et al.,* 2002; Stear *et al.,* 2009; Shaw *et al.,* 2012; Arsenpoulos *et al.,* 2017).

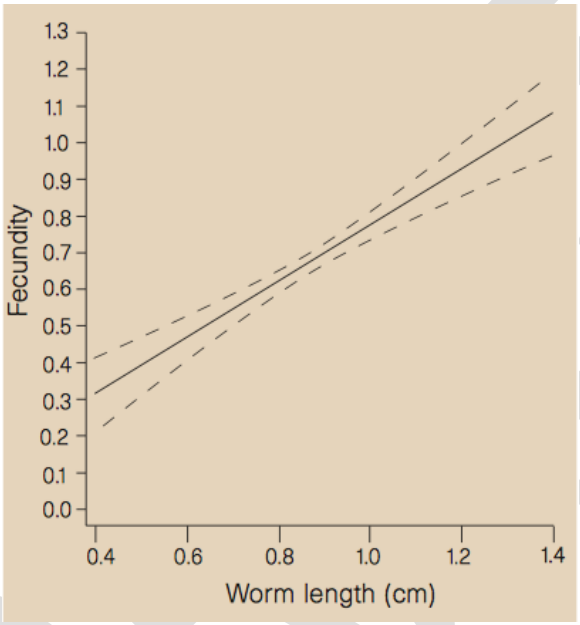

Figure 1.Relationship between worm length (growth) and fecundity. The dashed lines represent the 95% confidence limits.

Source: Stear *et al.,* 1997

Stear *et al.* (1999b) identified three factors affecting worm length variation: strength of the IgA response, specificity of this response and the density dependence influence of worm numbers. The combination of these three factors account for 93% of variation in worm length (Strain, 2001). Whilst it has not been proven that IgA is the controlling mechanism for worm length, these results suggest that if this is not the case then IgA must be closely associated with an alternative mechanism and can be used as a marker (Strain, 2001).

3.3.2 IgA amount and specificity

The more IgA produced, the stronger the immune response; Stear *et al.* (1995) found the correlation between worm length and peak IgA, on the sixth day post infection, to be 0.96. However, adult sheep have shorter worms than lambs, therefore by including all age groups of sheep the correlation will overestimate the relationship for lambs.

Supplementation with protein has been proven to increase IgA production and enhance resistance to nematodes; the production of IgA requires amino acids and therefore adequate protein in the diet (Stear *et al.,* 1999c; Strain and Stear, 1999; Arsenpoulos *et al.,* 2017).

The specificity of an IgA response varies enormously between individuals; using Western blot, Stear *et al.* (1999) examined over 100 bands of L3 and L4 with no individual sheep being able to recognise them all. Furthermore, Strain, (2001) explains that only four bands from a total of 99, associated with T. Cicumcincta, are associated with resistance. Therefore, for a lamb to be resistant it must not only produce a sufficient amount of IgA but it must also be antigen specific.

3.3.3 Density Dependence

The density of the worm burden is also associated with worm length and therefore fecundity (Stear *et al.,* 1999b; Strain, 2001; Stear *et al.,* 2009). For every extra 1000 worms, the worm length declines by an average of 0.1mm. The exact reason for this relationship is unclear, however it is hypothesised that the competition for resources or a further increase in immune response from the host may reduce worm length (Stear *et al.,* 2009).

3.3.4 Heritability

The heritability of IgA ranges from 0.46-0.67 (Davies *et al.,* 2005; Strain *et al.,* 2002; Beraldi *et al.,* 2008; Stear *et al.,* 2009; Shaw *et al.,* 2012), which is higher than FEC, at 0.14-0.33 (Stear *et al.,* 1997; Bishop *et al.,* 1996; Beraldi *et al.,* 2008; Mpetile *et al.,* 2017). Therefore by selecting for IgA instead of FEC genetic improvement should be faster, as the model in figure 2 demonstrates (Davies *et al.,* 2005).

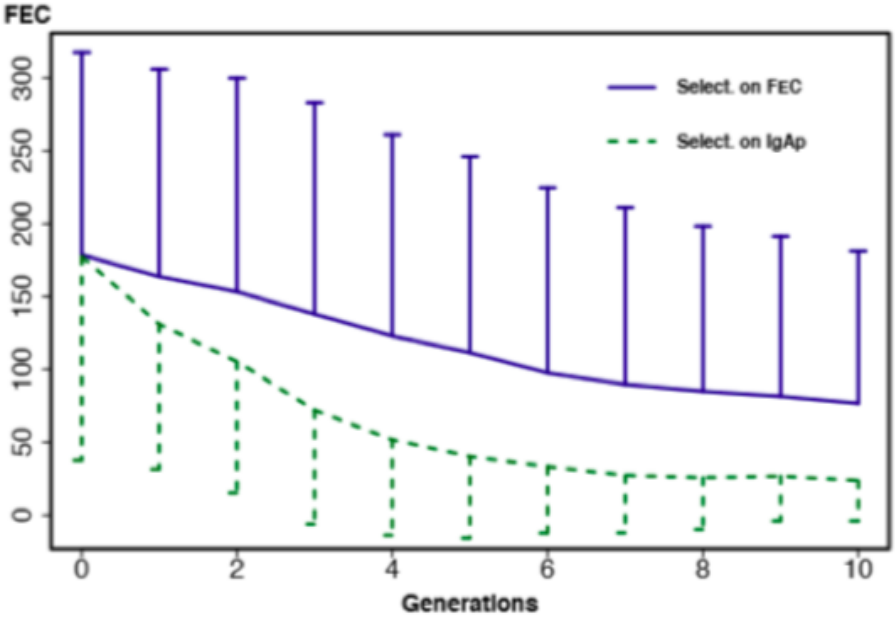

Figure 2. The decline in faecal egg counts of 10 generations based on selective breeding for FEC and IgA.

Source: Stear and Nakielny, 2015

Heritabilities for FEC – S and saliva IgA in the Lleyn breed are currently at 0.07 and 0.11 (2018) respectively and these have fallen compared to the previous year when they were 0.08 and 0.16.

This was disappointing as it would be expected that as more data is gathered heritability should improve (Signet Breeding Services).

3.3.5 IgA and FEC Correlation

Shaw *et al.* (2012) and Stear and Nakielny (2015) found a negative correlation between salivary IgA and FEC of -0.45 and -0.81 respectively and therefore suggest that IgA is a useful marker for resistance (Venturina, *et al.,* 2013). The reason for such a difference between the two studies is unknown but could be due to breed, method or year. Studies by Strain *et al., (*2002) and Mpetile *et al.* (2015), demonstrate that year has a significant effect on faecal egg counts. Currently, research is still in its infancy and therefore both IgA and FEC are still required to identify more resistant animals. However, in the future there is hope that the marker will replace FEC as an indicator of resistance in sheep.

3.3.6 Sampling method

IgA can be measured in gastric mucus, saliva (Shaw *et al.,* 2012), blood or plasma (Stear *et al.*, 1999). It should be noted that most of the research papers report on plasma, mucus or blood IgA with very little evidence of use of saliva. Henderson and Stear, (2006) found a significant correlation of 0.66 between plasma and mucosal IgA. A review of the use of saliva to measure IgA in humans suggests that there are a number of variables that influence the levels of antibodies in oral secretions. These include difficulties with reproducibility and standardisation of immunoassays, the impact of flow rate, acute and chronic stress and protein loss during sample handling (Brandtzaeg, 2007).

Compared to FEC, measuring IgA may be a more hygienic, safer and simpler method to estimate resistance (Stear *et al.,* 2009). Additionally, IgA could be more sensitive than FEC at detecting infection as high IgA levels could still identify an infected sheep despite no detectable eggs. Sheep can still be IgA sampled after anthelmintic treatment making it an easier procedure to integrate into flock management (AHDB, 2015; Signet, 2015). Finally, the production loss associated with FEC due to the necessity to wait until a sufficient worm burden has built up may not be as great with the use of IgA, although animals will still need to be challenged before sampling.

The ability to use saliva samples to assess IgA levels further simplifies sampling procedure as it is less invasive than blood sampling and can be done by the farmer without veterinary assistance therefore reducing costs (Shaw *et al.,* 2012). Additionally, compared to collection of individual faecal samples, Shaw *et al.* (2012) estimate that it takes approximately two thirds of the time to take saliva samples. However, a recent study published by AHDB, (2015) indicated that on average it was quicker to collect faecal samples, although without prior experience of either faecal or saliva sampling it was the latter that was indeed quicker. Additionally, when the sheep is selected for sampling there may not be an available faecal sample, whereas, a saliva sample will always be possible, therefore reducing the handling time required (AHDB, 2015).

3.3.7 Factors correlated with selection for IgA

For breeding for resistance to be successful, the sheep produced must remain productive. There are various factors that are correlated with parasite immunity that will be discussed.

Sex

Research suggests that females are more resistant than males with Strain (2001) and Stear *et al.* (2004) finding that females have higher IgA activity than ram and wether lambs and many papers proving that females show lower FEC than males (Berger, 1993; Pollott *et al.,* 2004; Abuargob and Stear, 2014; Mpetile *et al.,* 2015).

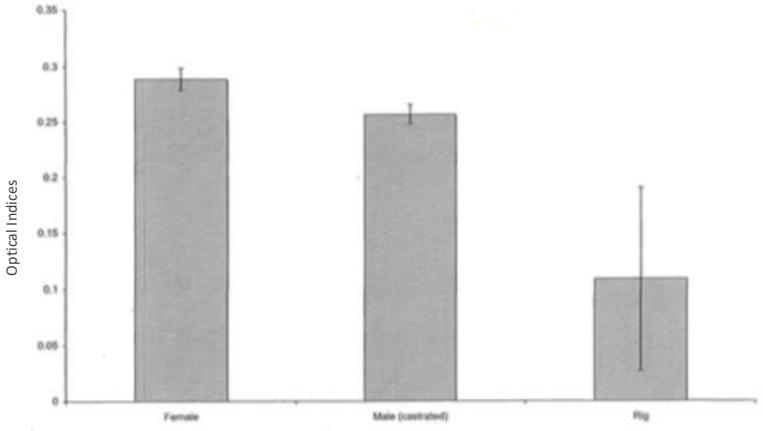

Figure 3. The effect of sex on parasite-specific IgA levels

Source: Strain, (2001)

It was thought that this difference was due to an immunosuppression effect of testosterone, however with more lambs being castrated very young this now seems unlikely (Berger, 1993); although it may have some effect as Strain, (2001) found that wether lambs had a higher response than rigs as figure 3 shows.

The difference could also be due to males having a higher appetite thus consuming a larger amount of vegetation and therefore ingesting more larvae (Abuargob and Stear, 2014), having a higher degree of stress due to mating and aggression and therefore more susceptible to infection (Strain, 2001) or could be a positive effect of female hormones (Berger, 1993). This latter hypothesis is supported by Idris *et al.* (2012) who suggests that sex may not have any effect on pre-pubertal lambs and others who did not find any difference between genders until lambs reached puberty at about six months old (Pollott *et al.,* 2004; Abuargob *et al.,* 2014).

However, not all research has found that gender causes a significant difference (Gauly and Erhardt, 2001; Strain *et al.,* 2001; Good *et al.,* 2006). This could be due to breed differences or age at which the experiment was conducted; Watt *et al.* (2016) found that IgA only differed between adult males and females.

Age

Parasitic immunity is acquired, not innate (Stear *et al.,* 1999b; Halliday *et al.,* 2007; Beraldi *et al.,* 2008). Therefore, as would be expected, lambs have significantly lower IgA levels and higher FEC results than older sheep (Good *et al.,* 2006; Watt *et al.,* 2016). Specifically, Douch and Morum (1993) and Smith *et al.* (1985) found that four month old lambs had significantly higher FEC than 28 and 10 month old sheep respectively and the magnitude of the immune response was lower.

As the lamb matures the animals have a greater capacity to develop immunity (Strain, 2001; Greer and Hamie, 2016); thus the genetic variation increases as the lambs' age (Strain, 2001) and can be expected to be present by six-eight months old (Strain, 2001; Greer and Hamie, 2016); however, even by one year old the antibody response will not be as high as in an older animal (Watson *et al.,* 1994).

Greer and Hamie, (2016) hypothesize that immunity is influenced by stage of maturity rather than age itself; this varied for each species however, the average stage of maturity was found to be 45% of expected mature bodyweight. This reasoning could further explain the difference between male and female susceptibility to GIN; when compared at the same age the males will be at a lower mature stage than the females as they would be expected to reach a heavier mature weight (Greer and Hamie, 2016).

Breed

Generally, smaller breeds are considered more resistant; this is the result of selection for immunity rather than production traits (Strain, 2001; Hielscher *et al.,* 2006). For example, Zaralis *et al.* (2008) found that Suffolk x Greyface lambs were more susceptible than the smaller Scottish Blackface lambs and upon infection develop anorexia faster and have a decreased intake of food by 13%. Additionally, Shetland sheep have been found to be more resistant than Southdown sheep (Golding and Small, 2009).

There have been few studies conducted on UK sheep breeds however, Good *et al.* (2006) found that Texel sheep are significantly ($P<0.001$) more resistant than Suffolk sheep. This was only significant once the lambs were over 14 weeks of age. Ahmed *et al.* (2015) found similar results and explain that the differences are likely to be due the fact the Texels produce more IgA and that Suffolk lambs have greater levels of tissue damage as indicated by the level of plasma pepsinogen.

Differences between breeds may also result from differences in grazing behaviour and the number of sites available for the parasites to colonize (Good *et al.,* 2006).

Growth rate

There is a very limited amount of research on the effect of parasite-specific IgA on growth rate and weight gain, however, correlations using FEC and growth rate can be used instead. Care must be taken as correlation between FEC and IgA can vary between studies and ranges from -0.45 to -0.81 as shown previously. Additionally, Shaw *et al.* (2012), found that salivary IgA was associated with weight gain whilst the relationship between FEC and growth was poor. Correlations vary within the literature with many studies finding a negative, and therefore beneficial relationship whilst others found a positive correlation between IgA or FEC on growth rate; these are summarised in table 2. The results highlight the effect that different breeds and ages can have. Other reasons for variation could also include year, location, time of sampling (Pollott *et al.,* 2004; Stear *et al.,* 2004; Abuargob *et al.,* 2014; Mpetile *et al.,* 2015), litter size, with twins having lower immunity (Stear *et al.,* 1996; Morris *et al.,* 2000; Stear *et al.,* 2009; Idris *et al.,* 2012) and sire and dam (Strain *et al.,* 2002).

Table 2. Correlation of growth rate and IgA, affected by breed and age

Breed	Age	Species	Correlation	FEC or IgA	Author
Scottish Blackface	6-7 months	T. Circumcincta	-0.8	FEC	Stear *et al.,* 1999
Scottish Blackface	3-6	T. Circumcincta	-0.85	FEC	Stear *et al,* 1996
Polish Long-wool sheep	7 months	T. Circumcincta, H. Contortus	-0.61	FEC	Bouix *et al.,* 1998
Merino	Weaning, 10 months, 16 months	Gastrointestinal nematodes	-0.20, -0.18, -0.26	FEC	Eady *et al.,*
Merino	16 months	Gastrointestinal nematodes	-0.14	FEC	Khusro *et al.,*
Merino	Up to 18 months	Gastrointestinal nematodes	0.12	FEC	Pollott and Greef, 2004
Texel	4-6 months 7-8 months	Strongyle species	-0.36 to -0.02 -0.47 to 0.18	FEC	Bishop *et al.,* 2004
Cross bred (Finnish Landrace x Texel) x Romney	Weaning - adult	Gastrointestinal nematodes	-0.52	IgA (saliva)	Shaw et al., 2013
Cross bred (Finnish Landrace x Texel) x Romney	Weaning - adult	Gastrointestinal nematodes	-0.07	FEC	Shaw et al., 2013
Romney	100 days	Trichostrongylus species	0.95	FEC	McEwan *et al.,* 1992

Romney	Yearling	Trichostrongylus species. Haemonchus Contortus	0.03-0.08	FEC	Morris *et al.,* 2000

Whilst the correlations in table 2 generally demonstrate a negative and therefore beneficial relationship between immunity and growth rate, care should be taken when breeding to not over select for immunity due to the associated costs (Greer, 2008; Greer and Hamie, 2016). Due to the production of cells that are largely proteinaceous in nature, immunity beyond a certain level could mean that protein is prioritized for immune cell production rather than growth and could result in a 15% loss of productivity (Greer, 2008), although supplying a high protein diet could reduce this. Additionally, Greer, (2008), hypothesizes that the ability to genetically express production traits could be reduced. However, a study conducted by Shaw *et al.* (2013) found that selection for IgA was favourably associated with live weight and could therefore be used as an important indicator for both resistance and growth; there was however, an unfavourable relationship between IgE and growth rate possibly due to reasons explained by Greer, (2008). Therefore, the selection for specific elements of immunity could indeed reduce productivity. There needs to be more research into this area.

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4.0 Project Proposal

4.1 On 13 commercial farms (2017 and 2018)

On farm sampling of lambs for FEC and saliva IgA when they are approximately 21 weeks of age. These samples were be taken by the farmers, experienced in the sample collection techniques and in sheep husbandry, for the purposes of their own flock improvement and happened irrespective of the work at HAU. Results have been used to strengthen the predicted EBVs for both FEC and saliva IgA to increase numbers for Signet evaluation.

Faecal grab (minimum of 4g/lamb) and saliva samples (by soaking dental swabs in saliva) were collected in tandem at each participating farm (13) when lambs were approximately 21 weeks of age. Estimated sample numbers of 3000 FEC and 3000 saliva each year. Lambs were restrained briefly, once to collect both samples (see sample collection methods in the appendix).

4.2 Sampling at HAU (2017)

4.2.1 Sampling of 2017 born lambs

To assess repeatability of both FEC and saliva IgA, 200, 2017 born Lleyn lambs at HAU were used for repeat sampling of faeces and saliva on two consecutive days when around 21 weeks of age. 400 samples for FEC and 400 for saliva IgA. The samples were taken by an experienced shepherd who had collected similar samples in the past.

4.2.2. HAU progeny test (mating 2017)

180 ewes were mated in groups of 50 to high or low saliva IgA or FEC Estimated Breeding Value (EBV) rams (see table 3). This should provide an early indication of the economic and physical impact of genetic selection for parasite resistance on commercial lamb performance. Lambs from the planned matings were weighed at birth and again at 8 weeks and 21 weeks of age. FEC and saliva samples were taken from over 200 male and female lambs at approximately 21 weeks of age.

Table 2. Selected sires

Sire Number	Ear Tag Number	EBV saliva IgA and FEC S	FEC EBV	Saliva IgA EBV
1	UK0307675 01401	High	-0.39	0.10
2	UK0307675 03008	Low	0.43	-0.02
3	UK0307675 03268	Low	0.21	-0.06
4	UK0309317 03749	Low	0.05	-0.13
5	UK0325635 00744	High	-0.55	0.12
6	UK0325635 00889	High	-0.55	0.09

4.2.2 Project variation (summer/autumn 2018)

The repeatability of FEC and saliva IgA was disappointing in year 1 when 200 lambs were sampled on two consecutive days, so the exercise was repeated on a further 84 lambs in 2018. Lambs were chosen from those born to the six selected sires in the spring of 2018 (HAU progeny test above) and included 10 female lambs and 4 wether lambs from each sire. It has been suggested that serum IgA may be a more reliable indicator of worm resistance so blood samples were taken to measure serum IgA to compare to results for saliva IgA. 12 male lambs were taken to slaughter on day 2 after initial FEC, saliva IgA and serum IgA samples were taken on day 1 and a further 12 were taken on day 25. Guts were transported from the abattoir (Euro Quality Lamb at Craven Arms) to Shrewsbury APHA for worm counting and speciation. The full sampling regime is shown in table 4.

Table 3. Sampling regime autumn 2018

	FEC	**Saliva IgA**	**Serum IgA**	**Worm Speciation**
	No. lambs	**No. lambs**	**No. lambs**	**No. lambs**
Day 1 *	84	84	84	
3/10/18	84	84		
Day 2				12
4/10/18				
Day 3	72	72	72	
5/10/18				
Day 17		72		
19/10/18				
Day 24	72	72		
26/10/18				
Day 25				12
27/10/18				
Day 31		60	60	
2/11/18				
Day 38	60	60	60	
9/11/18				
Total	372	504	276	24

*Day 1 - one FEC sample taken and divided into two for analysis or two consecutive samples taken.
Day 1 - two dental swabs soaked in saliva for duplicate analysis.

The lambs were weighed on five occasions from birth up to the first day of sampling in order to calculate a daily live weight gain (DLWG). They were weighed using a Tru-Test XR5000 which was calibrated before each group weighing.

Table 4. Lamb weighing

Date	**Age of Lambs**
12/02/2018 – 18/03/2018	0 months
30/04/18 – 02/05/2018	2 months

30/05/19 – 01/06/18	3 months
25/07/2018 - 30/07/2018	5 months
03/10/2018	7 months

Before sampling could begin FEC tests were carried out to check that lambs had been adequately challenged by worms.

Lambs had been significantly challenged by nasal bot flies through the summer of 2018 and hence needed treatment with ivermectin to resolve the problem. The last treatment was given on 25 July and sampling for this project began on 3 October.

5.0 Methods

5.1 Sample collection and laboratory analysis

FEC testing: Individual grab samples from the rectum of each sheep were placed in sealed plastic bags with all air removed and sent to Techion Group Ltd for analysis. Sampling aimed to gather at least 4 g of faeces per sample to allow for accurate analysis. Sheep that did not produce a large enough sample were segregated so that they could be sampled again.
In the laboratory, each sample was weighed and mixed with water at a ratio of 1:3. The mixture was then poured into a sedimenter until the 'slurry' line was reached and water was added to the 'water' line. The mixture was shaken and left to stand for 30 minutes. The mixture was poured out leaving approximately 15ml of sediment to which 80ml of saline was added. The solution was then further mixed and filtered before being pipetted into a FECPAKG2 cassette. Using a FECPAKG2 machine, digital images of the cassette were taken allowing eggs to be counted remotely. Results were presented as strongyle eggs per gram and were returned via email.

IgA testing
Individual saliva samples were collected using a dental swab and forceps. This was inserted between the cheeks and gums and gently manoeuvred for approximately ten seconds to ensure an adequate quantity of saliva was collected. Each sample was placed in a 15ml vial and sent to Glasgow University laboratories (2017) or Moredun Institute (2018). A similar method to a study by Shaw *et al.* (2012) was used in this study: saliva was extracted using centrifugation and an enzyme linked immunosorbent assay (ELISA) measuring optical density (OD) was conducted for the detection of IgA response to *T.circumcincta* L3 antigens (see appendix 3 for method). Serum samples (2018) were analysed using the same method.

A total of 24 male lambs were sent to slaughter in October 2018 and their guts were examined for worm numbers and speciation at APHA Shrewsbury and Carmarthen.

5.2 Statistical analysis

Data was analysed in Microsoft Excel and Genstat (18th ed). A paired t-test was used to assess the reliability and repeatability of FEC and saliva IgA samples. Regression analysis was used to observe the relationship between variable means over the sampling days. Finally, to analyse repeatability on

a more individual basis, Spearman's Rank Correlation was used. One-way analysis of variance was performed to determine if sire or EBV had an effect on DLWG, FEC, saliva IgA and serum IgA levels.

Regression analysis was used to determine whether there was a relationship between FEC, saliva IgA and serum IgA on DLWG from birth to seven months. Results from the first day of sampling, including the mean FEC and saliva from the duplicate samples taken, were used as this was when IgA levels and FEC results were predicted to be at their highest before worming on day 3.

6.0 Results

6.1 Results from OG members farms

Members of the Operational Group took samples from their own sheep in 2017 and 2018. The numbers recorded are shown in table 6.

Table 5. Samples taken on OG members farms

Numbers of samples taken on OG member farms	FEC	Saliva
2017	2660	3197
2018	1971	2084

The Lleyn breed has supplied the vast majority of samples for both FEC and IgA for use in Signet genetic evaluations. Figure 4 shows the numbers of results submitted up to 2019 across breeds.

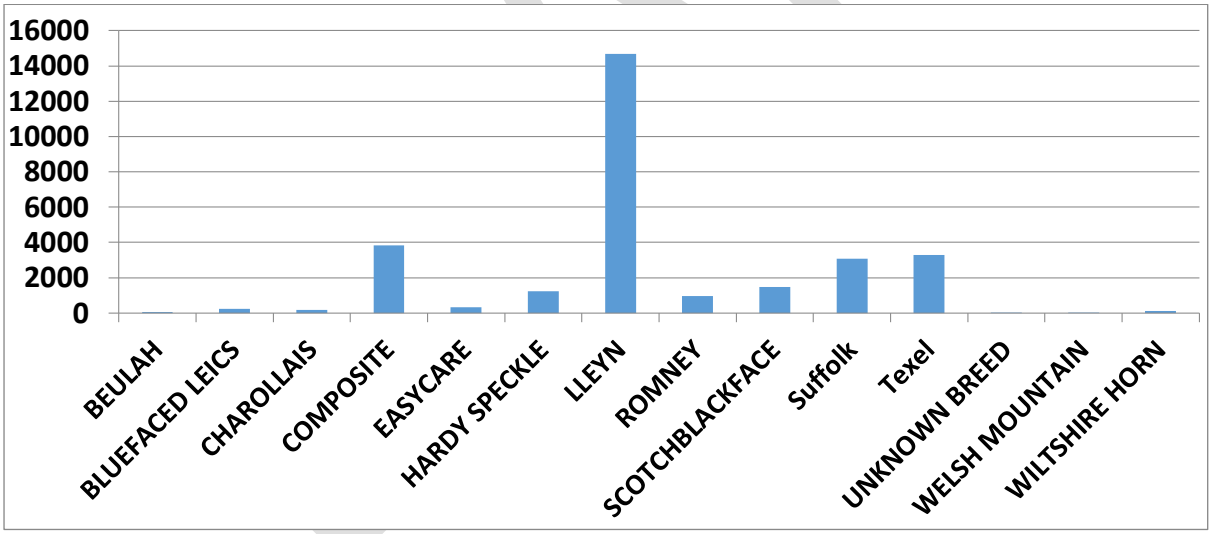

Figure 5. FEC samples provided to Signet per annum

Source: Signet Breeding Services, 2019

The planned matings at HAU have improved the connectedness of the HAU flock to other Lleyn flocks in the OG. The data to support this is shown in appendix 4.

6.2 Sampling at HAU in 2017

At HAU FEC and saliva samples were collected on the 17 and 18 October 2017. On the first day of collection each sample was labelled with the lamb EID tag number, whilst on the second, to ensure that laboratory bias did not occur, samples were labelled 001 - 200 and a record was kept of the corresponding EID tag number so that samples could be matched to the individual sheep. Saliva and FEC samples were refrigerated prior to postage to the laboratory the next day. Lambs were collected from a grass field and held in a closed barn for the duration of sampling. Water, but no food, was provided. Lambs were returned to the same field after the first day of sampling. Animals were able to graze freely overnight and returned for further sampling the next morning. A race was constructed using a Prattley sheep handling system that allowed approximately eight sheep to be sampled in a group. Lambs were retained inside for approximately 8 hours while sampling was completed for the whole group.

Table 7 shows the mean results from FEC were significantly different (P<0.001) between day 1 and day 2 (on average by 182 epg) with higher FEC on day 2. 54% of results varied by >180 epg and 46% varied by <180epg. For IgA there was no significant difference between the means with 28% varying by <0.1 and 72% varying by >0.10.

Table 3. T-test analysis of the effect of day of sampling on FEC and saliva IgA of ewe lambs

Sample	FEC (EPG)(log10)	Saliva IgA (OD)
Day 1 sample	356 (2.366)	0.544
Day 2 sample	538(2.611)	0.559
S.E.D.	38.28 (0.0401)	0.0324
P value	<0.001	0.646 (NS)

A moderate correlation was found between FEC on days 1 and 2 and between saliva IgA on days 1 and 2 (table 7). However there was no correlation between FEC and IgA on either sampling day. R^2 values were all low showing that only 36% and 27% of the variation in sampling days for FEC and IgA respectively can be explained by the correlation. Figure 5 shows the results graphically.

Table 7. Correlation between sampling days for FEC and IgA

	Correlation	R^2	P
FEC $\log_{10}$ day 1 – FEC $\log_{10}$ day 2	0.596	0.355	<0.001
IgA day 1 – IgA day 2	0.516	0.266	<0.001
FEC log10 day 1 – IgA day 1	0.019	0.0003	0.797
FEC log10 day 2 – IgA day 2	0.013	0.0002	0.854

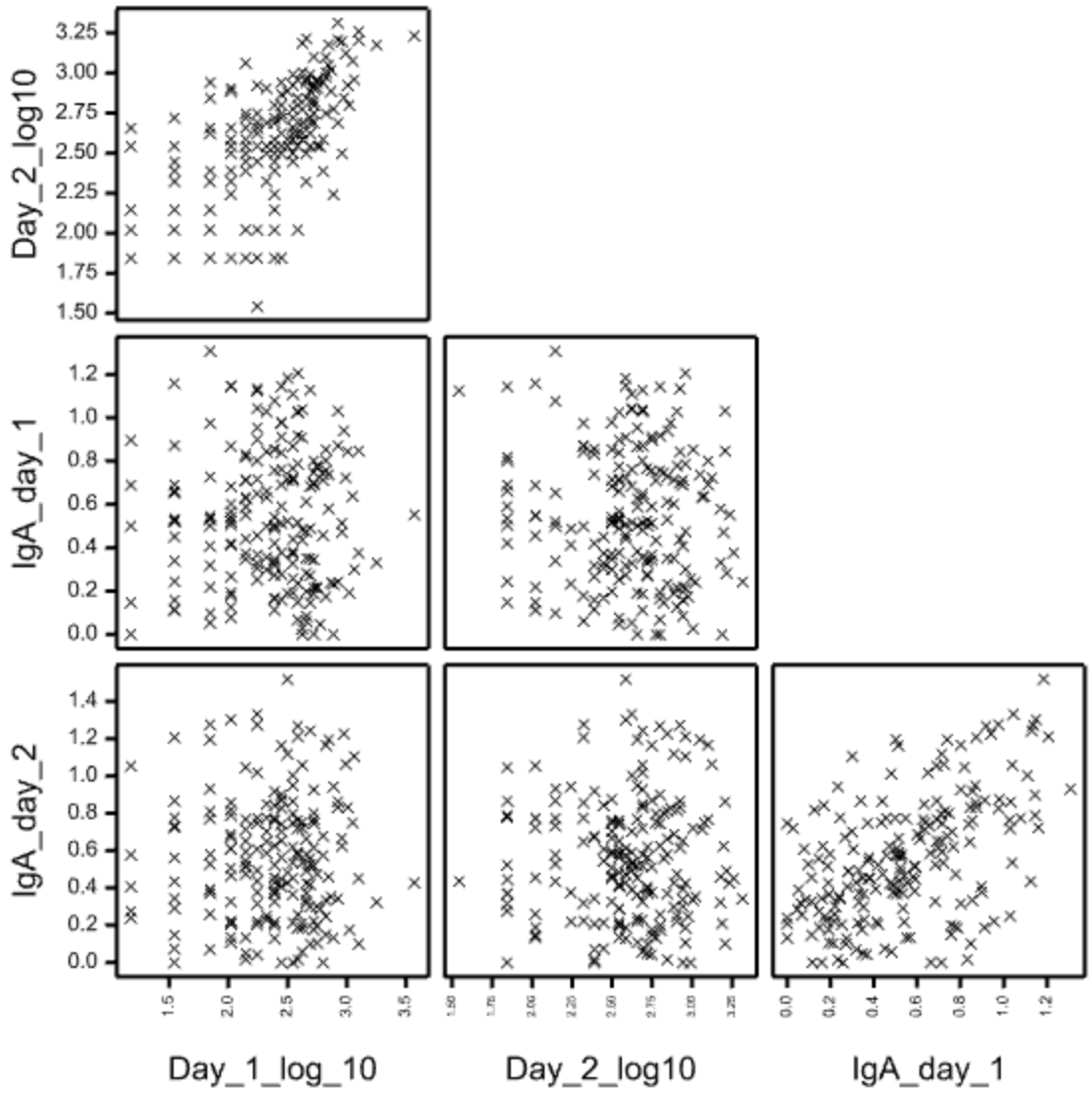

Figure 5. Correlation matrix between FEC log10 and saliva IgA.

Spearman's Rank Correlation

Spearman's rank correlation was used to assess whether results remained in the same order from one day to the next. The relationship was stronger for FEC than for IgA (table 9).

Table 8. Spearman's Rank Correlation for FEC and IgA results from day 1 to day 2.

	Spearman's Rank Correlation Coefficient	**P value**
FEC (EPG) - day 1 vs day 2	0.625	<0.001
Saliva IgA (OD) - day 1 vs day 2	0.482	<0.001

Regression analysis was carried out to explore the relationships between FEC, IgA and other performance parameters (tables 10 and 11).

Mean FEC (days 1 and 2) appeared to have a significant relationship with 8 week weight but had no effect on any other performance parameters. Mean IgA appeared to show a significant relationship with muscle depth and DLWG to 8 weeks.

Table 9. Regression analysis – relationship between mean FEC log10 and growth and body composition.

	8 week DLWG (kg/day)	Scan wt DLWG (kg/day)	Muscle depth (mm)	Fat depth (mm)
Significance	0.010	0.84	0.263	0.141
Standard error	0.0484	0.0284	2.38	1.12
R^2	0.29	0	0.10	0.70
	Y=0.342-0.0279x		Y=20.95 + 0.622x	Y=2.969 – 0.384x

Table 0. Regression analysis – relationship between mean IgA and growth and body composition

	8 week DLWG (kg/day)	Scan wt DLWG (kg/day)	Muscle depth (mm)	Fat depth (mm)
Significance	0.017	0.669	0.049	0.397
Standard error	0.0489	0.0287	2.37	1.11
R^2	0.24	0	0.016	0
	Y=0.255 + 0.0301x		Y=23.24-1.256x	

Effect of sire

Nine sires were used in the flock although total lambs per ram varied widely from only 4 to a maximum of 60. Table 11shows that there were significant differences between sires in regard to both IgA and FEC log10.

Table 1. Analysis of variance between sires

	DLWG to 8 weeks	DLWG to scan	Mean IgA	Mean FEC log10
Significance	0.001	0.001	0.006	0.001
CV%	16.87	12.4	49.33	12.27
SED	0.0179	0.1114	0.0930	0.1076

6.3 Results for autumn 2018 sampling

An initial mob FEC test taken from a group of over 200 ewe lambs (including the trial lambs) revealed 300 epg 2 weeks before the trial started. This was a low to moderate challenge, but time pressures on analysis dictated that the trial should begin on 3 October.
Faecal and saliva samples were taken from 235 lambs on 3 October. This included lambs that simply had one sample for FEC and one for saliva as well as the 84 lambs that were sampled in duplicate for FEC and saliva and on day one and had a blood sample taken for serum IgA analysis. For all FECs reported as 0 these were corrected to 15 epg given that the lowest level of detection is 35 epg.
For the 235 lambs the average FEC –S was 230 epg ranging from 15 to 4165 epg in individuals. The average IgA was 0.490 ranging from 0 to 1.349 OD units.

There was large variation in FEC between individuals for the 84 lambs taken on for further sampling, as table 13 shows.

Table 4. The variation in FEC results (EPG) taken over all sampling days. (Note lambs given a wormer day 3).

Sampling day	Average EPG	Minimum EPG	Maximum EPG
1	187	0	805
3	160	0	1020
17	167	0	735
24	40	0	840
31	28	0	105

A paired, two tailed t-test was performed to evaluate the reliability of duplicate sampling of FEC (transformed to $\log^{10}$ of the FEC strongyle – FEC-S) and saliva IgA taken on day one for the 84 lambs. As table 8 shows, the FEC tests were low and there was no significant difference between the mean FEC (P=0.205) or saliva IgA (P=0.226) samples taken on the same day. The t-test shows consistency between group sampling but does not evaluate on an individual basis (table 14).

Table 5. Paired t-test analysis of duplicate FEC and saliva IgA samples taken on day one

Treatment	FEC (EPG)(log10)	Saliva IgA (OD)
Day 1 sample 1 (A)	187 (2.065)	0.50
Day 1 sample 2 (B)	160 (1.959)	0.45
S.E.D.	0.0734	0.049
P value	0.150	0.265

A paired, two tailed t-test was also performed to evaluate the variation between mean FEC (day 1), mean saliva IgA (day 1) and serum IgA taken on sampling days one and three. Saliva IgA decreased by 21.3% between day one and day three (P=<0.001). FEC decreased by 4.2% by day three, and this

was not significantly different to day 1 (P=0.446). There was no significant difference between serum IgA results on day one and day three (P=0.294) (table 15).

Table 6. Paired t-test analysis of FEC, saliva IgA and serum IgA samples taken on day one and day three

Treatment	FEC (EPG)(log10)	Saliva IgA (OD)	Serum IgA (OD)
Mean day one	173 (2.012)	0.47	0.61
Day three	167 (2.009)	0.37	0.62
S.E.M.	17.56	0.032	0.020
P value	0.446	<0.001	0.294

Ranking

Techion UK Ltd advised that when two samples give different results then they can be different by 180 epg and still be considered to be the same (personal communication from E. Thomas, Techion Uk Ltd). This allows for the inherent errors within the sampling and egg counting methodology. When assessing the FEC results in the light of the +/- 180 epg variation then this shows that 21 out of the 84 (25%) duplicate samples taken on day 1 varied by more than 180 epg.

Dr Tom McNeilly (personal communication from Moredun Institute) calculated the coefficient of variation for the IgA testing. Intra- and inter-assay coefficients of variation (Cv) were calculated based on data generated in 2018. Intra-assay Cv was estimated based on paired well OD readings from 2225 samples. Inter-assay Cv was calculated based on OD index for the same sample (n=9) analysed on separate ELISA plates.

Table 7. Coefficient of variation for IgA testing

	Average % Cv	STDEV	Range
inter-assay % Cv	8.60	7.57	1.46-23.62%
intra-assay % Cv	2.66	2.95	0.04-38.02%

This indicates that samples done on different dates could vary by 8.6% so for an OD of 0.5 it could vary by +/- 0.043. Applying this Cv to the day one samples showed that of the 80 reliable duplicate samples taken, 35 (44 %) of these varied by less than 0.1 OD units. This confirms that both methods show significant variation between duplicate samples.

Spearman's Rank Correlation Coefficient

This correlation coefficient is a measure of association between the ranks for a pair of samples. As table 17 shows, there was a modest correlation between most FEC and saliva variables (0.415-0.530). However, a much stronger correlation was found between serum IgA samples taken on the different sampling days (0.642 to 0.878).

Table 8. The correlation coefficient between FEC S, saliva IgA and serum IgA.

	Spearman's Rank Correlation Coefficient	**P value**
FEC (EPG) - day 1 duplicates All lambs	0.415	<0.001
Saliva IgA (OD) - day 1 duplicates All lambs	0.530	<0.001
Females only		
FEC (EPG) – mean day 1 vs day 3	0.415	<0.001
Saliva IgA (OD) – mean day 1 vs day 3	0.463	<0.001
Saliva IgA day 3 vs day 17	0.480	<0.001
Saliva IgA day 3 vs day 24	0.377	0.004
Serum IgA (OD) - day 1 vs day 3	0.878	<0.001
Serum IgA (OD) – day 1 vs day 31	0.673	<0.001
Serum IgA (OD) day 1 vs day 38	0.642	<0.001
Serum IgA (OD) day 3 vs day 31	0.722	<0.001
Serum IgA day 31 vs day 38	0.793	<0.001

There was a strong correlation for serum results between day 1 and day 3 which was highly significant, meaning that lambs remained very largely in the same order/rank over different sampling days suggesting that serum IgA is more reliable and consistent than the other measures employed here. The correlations were also high for all other serum samples taken. The relationship was not as strong for all other parameters and comparisons with weaker but highly significant correlations.

Regression Analysis

The variable means for males and females were calculated and plotted over sampling days to observe the relationship. As shown in figure 6, all females were treated with monepantel (Zolvix) on sampling day three, hence the significant reduction in FEC by sampling day 24. Three out of twelve males were treated with monepantel on day three as they were scouring which was assumed to be an indication of a heavy worm burden. The remaining nine males were left untreated, thus FEC slightly increased by day 24.

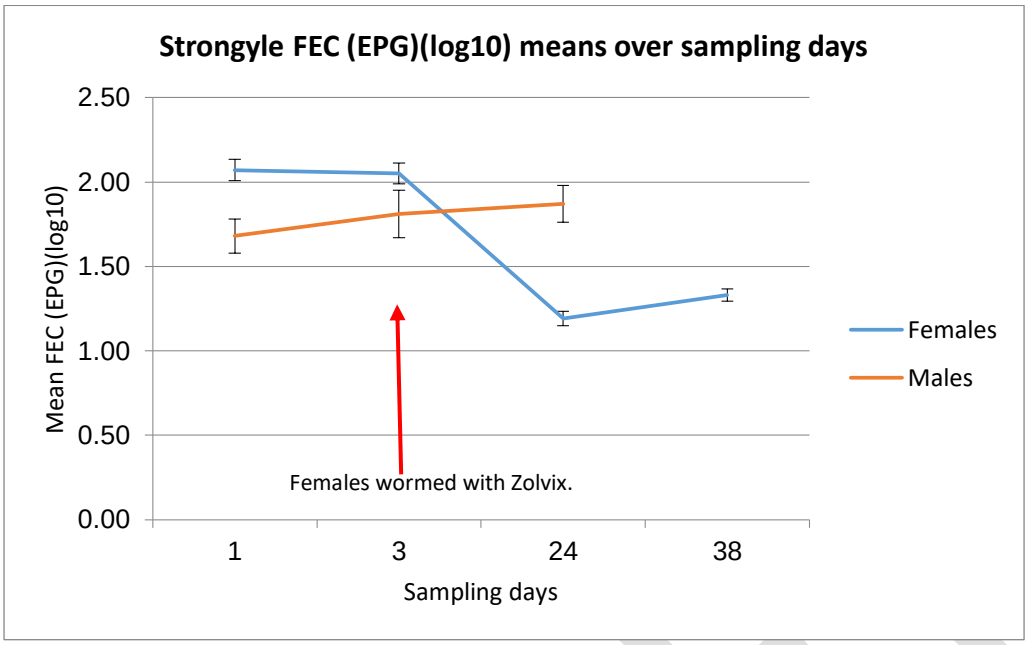

Figure 6. FEC Strongyle means over sampling days

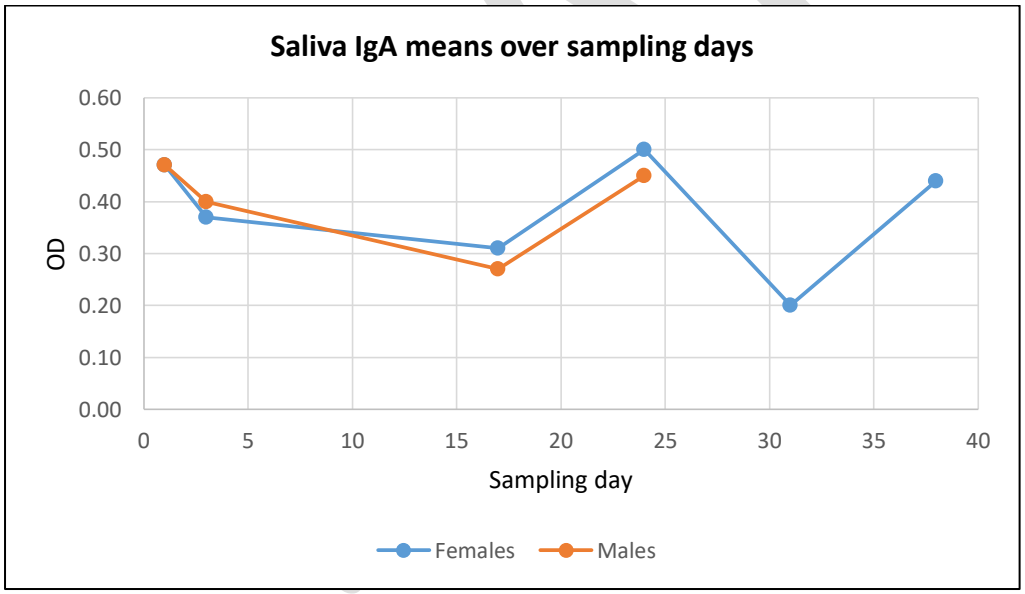

Figure 7. Saliva IgA means over sampling days

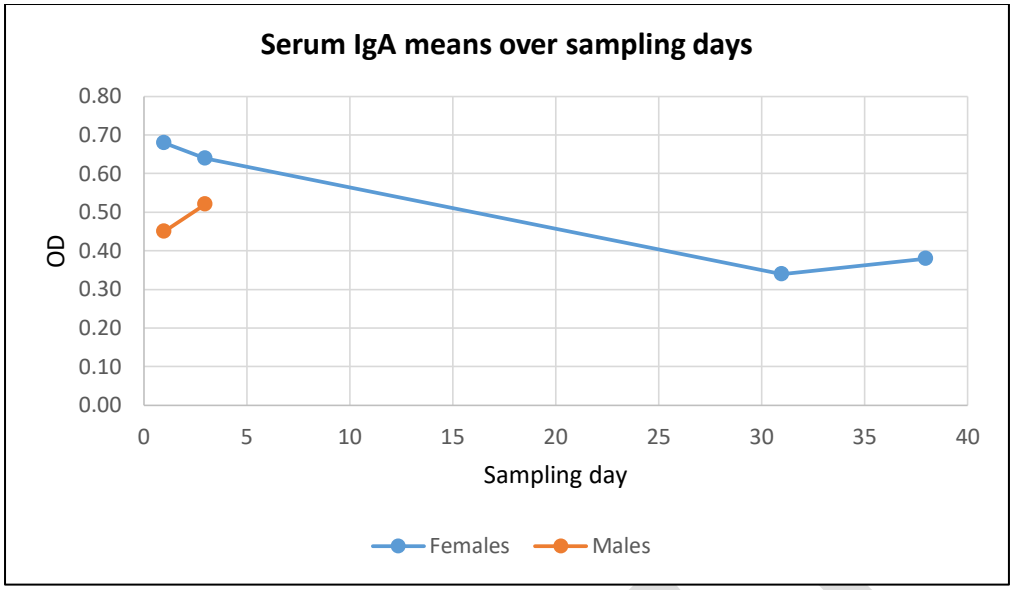

Figure 8. Serum IgA means over sampling days

The variable means for males and females have been plotted against each other in figures 4 and 5 to observe the relationship between them over the sampling period.

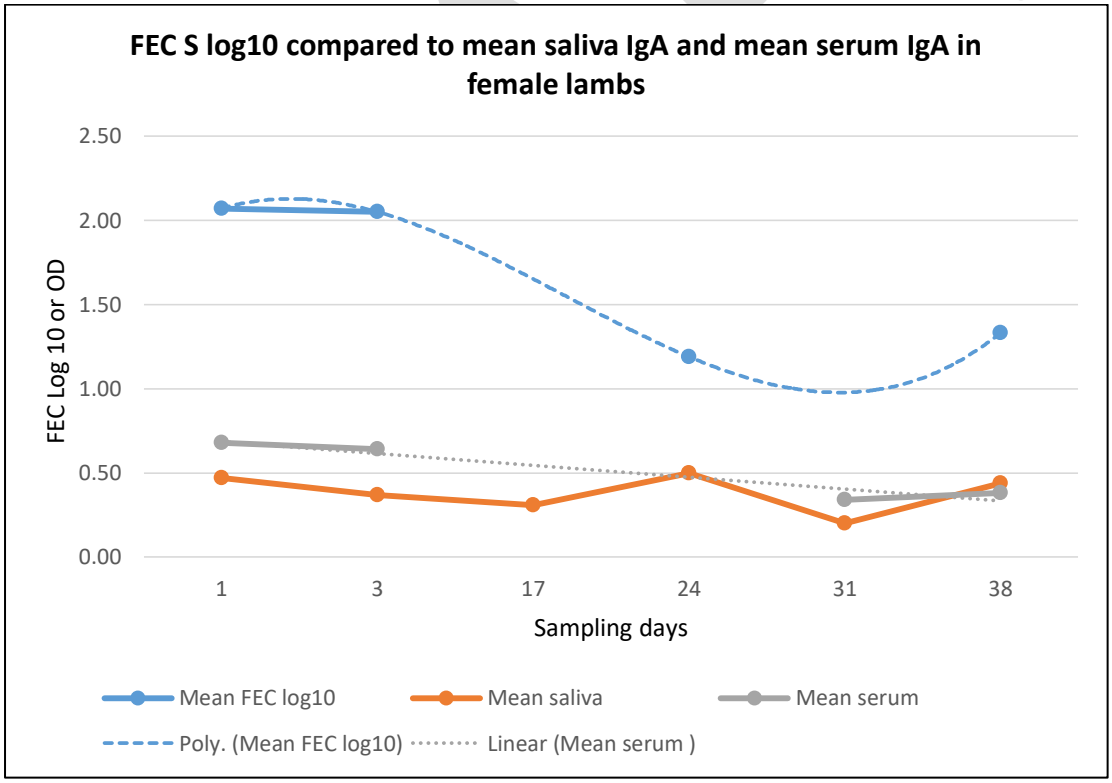

Figure 9. Relationship between female FEC strongyle, saliva IgA and serum IgA means over sampling days.

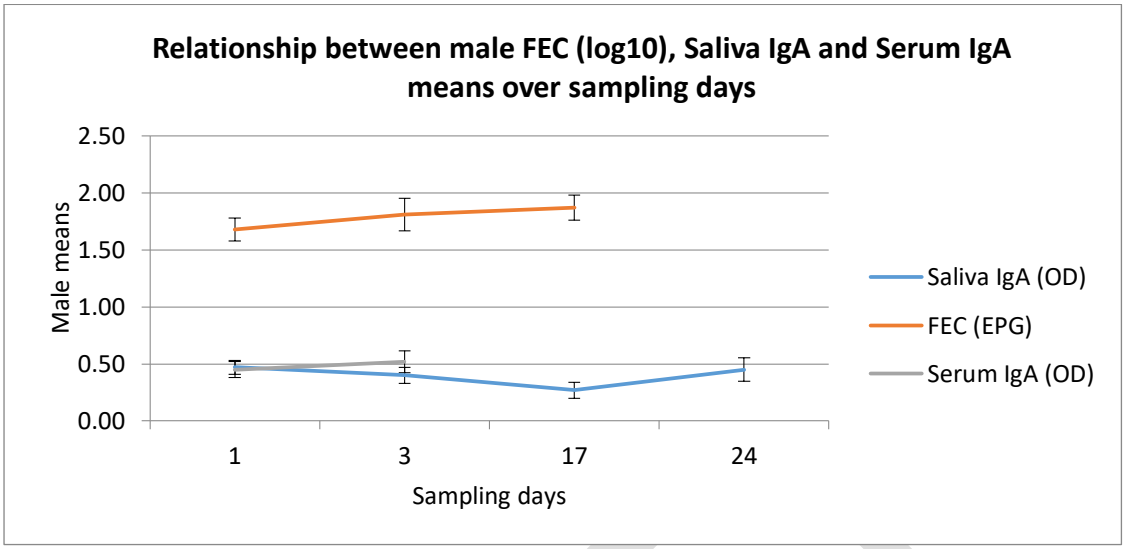

Figure 10. Relationship between male FEC strongyle, saliva IgA and serum IgA means over sampling days.

Correlation

To estimate correlation, all FEC samples were transformed to log10, adding 15 epg to any results less than 35 (i.e. those reported as zero). The sensitivity of the technique is 35 epg and 15 was considered a sensible figure to take as an indicator of <35 epg rather than assuming zero epg. This practice is recommended in parasitology to gather more realistic and accurate results. There were no significant correlations between FEC S and saliva or serum IgA on day 1.

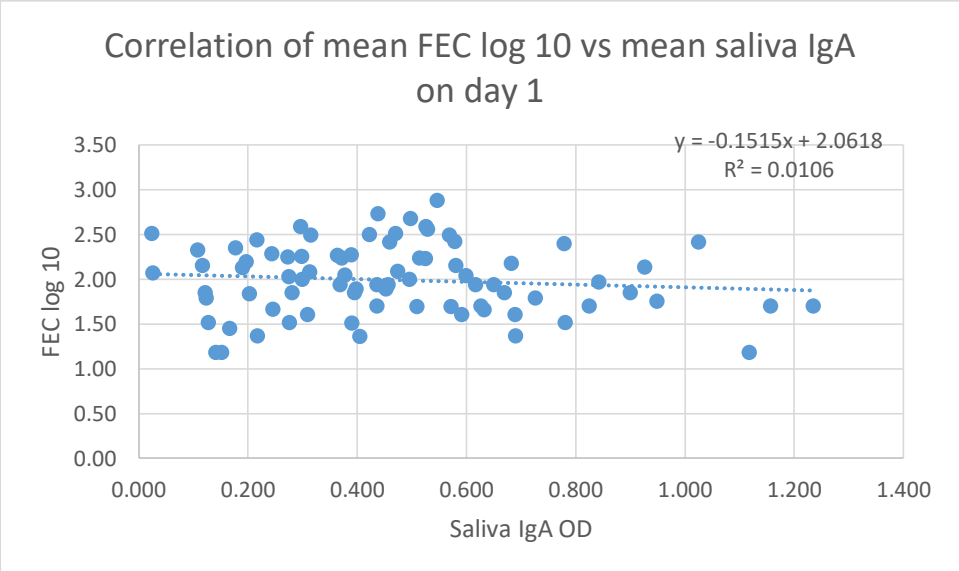

Figure 11. Correlation between FEC (log10) and saliva IgA means on day 1.

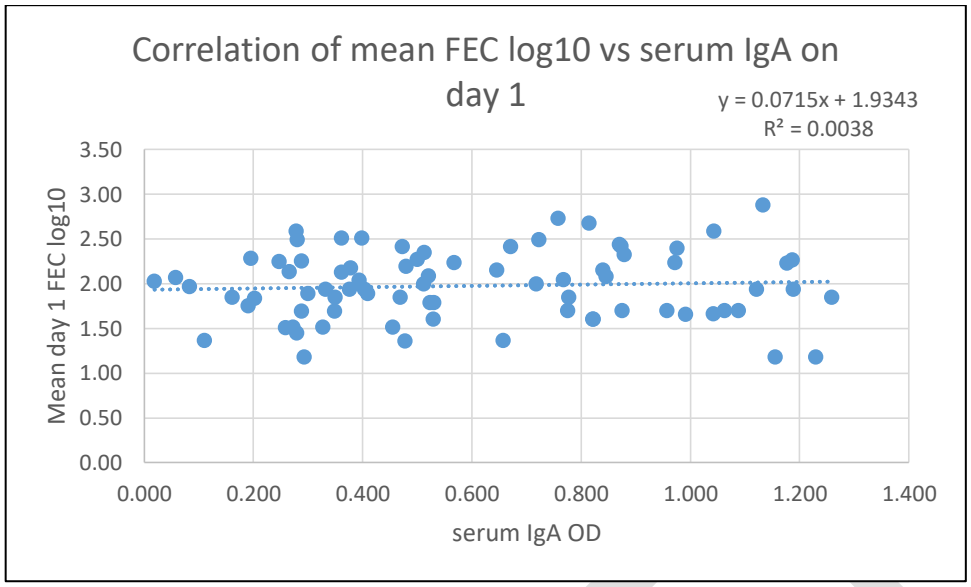

Figure 12. Correlation between mean FEC log10 and serum IgA on day 1.

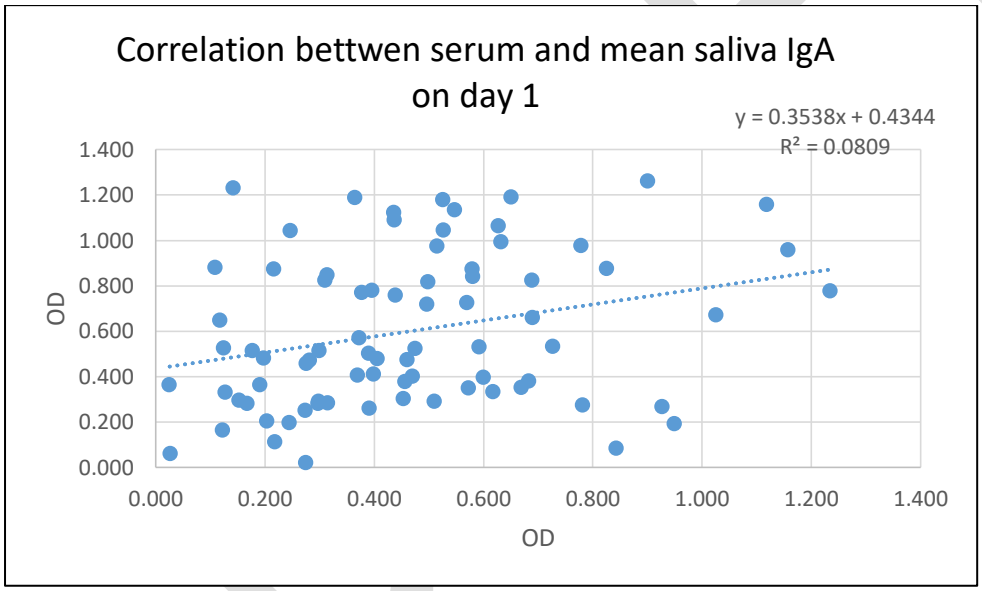

Figure 13. Correlation between mean saliva IgA and serum IgA on sampling day one.

Table 18 shows a strong correlation between sampling days for serum IgA. However, there was a very weak correlation between FEC and saliva IgA, and FEC and serum IgA on sampling day one.

Table 9. Correlation between variables

	Correlation	Relationship	P
FEC duplicates - day 1	0.416	Modest	<0.001
Saliva IgA duplicates - day 1	0.430	Modest	<0.001
Mean FEC - day 1 vs day 3	0.531	Modest	<0.001
Mean saliva IgA - day 1 vs day 3	0.461	Modest	<0.001
Serum IgA - day 1 vs day 3	0.885	Strong	<0.001
Mean FEC vs mean saliva IgA - day 1	-0.065	Very weak	NS
Mean saliva IgA vs serum IgA - day 1	0.284	Weak	0.012
Mean FEC vs serum IgA - day 1	0.181	Very weak	NS

6.4 Worm counts and speciation

Tables 19 and 20 show the results for worm counts and speciation present in the gut of 24 males in comparison to their FEC, saliva IgA and serum IgA levels. The 12 males sent to slaughter on day two had not been treated with an anthelmintic since 25 July 2018. The worm speciation results clearly show evidence that sheep were infected with *Teladorsagia*, and very significantly in some animals, however infection was not confirmed by all the individual FEC results. For example, lamb 6039 had 2200 adult *Teladorsagia* and 600 immature L4 present in the gut, however had a FEC of zero the day before slaughter. This could indicate that the worms were present but were not producing eggs that were evident in the faeces. However lambs with high numbers of *Teladorsagia* (>10,000) in the abomasum (lambs 5934, 6280 and 6320) tended to have higher FEC than other lambs. Given the low average FEC results for all the lambs on day 1 it was reassuring to see that many animals had a significant worm burden which should have been sufficient challenge to elicit an immune IgA response in the lambs.

Table 10. Worm counts and speciation from 12 males sent to slaughter on day 2

EID	Abo – *Teladorsagia*	Abo - Immature/ L4	Mean FEC (day 1)	Mean saliva IgA (day 1)	Serum IgA (day 1)
5934	13900	11200	315	0.331	0.281
6008	700	2600	42.5	0.782	0.273
6036	100	400	70	0.727	0.531
6039	2200	600	0	0.153	0.293
6040	5000	8900	70	0.125	0.524
6063	2200	2400	33.5	0.168	0.28
6081	4400	5200	175	0.274	0.248
6169	1000	400	210	0.027	0.058
6280	15400	5700	280	0.461	0.473
6300	1000	700	157.5	0.641	0.683
6305	1000	1200	60	0.593	0.53
6320	14400	4000	192.5	1.036	0.371

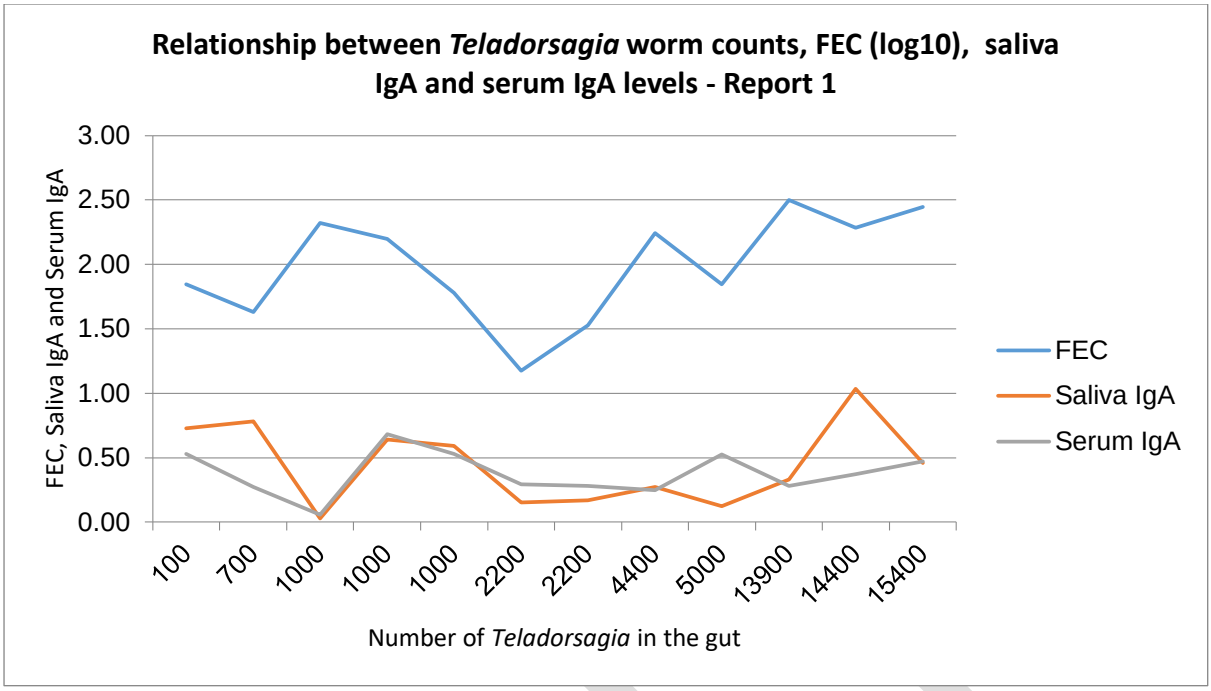

Figure 14. Relationship between number of *Teladorsagia* in the gut FEC (log10), saliva IgA and serum IgA for lambs slaughtered on day 2

Table 11. Worm counts and speciation from 12 males sent to slaughter on day 25

EID	Abo - *Teladorsagia/ Ostertagia*	Abo - Immature / L4	FEC (EPG)	Saliva IgA (OD)	Treatment (Zolvix) on day 3
5883	2600	0	140	0.460	-
5908	900	16700	35	0.287	-
5911	4300	46	0	-	Wormed
5933	-	-	0	0.481	Wormed
6015	500	-	0	0.487	Wormed
6046	300	-	0	0.048	-
6084	1100	2500	840	0.390	-
6107	4200	3000	0	0.340	-
6135	10500	4200	105	0.146	-
6165	500	500	280	0.341	-
6368	300	200	210	0.369	-
6383	1600	100	245	0.594	-

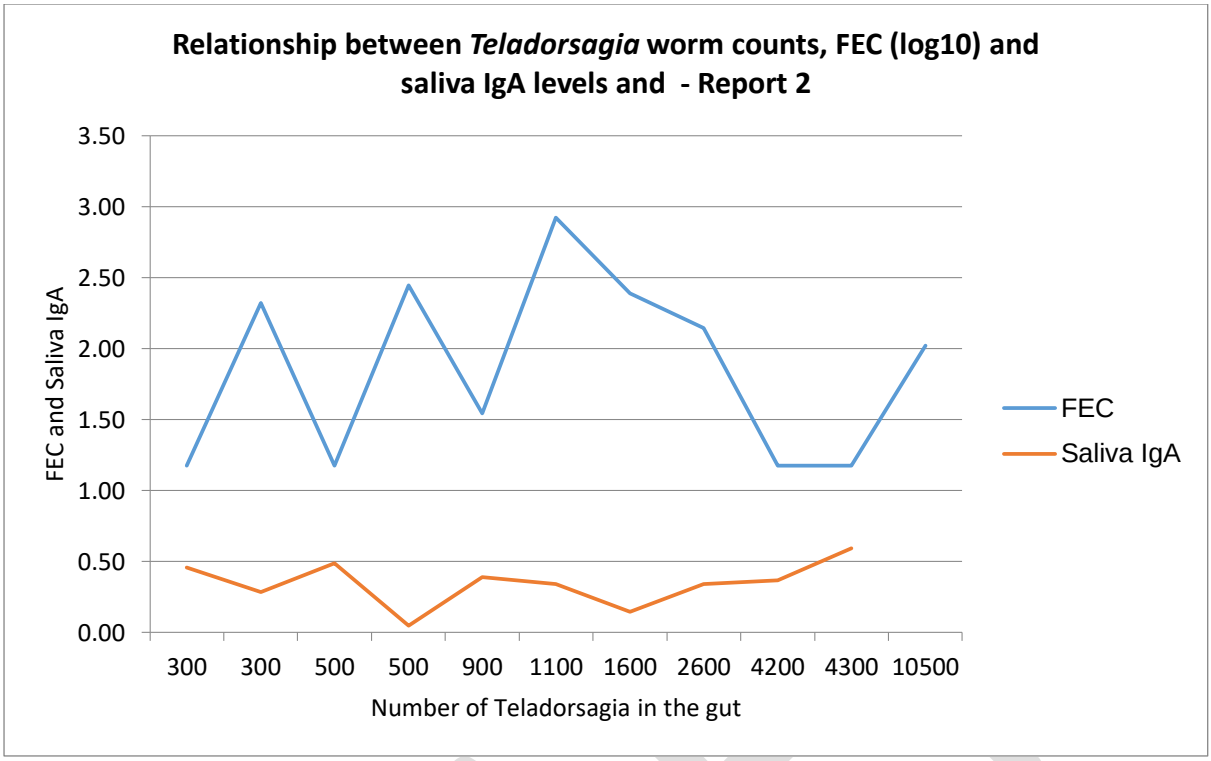

Figure 15. Relationship between number of *Teladorsagia* in the gut, FEC log 10, and saliva IgA for lambs slaughtered on day 25.

Due to unforeseen circumstances there is no confidence in the results presented in table 20 and figure 15 due to suspected confusion of lamb identification at the abattoir.

6.5 The influence of sire

Performance data for all 84 lambs

Table 12. Birth weights and daily live-weight gains for all lambs

	Mean birth weight (kg)	DLWG birth to 67 days	DLWG birth to 98 days	DLWG birth to 154 days	DLWG birth to 222 days	Weight on 3/10/18
All lambs	5.80	0.211	0.223	0.203	0.163	42.04
Sire 1	5.86	0.208	0.216	0.203	0.160	41.6
Sire 2	5.57	0.203	0.208	0.195	0.162	42.3
Sire 3	5.50	0.193	0.213	0.198	0.160	41.1
Sire 4	5.71	0.202	0.224	0.206	0.162	41.5

Sire 5	6.25	0.244	0.246	0.214	0.170	43.8
Sire 6	5.93	0.216	0.231	0.202	0.164	42.0
P	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**
SED	**0.347**	**0.0219**	**0.0157**	**0.0107**	**0.0055**	**1.305**

There were no significant differences between sires in terms of daily liveweight gain between birth and the four weighing dates, although sire 5 appeared to have lambs with the highest DLWG to the first two weighing dates. Performance of lambs between weighings three (end of July) and four (early October) was poor with lambs only growing at an average of 76 g/day. This was possibly a consequence of the infection with nasal bots and the very dry summer.

Data for ewe lambs only

There were no significant differences between sires for DLWG to 3/10/18, mean saliva IgA on day 1, overall saliva IgA or overall serum IgA (across all sampling days) although there was a trend towards significance for serum IgA, with high saliva IgA EBV sires appearing to produce lambs with higher IgA, in particular sire 5. Likewise sire 5 had the highest mean serum IgA on day 1.

Table 13. The influence of sire on DLWG to 22 weeks, mean saliva IgA on day 1 and mean saliva IgA and serum IgA over all sampling days for female lambs

Sire	DLWG birth to 22 weeks (g/day)	DLWG birth to 3 October	Mean saliva IgA day 1 (OD)	Serum IgA day 1 (OD)	Overall mean saliva IgA (OD)	Overall serum IgA (OD)
1	0.209	0.161	0.618	0.729	0.417	0.569
2	0.202	0.163	0.422	0.699	0.401	0.491
3	0.207	0.158	0.365	0.548	0.303	0.440
4	0.219	0.165	0.489	0.517	0.371	0.352
5	0.220	0.166	0.469	0.893	0.404	0.699
6	0.208	0.161	0.436	0.659	0.383	0.477
P value	0.637	0.825	0.396	0.164	0.780	0.090
SED	0.0123	0.0064	0.1180	0.1914	0.0825	0.1179

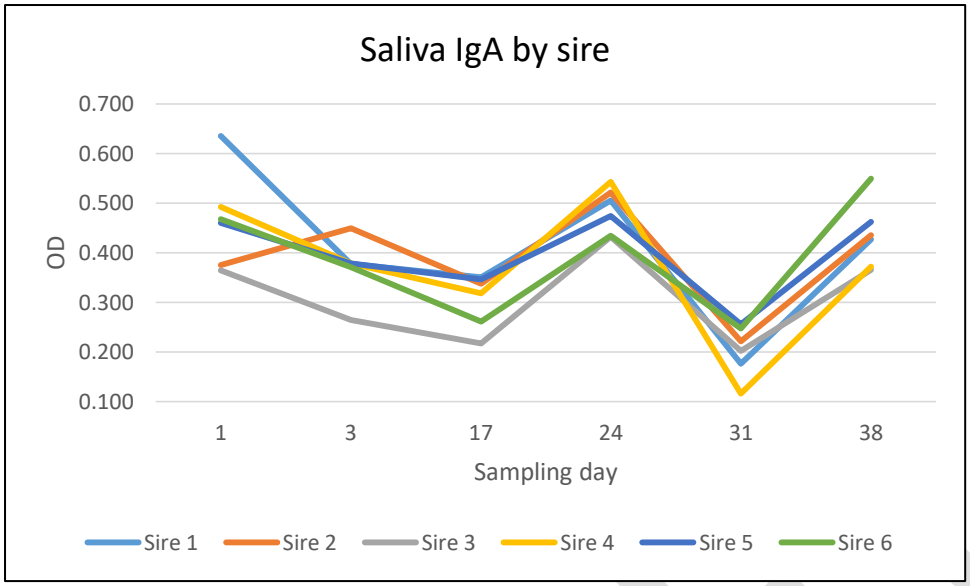

Figure 16. Saliva IgA by sire over all sampling days

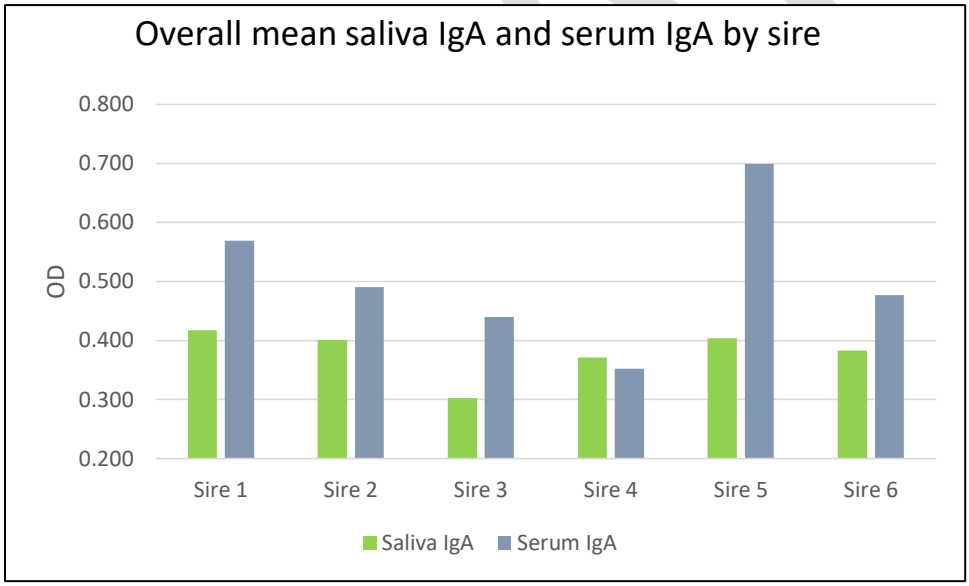

Figure 17. Overall mean saliva IgA and serum IgA by sire

7.0 Discussion

Results from 2017 indicated poor repeatability of both FEC and saliva IgA from one day to the next. Mean FEC increased but IgA did not. The differences in terms of FEC could partly be explained by the influence of food intake, the long holding period of some animals before sampling, and stress, as well as the inherent problems of variable faecal egg output and faecal dry matter. Storey, (2015) suggested that handling and absence of food can disrupt gut flow which would affect the ease of sampling, size of the sample and concentration of eggs. For example, a smaller faecal sample may result in a higher concentration of eggs and therefore a higher FEC result.

In 2018 efforts were made to minimise these variables, with animals retained inside for a much shorter period of time and offered both food and water. The results for FEC in 2018 were mostly low, with mean counts of 173 and 167epg on days one and three of the trial respectively. The twelve animals sent for slaughter on day 2 of the trial all had a burden of *T. circumcincta* worms, with 4 of 12 lambs having a significant burden of 5000 to 15,400 adult worms. 4 of 12 lambs also had 5000 or more larval worms in the abomasum. Lambs with high burdens of adult worms had low egg counts of 70 to 315epg. A possible explanation is that the adult worms were mature enough to be identified as *T.circumcincta* but not fully mature and not yet laying eggs.

During May, June and July 2018 the lambs suffered from nasal bot fly and subsequently received repeated anthelmintic treatments with oral ivermectin. This treatment could have affected the development of GIN in lambs in the trial, although the last anthelmintic treatment was given on 25 July, allowing over 10 weeks for reinfection to have taken place. McRae et al (2014) found that when Scottish Blackface lambs were challenged with 30,000 *Teladorsagia* larvae (L3), eggs were not observed in faeces until 28 days post infection. This could suggest that if lambs were treated in July it would have taken a couple of weeks before reinfection and then a further 28 days until eggs might be observed in the faeces. Furthermore, the dry conditions in the summer of 2018 could have delayed reinfection after the last ivermectin treatment. This would support the suggestion that the worms present in these lambs may only just have become sufficiently mature to start laying eggs.

It was estimated that around 15% of the lambs sampled in 2018 had liquid faeces and this is another factor that may have caused the low egg counts.

The faecal samples were all sent to Techion UK Ltd. Techion do not use a standard technique like McMaster, instead they use image based FECPAK^{G2}. One limitation of this technique is the possibility that the camera is not picking up all of the eggs present. Also, the FECPAK ^{G2} system involves a sedimentation process, following which the supernatant is tipped away. With liquid samples, it is suggested that the tipping away of the supernatant may also tip away worm eggs, artificially reducing the number of eggs then counted.

However, the FECPAK ^{G2} system is being used by the PRLB for all FEC testing in order to maintain consistency across participating farms. Several studies show wide variability among individual FECs (Bishop and Morris, 2007; Stear et al., 1999). Bishop et al (1996) attributed one third of the variation found for individual egg counts to measurement error. They also concluded that this level of variability in FEC means that selection decisions can be made more accurately using multiple egg counts for each animal rather than a single count. This is a practical option but perhaps one that would be prohibitive on cost.

There is very little literature to support poor repeatability of saliva IgA and the general consensus is that IgA is a more reliable and repeatable marker than FEC (Roeber et al., 2012; Storey, 2015; Fairlie-Clarke et al., 2019). However, individual animals appear to vary in their ability to produce IgA and IgA levels in saliva do not necessarily reflect the level found on the mucosal surfaces of the site of infection (Shaw et al., 2012; Bowles et al., 1995). McRae et al, (2014) showed that IgA peaked at 7 days post infection and then declined. In field infections it is impossible to know the stage in the life cycle in relation to when the lambs were challenged. Although the lambs in this study had been managed together, it cannot be assumed that they will all have picked up worms at the same time.

A reassuring finding from this work was the trend for high saliva IgA sires to produce high serum IgA offspring indicating a relationship between the two traits, however saliva IgA seemed to have no effect on lamb performance. These results are supported by the findings of Fairlie-Clarke et al., (2019) and Shaw et al., (2012) who both found saliva IgA to be heritable. Fairlie-Clarke et al., (2019) also found that the relationship between sire EBV and progeny IgA in Lleyn lambs across the UK suggested that sires with higher EBVs for IgA will produce offspring with greater IgA responses to *Teladorsagia* infection.

Variation in saliva flow is a limiting factor since it is influenced by time of day, feeding, drinking and stress. To overcome these problems there would need to be considerable effort made to standardise pre-sampling conditions and perhaps to measure saliva flow rate (as is done in horses) although over-complication of the method and the cost of sampling are likely to be prohibitive for most sheep producers. PRLB members have taken samples when they have been able, to suit their flocks and work load but they were not given specific instructions to standardise pre-sampling conditions of feed, drink or time of day and this may have been a possible refinement of the technique that could be implemented in future. The sampling for saliva over the past 4 years by the PRLB has been valuable but genetic progress will have been slow due to the inherent variation in results.

Serum IgA replicates were not taken on the same day however serum IgA results did not differ significantly from day one to day three, increasing by only 1.6%, and therefore appeared to be highly repeatable between sampling days. Davies et al., (2005) found plasma IgA to be highly heritable and repeatable which supports these findings as plasma and serum both come from the liquid portion of blood. The trend to a significant difference between sires in overall serum IgA was reassuring with high saliva EBV rams tending to produce lambs with high serum IgA.

The more reliable results found here for serum IgA, with generally consistent ranking of individuals on each sampling day show much more promise with the possibility of much more repeatable results that would ultimately produce more reliable EBVs and higher heritability for the trait. This would involve veterinary surgeons taking the samples which will increase cost but if a more reliable measure of worm resistance is established then that investment would seem justified.

8.0 Conclusions

1. FEC testing on a proportion of animals proved to be very variable and inconsistent when samples were taken on two consecutive days or when duplicate samples were taken on the same day from Lleyn lambs in the HAU flock. This puts into question the accuracy of using a single FEC as a measure of worm resistance in Lleyn sheep. EBVs are generated from data from a number of related animals as well as the animal itself but using a test that is more reliable will increase the rate of genetic progress.

2. Saliva IgA testing, likewise proved to be very variable on consecutive days or when duplicate samples were taken on the same day. Again this suggests that the current methodology needs modification to standardise pre-sampling conditions or that an alternative test needs to be developed. Saliva IgA EBVs are generated from related animals as well as the animal itself but faster rates of genetic gain could be achieved if the foundation testing of individuals was more accurate and repeatable. It also suggests that taking one sample on one day is unlikely to give a reliable indication of host resistance to parasites.
3. Ranking of individuals by FEC and saliva IgA showed some consistency between sampling days but was relatively weak indicating a potentially unacceptable degree of error for both tests.
4. Serum IgA results appeared to be much more consistent than either FEC or saliva with the majority of animals staying in the same rank order on each sampling occasion.
5. Despite the variability it appeared that there was a relationship between sire saliva EBV and serum IgA in their lambs, with high sires tending to produce high serum IgA offspring.
6. It is suggested that the PRLB look to take serum samples from their stock this autumn to investigate serum IgA as a more reliable measure of host resistance to internal parasites. Anticipated sample numbers will be between 500 and 1000 in 2019 and PRLB are looking to secure support from Signet/AHDB and elsewhere if possible. If concurrent FEC and saliva samples are taken then faecal consistency should be recorded and time of day. It may also be prudent to run McMaster FEC testing on a proportion of samples. The relatively small numbers of animals tested over the two years of this project suggest that this work should perhaps be repeated on a larger number of animals before firm conclusions can be drawn.
7. Heritabilities for FEC – S and saliva IgA in the Lleyn breed are currently at 0.07 and 0.11 (2018) respectively and these have fallen compared to the previous year when they were 0.08 and 0.16. This work has perhaps highlighted why heritability has been falling and that this may largely be due to variability in sampling and methodology.

9.0 How the project built bridges between research and the agricultural industry

PRLB is a group of pure-bred and pedigree Lleyn sheep breeders who all performance record their flocks through Signet Breeding Services. Signet provides genetic evaluations to livestock producers to improve the genetic potential of their stock. This project built on a previous project partnership between PRLB, AHDB and Glasgow University looking to improve selection of breeding sheep for worm resistance using saliva IgA. The PRLB initially started taking saliva samples in 2014 for a project funded by AHDB under their Farm Innovation Grant Scheme. This was a £5000 grant to begin the work. Subsequently the group members have continued to take samples from their stock but were keen to investigate the reliability of the testing and to use saliva IgA more extensively as a selection tool. An ongoing and very beneficial relationship has been sustained between the group and Sam Boon and his team at Signet. This project came about through Harper Adams University joining the PRLB and offering the capability to do more intense testing on the university Lleyn flock. Glasgow University was the only organisation offering saliva IgA testing at the time so all samples were sent to them until 2018/19 when saliva and serum samples were sent to the Moredun Institute. This has linked the group to the parasitology team at Moredun (Dr Tom McNeilly in particular) and this has led to an ongoing relationship, discussing and debating the results of this work.

In 2017 and 2018 FEC samples were sent to Techion UK Ltd for testing through their FECPAK G2 system and staff there have been very happy to discuss results and interpretation, with several meetings taking place to share knowledge.

IgA testing is now being moved to 'Biobest Laboratories Ltd' a commercial laboratory in Edinburgh, a specialist veterinary virology, serology and DNA diagnostics business. Dr Rebecca Mearns has been in discussions with the group to plan testing for 2019 and is currently doing preliminary work to set up the assay.

10.0 Additional or unexpected benefits or detriments of the project

FEC testing is encouraged and used widely in the sheep industry as a means of detecting infection by internal parasites. It is the foundation of the Sustainable Control of Parasites in Sheep (SCOPS) advice on monitoring worm burdens and gives an indication of when farmers should treat their animals. FEC testing is carried out by farmers themselves, by vets and commercial laboratories. It has proved very valuable in detecting wormer resistance and has helped to guide the use of anthelmintics. It has also helped many farmers to reduce the use of anthelmintics without losing livestock performance. We do not want the results of this study to undermine the use of FEC as a monitoring and diagnostic tool, so great care needs to be taken to ensure the message from this work is clear.

PRLB members have invested a large amount of their own money in FEC and saliva testing in fact over £1,2000 over the last two years as well as time and paid labour to assist with sampling. Although saliva IgA seems to have some value, the rate of genetic gain will be relatively slow with the current low estimate of heritability for this trait and the high variability in results on individual animals. The results of this project have been disappointing in terms of the value of saliva IgA as a reliable indicator of worm resistance. Despite the findings of this project PRLB is pleased to have

discovered the limitations of the saliva IgA despite significant investment and is keen to move to the next phase in selecting superior breeding stock based on serum IgA, where estimates of heritability appear to be much higher.

A further project is planned starting in April 2020 to investigate serum IgA, saliva IgA and FEC in 50 yearling ewes (sampled in this project in the autumn of 2018) lambing for the first time. This follows discussions with Dr Reinard Everts from the Netherlands at the Sheep Breeders Round Table Conference in November 2019, who has been testing ewes post lambing rather than testing lambs – finding this approach more reliable. This will look at the peri-parturient increase in worm egg output often seen in ewes post lambing and how this relates to IgA. This work will be funded by Signet Breeding Services.

Development and maintenance of good working relationships with the Moredun Institute, Signet Breeding Services, Techion UK Ltd and Biobest are very positive outcomes from this project.

Appendices

Appendix 1. Operational Group Members

Name		Address
John Peregrine Aubrey		Eastergrounds Farms, Slapton, Kingsbridge, Devon TQ7 2rb
Trevor and Chere Border	Chere – secretary 2019	Moonshine Farms, Will's Farm, Hollygate Road, Ridlington, Rutland,LE159AS
Marcus Bullock		Bank Farm Partners, Bank Farm, Abberley, Worcs, WR6 6BQ
Edward Collins	Secretary 2017-2018	Bearwood Farm, Leominster, Herefordshire, HR6 9EE
George Cullimore	Technical secretary	Coombe Barn, Kelston, Bath BA1 9AJ
Richard Evans	Chairman	Stonehouse Farm West Harling, Norwich, Norfolk NR16 2SD
Matt Heydon		JCB Farms Ltd Cote Farm, Farley, Oakmoor, Stoke-on Trent, Staffordshire ST10 4BQ
Scott Kirby	Applicant for EIP project	Harper Adams University, Newport, Shropshire TF10 8NB
Bethan and Dominic Klinkenberg		Glebe House, Up Marden, Chichester, West Sussex. PO18 9JR
Duncan Nelless		Thistley Haugh Farm, Longhorsley, Morpeth, Northumberland NE65 8RG
Tim Roberts		Longlands, Whitbourne, Worcester, Worcs, WR6 5S
Simon Thompson		Culland Hall Farm, Brailsford, Ashbourne, Derbyshire, DE6 3BW

Appendix 2.

Performance Recorded Lleyn Breeders Group

Terms of Reference for the Operational Group – 6 March 2017

1. PURPOSE
   The group was set up to bring together like-minded Lleyn breeders who are performance recording their flocks, and to promote breed improvement. In terms of the RDPE EIP project application the group agreed to collect faecal samples for worm egg counts and to take saliva samples for saliva IgA analysis. The aim of the project is to:
   - Identify Lleyn sheep that are more resistant to worms and to compare saliva IgA analysis to FEC EBVs and the means of doing this.
   - Develop existing ultrasound scanning technologies designed to measure muscle depth across the loin to provide a measure of total muscle area as an alternative to expensive CT scanning.
   - Using ewes at Harper Adams University to further validate the tests and investigate heritability of worm resistance by bit methods.
2. ROLES AND RESPONSIBILITIES
   The group will design, manage and lead delivery of the project. This will include:
   - Group members to collect samples (FEC and saliva IgA) from their own lambs at around 21 weeks of age and this data will be submitted to Signet Breeding Services for the purposes of the genetic evaluations.
   - Scott Kirby of Harper Adams University (HAU) is the applicant and HAU will manage the paying of invoices, tracking spend and bank rolling.
   - Kate Phillips of HAU and George Cullimore of PRLBG – will act as agents, oversee the management of the project, liaise with Defra over progress reports and claims/audits and provide group meeting administration and coordination.
   - Technical lead on genetic evaluations will be Sam Boon of Signet Breeding Services.

   The group is accountable for:
   - working cooperatively
   - maintaining a focus on the agreed aims and outcomes

   The membership of the group will commit to:
   - attending all scheduled group meetings, or nominate a deputy
   - championing the project
   - share information across the group
   - make timely decisions
   - notify members of the group if any matters arise which negatively affect the project

   Members of the group will expect:
   - that each member of the group will participate fully, and provide information/ data in a timely manner
   - open and honest discussion about the direction of the project
   - to be alerted to risks and issues that could impact on the project

3. GOVERNANCE AND REPORTING
   The group is be chaired by Richard Evans who will provide leadership, develop consensus and conflict resolution. Secretariat will be provided by Edward Collins with agendas and reports circulated a week in advance. An annual review of the ToR will be conducted to ascertain if they are relevant and fit for purpose. Any conflicts of interest must be declared

at the outset, the proposer of any conflict of interest shall not be allowed to participate in any vote or discussion whereby their interests are, or could be, different from the best interests of the operational group itself.

4. CORE MEMBERSHIP AND FUNDING

The founding members of the operational group are as follows. Members should attend regularly and deputies put forward where they are unable to attend.

Name	Address
John Peregrine Aubrey	Easterground Farms, Slapton, Kingsbridge, Devon TQ7 2rb
Trevor Border	Moonshine Farms, Will's Farm, Hollygate Road, Ridlington, Rutland,LE159AS
Marcus Bullock	Bank Farm Partners, Bank Farm, Abberley, Worcs, WR6 6BQ
Edward Collins	Bearwood Farm, Leominster, Herefordshire, HR6 9EE
George Cullimore	Coombe Barn, Kelston, Bath BA1 9AJ
Richard Evans	Stonehouse Farm West Harling, Norwich, Norfolk NR16 2SD
Matt Heydon	JCB Farms Ltd Cote Farm, Farley, Oakmoor, Stoke-on Trent, Staffordshire ST10 4BQ
Ian Horsley	Downhayne, East Village, Crediton, Devon, EX17 4DN
Scott Kirby	Harper Adams University, Newport, Shropshire TF10 8NB
Bethan and Dominic Klinkenberg	Glebe House, Up Marden, Chichester, West Sussex. PO18 9JR
Graham Matravers	G.R. & V. M. Matravers, Manor Farm, Long Whatton, Loughborough, Leicestershire. LE12 5DF
Duncan Nelless	Thistley Haugh Farm, Longhorsley, Morpeth, Northumberland NE65 8RG
Tim Roberts	Longlands, Whitbourne, Worcester, Wocs, WR6 5S
Simon Thompson	Culland Hall Farm, Brailsford, Ashbourne, Derbyshire, DE6 3BW

Chris Wilkinson	Oakwood Farms, Wildfell Farm, Chapel Road, Ramsey Heights, Huntingdon, Cambridgeshire, PE26 2RS

To match fund the project the group commit to providing the following indicative sums:

Name	Year 1	Year 2	Year 3
All farmers - FEC samples -	4500		
All farmers IgA samples	3000		
All farmers ultrasound scan	5250		

The terms of reference are effective from 1/07/2017 and will run to cover the period of the development, running and final monitoring of the grant aided project, to 31/08/19

5. MEETING SCHEDULE AND OUTPUTS
   - The operational group will meet at least twice each year or as and when required, the location to be agreed as being convenient to the majority of members.
   - Teleconferences may be used as alternatives (use of Signet Breeding Services System).
   - Meetings will be chaired and include feedback from group members as to progress and any issues identified and management reports will be prepared.
   - Decisions made by consensus, but if not possible a vote will be taken where the decision is with the majority, if not, then the chairman will have the final decision.
   - Non-members / observers will be invited to group meetings where they have specific expertise relevant to the project (e.g. Karen Fairlie Clarke from Glasgow University or Eurion Thomas from Techion Ltd).
   - Confidential data will be circulated only in password protected files to protect business data and any financial information.

Ownership of outputs

- Each member will receive more robust Estimated Breeding Values for Saliva IgA and FEC for their sheep that will enable them to make better informed decisions when breeding for worm resistance.
- The updated versions of these will be made available four times per year as a result of the usual Signet Breeding Services 'BLUP runs'. They will receive them in printed form and can access them via the BASCO website.
- They should also see improved accuracies for the EBVs for all traits measured as the result of the establishment of the central progeny testing at HAU, helping to improve their rates of genetic progress.

Sharing of information

We confirm that the Operational group members agree that all the results and outputs of the project can be shared freely with non-group members.

6. DECLARATION

We the undersigned are members of the Performance Recorded Lleyn Breeders Group and agree to act in accordance with the working arrangements contained within the Terms of Reference.

Name	Signature	Date
TREVOR BORDER	T. Border.	6.3.17
GRAEME MATHIESON	G. Mathieson	"
CHRIS WILKINSON	C. Wilkinson	"
Dominic Klinkenberg	D. Klinkenberg	"
MATTHEW HAYDON	M. J. Haydon	"
CLARE HILL	C. Hill	"
[illegible]	[illegible]	"
Marcus S. Bullock	M. Bullock	"
John Peregrine Athy	J. P. Athy	"
EDWARD COLLINS	E. Collins	"
RICHARD EVANS	R. Evans	"
GEORGE CULLIMORE	G. F. Cullimore	"
IAN HORSLEY	I. Horsley	"
SCOTT KIRKBY	S. Kirkby	"
Name	Signature	Date
DUNCAN NELLESS	D. Nelless	14-2-17
Name	Signature	Date
T. Roberts	T. Roberts	27/2/17

Appendix 3.

Linkage between flocks (Signet Breeding Services)

NAME	PREFIX	FLOCK CODE	MLC MEMBER ID	Sept 2017 Analysis Average Linkage	Jan 2019 Analysis Average Linkage
Harper_Adams_University	HARPER_ADAMS	1903	16034	0.022	0.03
Bank_Farm_Partners	0	619	22374	0.085	0.094
Mr_G_Cullimore	SUNDANCE	1932	30077	0.053	0.055
H_G_Geen_&_Son	0	1153	30376	0.045	0.046
Manor_Farm_East_Marden	APPLEDOWN	2259	30423	0.063	0.069
E_S_J_Collins	BEARWOOD	981	20145	0.063	0.066
Luscious_Lamb	KIRTLINGTON	291694	30619	0.029	0.036
Performance_Lleyns_(_J.P.Aubre	SLAPTON	30250	30250	0.039	0.041
Jason_Spence	NUTSHELL	2255	30442	0.038	0.041
R_&_S_Evans	0	1547	30056	0.045	0.051
Leighfield_Lleyns	LEIGHFIELD_LLEY	1704	30511	0.019	0.021
E_&_D_Jones	LLUEST	599	21599	0.053	0.05
Simon_Thompson	CULLAND	1448	30149	0.031	0.032
H_L_Nelless	THISTLEYHAUGH	884	22485	0.036	0.04
Hamish_Goldie	GOLDIES	1312	22511	0.054	0.053
Finlay_McGowan	INCHEOCH	894	22149	0.044	0.044
F_D_&_M_E_Candy_&_Sons	CANBURRY	1358	30507	0.039	0.039
D_N_Bennett_&_Son	PLASUCHA	893	30026	0.063	0.056
JCB_Farms_Ltd	WOOTTON	167577	30711	0.022	0.027
H_F_Mills	0	1429	22300	0.035	0.036
E_&_P_Evans	0	10	2647	0.028	0.03
Mr_Reuben_Saunders	BOAKLEY_LLEYNS	2309	30536	0.034	0.036
Garton_Hardy_Farming	GARTON_HARDY	2536	30727	0.019	0.022
W_W_&_J_A_McCurdie	MAC	205478	30731	0.015	0.022
S._L._Fletcher	0	2642	30666	0.022	0.023
Derek_J_Steen	WHITCASTLES	809	22436	0.039	0.039
S_J_&_C_J_Steel	0	754	19486	0.021	0.022
D_C_&_C_M_Evans	OLDAPORT	150	13639	0.019	0.019
Mr_Nick_Walter	BALTHAYOCK	542626	30068	0.031	0.031
G_&_A_Fort	BRIGHTONHOUSE	1299	30217	0.028	0.032
Laga_Farms_Ltd	LAGA	971	20621	0.029	0.027
C_G_&_J_F_Phillips	MACARONI	1229	30301	0.025	0.022
J_M_&_G_C_Adams	ROSELAND	847	22642	0.031	0.027
Crosby_Cleland	BROOKMOUNT	1375	22120	0.007	0.007
Mr_Robert_Johnston	0	1480	30155	0.022	0.022
SRUC_Kirkton_Farm	542768	542768	30331	0.023	0.023
Malcolm__&_David_Corbett	0	1308	21533	0.008	0.009
J_Campbell_&_Co	TOPHOLES	21968	21968	0.009	0.009
Mr_Deri_Morgan	TYNEWYDD	2437	30632	0.011	0.009
G_&_R_M_Roobottom	COWLEY_HILL	2662	30798	0.016	0.016
James_Riddell_&_Co	NETHER_COULLIE	1314	22386	0.011	0.009

Thresholds:

Green (acceptable, comparisons can be made

between sheep in different flocks with confidence) = values over 0.025

Amber (low linkage, comparisons between sheep should be made with caution) = values over 0.0125

Red (poor linkage) = values below 0.0125 - EBVs should not be compared between flocks (although within flock rankings will be accurate)

Data was considered for animals born between and including 2000 & 2019

An extra 10 years pedigree was considered with ped from 1990 onwards allowed

Appendix 4.

Dissemination

Promotion of Worm Resistance Project (EIP)						
Date	**Event**	**Venue**	**Person**	**Title**	**Delivery**	**People**
05/11/2017	Breed Development Committee of Lleyn Sheep Society	Holiday Inn, Runcorn	G. Cullimore	Saliva IgA project	Talk	12
17/11/2017	Sheep Breeders Round Table	Eastwood Hall, Notts NG16 3SS	S. Boon (Signet)	Signet Update	Talk to AHDB Progressive Sheep Group	100
18/11/2017	Sheep Breeders Round Table [1]	Eastwood Hall, Notts NG16 3SS	G. Cullimore and Karen Fairlie-Clarke	Getting better at collecting what is required	Talks	100
05/06/2018	PRLB annual farm walk	Stowell Farm Wiltshire	G. Cullimore	Update on saliva project	Talk	40
21/06/2018	EIP Agri	York	K. Phillips	Breeding for worm resistance in sheep	Talk	40
05/07/2018	Bank Farm Lleyns Open Day for Nuffield Scholars	Bank Farm Abberley, Worcester	K. Phillips	Breeding for worm resistance in sheep	Poster	25
11/07/2018	Challenge Sheep Discussion Group meeting	HAU Steeraway Farm	K. Phillips	Breeding for worm resistance in sheep	Part of talk	19
18/07/2018	National Sheep Event [4]	Three Counties Showground, Malvern	K. Phillips	Breeding for worm resistance in sheep	Poster on HAU stand	
20/09/2018	Maternal Sheep Group Visit to Harper Adams University	HAU	K. Phillips	Breeding for worm resistance in sheep	Talk	9

22/05/2018	Sheep Vet Conference - Aberystwyth	Aberystwyth	E. Steele (Signet)	What have we learned about breeding for parasite resistance in sheep in the UK?	Presentation to vets	75
18/10/2018	DEFRA Scoping Workshop (part 2)	Stoneleigh	G. Cullimore	Experiences breeding for worm resistance in sheep	Presentation to farmers and the supply chain	50
21/11/2018	SHAWG Conference [2]	Drayton Manor Hotel, Staffordshire	G. Cullimore	Breeding more resilient sheep	Presentation to farmers	130
06/01/2019	South West Region Lleyn Breeders Club	Tedbury St Mary Village Hall, near Exeter	G. Cullimore	Breeding sheep for worm resistance	Talk to farmers	30
08/01/2019	Moredun Sheep and Cattle Health day	Harper Adams University	K. Phillips	Breeding sheep for worm resistance	Presentation to farmers SQPs, vets etc	100
06/02/2019	Kent Sheep Study Group		E. Steele (Signet)	Breeding sheep for worm resistance	Talk to farmers as part of wider Signet presentation	
08/05/2019	Grosvenor Sheep Group	Harper Adams University	K. Phillips	Breeding sheep for worm resistance	Talk to visiting farmer group	16
10/05/2019	PRLB meeting	Bank Farm, Abberley, Worcs	K. Phillips		Update small group of PRLB on results	5
08/06/2019	Annual PRLB farm walk	Mr P Aubrey, Slapton, Devon	KP and GC	Plan to share analysed results	Short talk and discussion of future approach	12
23/07/2019	Shepton Flock Health Club	The Old Down Inn, Emborough, Radstock BA3 4SA	George Cullimore	Breeding sheep for worm resistance	Short talk as part of wider meeting	40
24/09/19	Farm walk for a group of Northern Irish farmers	Bank Farm, Abberley, Worcestershire	KP and GC	Breeding sheep for worm resistance	Presentation of project results	14
22/10/19	Farm walk for a group of Northern Irish Farmers	Bank Farm, Abberley, Worcestershire	KP and GC	Breeding sheep for worm resistance	Presentation of project results	19

16/11/2019	Sheep Breeders Round Table 2019 [3]	Radisson Blu Hotel, East Midlands Airport	KP and GC	IgA as an indicator of worm resistance in sheep	Conference presentation	200

Web links to presentations given

[1] Sheep Breeders Round Table 2017:

https://www.nationalsheep.org.uk/workspace/pdfs/11-cullimore-and-fairlie-clarke.pdf

[2] SHAWG 2018

http://beefandlamb.ahdb.org.uk/wp-content/uploads/2018/11/SHAWG-Conference-2018-Presentation-Slides-Session-2.pdf

[3]Sheep Breeders Round Table 2019:

https://www.nationalsheep.org.uk/workspace/pdfs/sat2-selecting-ewes-for-resilient-lamb-production-kate-phillips-harper-adams-university.pdf

[4]Poster used at the National Sheep Event at Malvern and at other meetings

Harper Adams University

Breeding for worm resistance in Lleyn Sheep

Performance Recorded Lleyn Breeders, Harper Adams University, Signet Breeding Services and Glasgow University.

PERFORMANCE RECORDED LLEYN BREEDERS

Introduction:

Anthelmintic resistance is a worldwide problem for the sheep industry and resistance has been reported to four of the five groups of wormer available in the UK.

Sheep farmers need to consider management options that reduce reliance on wormers, through following best practice advice from SCOPS and through improved grazing management and genetics.

PRLB has been investigating breeding sheep that are resistant to worms, and have through Signet Breeding Services developed estimated breeding values (EBVs) for faecal egg count and saliva IgA. Saliva immunoglobulin A is produced in response to infection by Teladorsagia circumcincta – a strongyle worm that is pathogenic in sheep.

Method:

Samples of faeces and saliva are taken from lambs that have a mob faecal egg count of over 500 epg (animals that have been challenged by worms and mount an immune response).

About 15 Lleyn breeders are involved in the project and Harper Adams is doing more detailed studies, looking at the repeatability of FEC and saliva tests and in planned breeding of low and high FEC and saliva IgA sheep.

Performance of the lambs and their FEC and IgA results will be used to improve our understanding of the mechanisms involved and improve the accuracy of the EBVs generated.

Results:

The repeatability studies have shown some consistency between results form one day to the next but the work will be repeated this autumn. Blood sampling will also be carried out to compare plasma IgA to saliva IgA and how these change over time. A positive relationship was found between saliva IgA and growth rate in 200 ewe lambs but this will be repeated with a further group of lambs this year.

Conclusions:

The selection of breeding stock for resistance to internal parasites is a worthwhile goal for the sheep industry, and will reduce reliance on chemical methods of parasite control and improve long term sustainability of the sector.

Funding for this research is provided by the European Innovation Partnership with support from Signet Breeding Services

Web address www.prlb.co.uk

Table 3.2: Factors affecting the unstimulated salivary flow rate in healthy subjects

Major factors*	Minor factors
Degree of hydration	Gender
Body position	Age (above 15 years)
Exposure to light	Body weight
Previous stimulation	Gland size
Circadian rhythms	Psychic effects - thought/sight of food
Circannual rhythms	Functional stimulation?

*Note: Most factors listed in the first column should be standardised during saliva collection