Ewes bred for resistance to gastrointestinal nematodes have lower parasite egg output during the peri-parturient period

Report by Eric Morgan, University of Bristol (Eric.morgan@bristol.ac.uk) for Peter Baber and Tim White, 25th October 2014.

Summary

Ewes with a range of estimated breeding values (EBV) for gastrointestinal nematode faecal egg count (FEC) were monitored during the peri-parturient period (PPP) by serial FEC for 8-11 weeks after lambing, on two farms. On the first farm (n = 47), ewes with lower EBV tended to produce fewer nematode eggs in the weeks following lambing, but differences in FEC between positive and negative EBV ewes, and correlation between EBV and FEC, were not significant. However, ewes in the bottom quartile for EBV produced significantly fewer eggs than those in the top quartile overall (38 % reduction), and at 8 weeks after lambing (69 % reduction). On the second farm, 53 twin-bearing ewes of similar age were compared. Ewes with low (negative) EBV produced significantly fewer eggs during the PPP than those with high (positive) EBV, with a 47 % reduction in peak counts and a 30 % reduction in average FEC over the PPP (50 % reduction in bottom compared with top quartile overall and at peak egg output). EBV in individual ewes was correlated with faecal egg output during the PPP ($r^2 = 0.056$). Neither EBV nor FEC were correlated with milk yield, as measured by lamb growth in the first eight weeks of age, with ewe weight loss over the same period, or with peak coccidial oocyst count. Results suggest that EBV predicts egg output during the PPP, especially within a comparable group of ewes. Selective breeding of ewes for low FEC has the potential to bring significant epidemiological benefits by reducing pasture contamination with nematode eggs, and alleviating the need for anthelmintic treatment of ewes and growing lambs.

Background

Ewes are known to increase shedding of nematode eggs in faeces around the time of lambing. This peri-parturient rise (PPR) in faecal egg counts (FEC) provides an important source of eggs to contaminate pastures and increase subsequent infection pressure for lambs. Selective breeding of ewes for attenuated PPR could reduce the need to treat ewes and lambs, and slow the development of anthelmintic resistance.

Breeding for nematode resistance has so far focused largely on the male line, through selection of rams with low (negative) estimated breeding values (EBV) for FEC. The value of EBV assigned to ewe lambs is of unknown value in terms of reducing future PPR. In addition, it is unclear whether enhanced immunity to nematodes following lambing could carry costs in terms of milk output or ewe weight loss, since the energy and protein costs of immunity are likely to trade off against those of growth and reproduction, or in their ability to resist other parasites and pathogens.
Improved understanding of the relationship between EBV for nematode FEC and the PPR could help to define targets for selective breeding, and quantify the expected epidemiological benefits of selective breeding strategies through reduced early season pasture contamination.

**Methods**

The study was conducted on two separate farms (Farm 1 and Farm 2) in the spring of 2014, both stocked with ewes of the Exlana sheep breed. Ewes were selected for inclusion across a wide range of EBVs for FEC, with similar numbers of high (positive) and low (negative) scores; all gave birth over a 10 day period, and were managed together for the duration of the study. In addition, on Farm 2, monitored ewes were all of similar age (two or three years), and gave birth to twins (with the exception of one triplet-bearing ewe). This restriction was intended to reduce variation in FEC due to, *inter alia*, unequal resource allocation trade-offs among ewes feeding different numbers of lambs, which could modify the expression of immunity and hence FEC during the PPR. Equivalently restricted selection of subjects was not possible on Farm 1 due to limited numbers of matching ewes.

Around the time of lambing, and on three further occasions in the next eight (Farm 1) and 11 (Farm 2) weeks, individual faecal samples were taken *per rectum* (although not all ewes provided a sample on each occasion). On Farm 2, ewes were weighed prior to and eight weeks after lambing, and lambs were weighed at around eight weeks of age as a measure of dam milk production. Samples were sent to the laboratory by post and stored at 8 °C, and FEC were conducted within five days using a modified McMaster method with a dilution factor of 10 eggs per gram (epg). Trichostrongyloid nematode eggs and coccidial oocysts were counted separately and transformed to eggs per gram (epg) and oocysts per gram (opg), respectively, for data presentation.

The relationship between EBV and observed FEC in individual ewes was assessed by Spearman rank correlation, using FEC at the peak of the PPR and average FEC per ewe over all sampling occasions. Differences between average FEC at each sampling point, and averaged over the duration of the study, were compared between high (positive) and low (negative) EBV groups using bootstrapping. Thus, individual FEC in each group were resampled with replacement, and sample size of the most numerous group truncated to match that of the smaller group. Individual counts in the low-EBV group were subtracted from those in the high-EBV group, and the average difference calculated. Monte Carlo simulation was used to generate 10,000 replicates, and a significant difference in average FEC was considered to be present where fewer than 5 % of replicates were equal to or below zero. This method was chosen to avoid bias introduced by uneven sample sizes in typically over-dispersed FEC data, and was repeated to compare FEC in the top and bottom quartiles of ewes for EBV. Spearman correlation was used to assess associations between EBV, FEC, and indicators of performance, i.e. mean lamb weight per dam at two months of age, and ewe weight loss between late pregnancy and the end of the study. This was done to check whether enhanced immunity against nematodes, manifest as reduced FEC, came at the cost of reduced resources for milk production or for maintenance of body weight. Finally, the potential for decreased immunity to micro-parasites in association with higher immunity to nematode infections in low EBV ewes was assessed by Spearman rank correlation of coccidial oocyst count and nematode egg count at peak PPR.
Results

The average of all available FEC within each group showed that faecal egg output was lower from ewes with low (negative) EBV for FEC, than from high (positive or zero) EBV ewes (Fig. 1). Based on this global average of individual FEC, 27 % fewer eggs were shed by low than high EBV ewes on Farm 1, and 34 % fewer on Farm 2. However, bootstrap resampling of average FEC over the whole study period did not show a significant difference between groups (Farm 1, $p = 0.13$; Farm 2, $p = 0.14$).

Figure 1. Mean nematode faecal egg count (FEC) over four sampling occasions from 0 to 11 weeks after lambing in 47 (Farm 1) and 53 (Farm 2) Exlana ewes, split into high (positive or zero) or low (negative) estimated breeding value for FEC. Error bars represent standard error. The range of observed individual counts was 0 to 3340 epg on Farm 1, and 0 to 1450 epg on Farm 2.
On both farms, average FEC increased after lambing, peaking at subsequent sampling occasions 5 - 6 weeks after lambing, before declining (Fig. 2). On Farm 1, peak FEC was between 19 and 22 % lower on average in the low EBV than the high EBV group, but the difference was not significant at any point (p > 0.10). On Farm 2, FEC was significantly lower in the low EBV group in week 5 (p = 0.041), and tended to be lower also in that group in week 7, though not significantly (p = 0.067). Peak FEC was, on average, 47 % lower in the low compared with the high EBV group.

Figure 2. Mean faecal egg count (FEC) in eggs per gram (epg) in Exlana ewes with high (positive or zero) and low (negative) estimated breeding value for nematode FEC, around the time of lambing and on three subsequent sampling occasions. Asterisk (*) represents a significant difference using bootstrap resampling (p = 0.041).

Average FEC over the sampling period for each individual ewe was positively correlated with its EBV on Farm 2 (rho = 0.316, n = 65, p = 0.022). However, the proportion of total variation in FEC explained by EBV was low (r^2 = 0.056). On Farm 1, the correlation between EBV and average FEC was positive but not significant (rho = 0.225, n = 47, p = 0.128).
When the extremes of the EBV range were considered, the lowest quartile of ewes for EBV generated significantly lower average FEC over the sampling period, on both farms (Fig. 3), and also specifically at 8 weeks on Farm 1, and at 7 weeks on Farm 2.

![Figure 3](image)

**Figure 3.** Mean faecal egg count (FEC) in eggs per gram (epg) in two groups of Exlana ewes, divided among quartiles (q1 to q4) of estimated breeding value for nematode FEC, around the time of lambing and on three subsequent sampling occasions. Asterisks represent significant differences in mean FEC between q1 and q4, using bootstrap resampling (*p<0.05, **p<0.01, ***p<0.001).

Percent reductions in faecal egg output during the PPR in the bottom compared with the top quartile of ewes based on EBV are shown in Table 1.

**Table 1.** Percent reductions in average faecal egg count (FEC) in ewes ranked in the bottom quartile for estimated breeding value (EBV) for FEC, compared with ewes in the top quartile. Bold type indicates sampling points at which the difference was found to be significant by bootstrap re-sampling, either between quartiles or between ewes with positive and negative EBV.

<table>
<thead>
<tr>
<th>Week</th>
<th>0</th>
<th>5</th>
<th>6/7</th>
<th>8/11</th>
<th>Average</th>
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<tr>
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<td>-72</td>
<td>31</td>
<td>26</td>
<td>69</td>
<td>38</td>
</tr>
<tr>
<td>Farm 2</td>
<td>37</td>
<td>48</td>
<td>52</td>
<td>11</td>
<td>50</td>
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On Farm 2, for which data were available, average FEC was not related to ewe weight loss or to lamb weight at two months of age \((p > 0.16)\). EBV was not correlated with ewe weight loss \((p = 0.65)\), but tended to be higher in ewes yielding lighter lambs \((\rho = -0.259, p = 0.064)\). Coccidial oocyst count peaked on this farm also in week 5 at 521 per gram of faeces \((\text{range 0 to 2640})\), and tended to be higher in ewes also shedding high numbers of nematode eggs \((\text{Spearman } \rho = 0.31, p = 0.058)\). On farm 1, peak coccidial oocyst counts were lower \((215 \text{ per gram, range 0 to 1200})\), and there was no correlation between them and nematode egg counts.

**Discussion**

This study shows that low EBV in ewes is associated with reduced egg output during the peri-parturient period \((\text{PPR})\). Peak egg output was reduced by as much as 50\% in low compared to high EBV ewes, and overall egg output during the PPR by 30-50\%. Moreover, individual EBV was significantly and positively correlated with average faecal egg output during the PPR \((\text{on Farm 2})\), and was therefore a significant predictor of this trait. The reduction in FEC in low-EBV ewes was not associated with any sacrifice in production in terms of lamb growth or ewe weight loss; in fact, ewes with low EBV tended to produce lambs that were heavier at two months of age, though this association was not significant. Ewes shedding fewer nematode eggs did not shed more coccidial oocysts on either farm.

Similar qualitative patterns were observed on Farm 1, though differences in FEC between high and low EBV ewes at times of peak egg output were more elusive. This could be due to the lower sample size \((47 \text{ versus 53})\), and especially the lesser inclusion criteria, with ewes not matched by age or number of lambs born, due to limited availability of matched ewes. Higher variability between ewes in factors affecting FEC during the PPR, such as individual fitness, nutrition or infection rate, or the resource demands of variable numbers of lambs, is likely to decrease the power of statistical comparisons between groups, and confound results. It is recommended that future studies as far as possible match ewes on potentially important criteria such as number of lambs, in order to increase study power. Effects of genetic resistance to nematode infection act through enhanced acquired natural immunity, and are likely to be most evident in relation to less resistant animals when infection pressure and nutritional stress are both high, and therefore magnified in ewes feeding higher numbers of lambs. Therefore twin-bearing ewes are probably the most suitable for studies on the effect of genotype on the PPR. On both farms, peak FEC was observed 5-6 weeks after lambing, and this would seem to be an ideal time for sampling the PPR in ewes.

Interestingly, however, differences in FEC between the top and bottom quartile ewes for EBV were most marked after the peak PPR, at 7 and 8 weeks after lambing. Unsurprisingly, comparisons between top and bottom EBV quartiles were more sensitive in detecting differences in FEC than comparisons between above and below average EBV ewes.

Extrapolating from differences in average FEC between groups on Farm 2, and making basic assumptions on faecal output rates in sheep \((2 \text{ kg per day})\), ewes with negative EBV for FEC are each predicted to shed on average 155 million fewer nematode eggs onto pasture than those with zero or positive EBV.
Conclusions

Results suggest that benefits from selective breeding for low EBV can be realised in the maternal line through reduced egg output in the peri-parturient period (PPP), with positive epidemiological consequences on infection pressure in lambs, and without negative consequences for other aspects of performance. Measuring FEC around 5 weeks after lambing appears to be an effective way of comparing egg output during the PPR in ewes.