Brief summary of research and results

Introduction and aims of the research

Additional data collected as part of the footrot project LK0668 'Breeding for resistance to footrot: Combining molecular and phenotypic approaches' and their detailed analysis are the subject of this bolt-on study. These data concern 'non footrot' hoof lesions and were initially analysed as part of a student thesis BSc Honours in Animal Science supervised by J. Conington entitled '*The prevalence of foot disorders in Texel and Scottish Blackface Sheep Within the UK'* by Clare Maxwell, 2008. This work showed that white line degeneration (shelly hoof) was a major problem for some flocks, with some farms having more than 50% (up to 76% for one flock) of their ewes with at least one hoof affected. Also, farmers often confuse footrot with shelly hoof, so correct diagnosis is important. As other SAC research that has been undertaken in Welsh Mountain sheep (funded by Genesis Faraday and Hybu Cig Cymru – Meat Promotion Wales) also showed a very high prevalence of shelly hoof, this study aimed to establish if a genetic basis for 'shelly hoof' exists. As there is some evidence in other species (e.g. horses and more recently in dairy cattle) that such hoof disorders occur mainly due to nutritional influences, this study also used electron microscopy to determine if this is also evident for shelly hoof in sheep.

Genetic studies

Methods

Currently, there are no reports in sheep of any genetic variation for shelly hoof and as such, the degree to which it is under genetic control is unknown. As we already collected data on shelly hoof from a large population of pedigreed animals as part of the previous LINK-funded Footrot project (LK0668), we used four data sets generated from this project and analysed them separately. These were Texel ewes (2,877), Texel lambs (1,816), Blackface ewes (3,277), and Blackface lambs (1199) in 17 and 5 flocks of Texel and Blackface respectively**.** All data sets included key environmental parameters (farm, ewe age, litter size etc.) as well as pedigree information. Two traits were generated from the data collected. These were firstly whether or not shelly hoof (SH) was present in any hoof ('yes' or 'no', coded '0' or '1'), and secondly, the number of feet affected with shelly hoof ('Nofeet'), coded 0-4. Multivariate linear REML analysis was used for Nofeet analyses using standard procedures for the analysis of this trait, and as the heritability results for the log transformed data were almost identical to the untransformed data, the latter was used for all Nofeet analyses in the four data sets. For SH, the data were transformed to underlying thresholds assuming a mean of zero, standard deviation of 1, and with the variance of π^2 /3. This methodology makes the assumption that the trait is controlled by many different genes that, on the underlying scale, are normally distributed and that it manifests in a phenotype which either does or does not display clinical signs. The expected response to selection on such a binary trait depends on the heritability of resistance and the prevalence. This can be calculated assuming a threshold ('all or none') model with an underlying normally distributed liability with heritability h_L^2, which depends on the heritability of the binary trait (h_{01}^2) as: $h_L^2 = p(1 - p)z^{-2} h_{01}^2$, where p is the prevalence of the binary trait and z is the ordinate of the standardised normal distribution corresponding to p. (This is relatively standard methodology for the genetic analysis of threshold traits, described by Falconer 1989 Chapter 18 and also by Robertson A., Lerner I.M. 1949, The heritability of all-or-none traits: viability of poultry. Genetics 34: 395-411).

Results

The prevalence of shelly hoof in the Texel breed was 24 % (ewes) and 7% (lambs). That for the Blackface was 47% (ewes) and 12% (lambs). The prevalence in the Texel breed ranged from 0% to 35%, with 7 flocks having 15% or more sheep affected. For the Blackface, shelly hoof was considerably higher overall, with the minimum number of sheep per flock affected being 20% and the highest with 76%. Interestingly, apart from the difference in prevalence of shelly hoof between lambs and mature ewes, unlike footrot, prevalence levels did not increase with increasing ewe age.

The heritability estimates shown in the table below range from 0.09 to 0.33. They are generally lower for the number of feet affected (Nofeet) and in Texel lambs the estimate is not statistically different from zero. Whether or not they get shelly hoof at all, is under moderate genetic control, and similar to some performance traits such as growth rate. Unlike for footrot, (where heritability levels were higher for flocks with higher prevalence levels), the results show that the heritability of shelly hoof is unaffected by prevalence levels.

Table 1: Heritability estimates for shelly hoof (s.e.)

Data set	**Nofeet**	**SH (0/1)**
Texel ewes	**0.10 (0.04)**	**0.29 (0.08)**
Texel lambs	**0.09 (0.08)**	**0.33 (0.07)**
Blackface ewes	**0.29 (0.05)**	**0.32 (0.06)**
Blackface lambs	**0.11 (0.04)**	**0.15 (0.07)**

Conclusions

Shelly hoof has high prevalence levels in Texel and Blackface sheep and the expression of clinical signs of shelly hoof is under moderate genetic control. Screening hooves for shelly hoof and selection using estimated breeding values (EBVs) for this trait would lead to a reduction in shelly hoof prevalence.

It is possible that this condition weakens the hoof and predisposes it to footrot, although the link between shelly hoof and footrot leading to lameness is still not understood. Separate analyses of shelly hoof and footrot for the derivation of EBVs are recommended unless further studies establish a strong genetic link between the two hoof conditions. As breeding for resistance to shelly hoof over time will lead to a permanent and cumulative improvement decline in this condition, it is a sustainable solution to the improvement of sheep welfare and productive efficiency.

Transmission Election Microscopy (TEM) studies (see Appendix for full details)

Methods

Samples of horn were taken in February 2009 from the hooves of 10 mixed-age, purebred Blackface ewes from the flock with the highest prevalence (76%) of shelly hoof reported by Maxwell, (2008). Three of the sheep had no clinical signs of shelly hoof, two had samples taken from hooves with and without shelly hoof, and 5 had samples taken from only from hooves with clinical signs. None of the sheep had active classical footrot or signs of previous footrot infection. The hoof samples were prepared for TEM by the University of Edinburgh TEM laboratory according to the protocol detailed in the Appendix. Ultra thin sections, (60nm thick) were cut from selected areas, stained in Uranyl Acetate and Lead Citrate then viewed in a Phillips CM120 Transmission Electron Microscope (FEI UK Ltd, Cambridge, England). Images were taken on a Gatan Orius CCD camera (Gatan UK, Oxon, England) for the samples showing clinical signs of horn degradation.

Results

The images of the cellular structure for the sheep with no clinical signs of shelly hoof on either sample are distinguishable clearly from those with shelly hoof. Figure 1 shows good horn structure with good keratinisation within the cells and smooth definition to the outer hoof wall. This uninterrupted almost polished appearance of the surface provides little opportunity for the entry of bacteria and other organisms responsible for the degradation of the hoof. The structure has cell membranes in a 'zip' formation that are packed closely together, again providing little opportunity for invasion of unwanted organisms (Fig 2). There are noteable signs of more melanin granules in some of the non-shelly hoof samples, (Fig 3). *Broken Dorsal wall:* The features of the horn that are characteristic of shelly hoof-affected sheep can be seen in Figures 4 to 8. The most striking feature of affected hooves was the jagged, broken edges of the dorsal wall horn (Figure 4) creating microscopic crevices (micro-fissures) leading deeper into the corum of the hoof (Figure 5). This was accompanied in most samples with a plethora of different bacteria and other organisms with a clear pathway to penetrate deeper into the hoof (Figure 6). *Poor cell membrane integrity:* The second characteristic feature of affected hooves was the separation of the cell membranes, or 'un-zipping'. This phenomenon is shown in Figure 7. The separation and breakdown of the cell membranes in this way, facilitates a weakness in the hoof that is vulnerable to the entry of unwanted bacteria, is more permeable to moisture and hence to water-soluble solutions that contribute to hoof breakdown. Where the unzipping has occurred, appears as between the cells in the TEM images, and in some instances the gaps are filled with bacteria and other organisms which further contribute to the degradation of the cell membranes and failure of their adhesion to one another. It is understood that calcium deficiency is responsible in part, to the breakdown of cell membranes in this way, although deficiencies in other minerals such as biotin (which is produced in the rumen) zinc, and proteins are also important. The specific proteins are those comprising sulphur amino acids (for keratinisation), the lack of which are responsible for poor horn structure and inadequate adhesion of the keratin protein structure to the wall of the membrane. This is manifested in a 'lace chain' appearance following the cell wall definition, which is shown clearly in Figure 6. Deficiencies in calcium are also associated with a lack of lipids to hold the horn cell membrane structures together. Lipids show up as forming darker colouration of the cell membrane on the TEM images. In some instances, distinct, well-formed cell membrane was presented but very poor keratinisation in the cells was evident that was particularly weak closer to the areas of cell membrane attachment, as shown on Figure 8 by paler colouration on the image. *Lack of keratinisation:* Obvious lack of complete keratinisation inside the horn cells is shown in Figure 8, with distinctive lack of structure to the cells. Failure of the horn to keratinise fully, leads to poor and weak horn formation that is prone to collapse and penetration by unwanted organisms. Together with poor cell membrane integrity leading to the 'unravelling', or 'unzipping' of the cells, the weight-bearing capacity of the animal is affected by having reduced flexibility and hence increases the chances of the animal becoming lame.

Conclusions

The possible involvement of nutrition in poor horn development leading to clinical signs of shelly hoof has been shown in this preliminary study. Interestingly, in the samples studied, there was no evidence of the degenerative effects of over-nutrition, which has previously been implicated in equine horn problems (Kempson, pers comm). However, this may be the case for some fast-growing terminal sire sheep with high levels of supplementary feeding, although this was not seen in the hill sheep samples studied. The evidence for poor horn development was seen in all samples from affected ewes. Some evidence of poor horn development was also detected in samples with no clinical signs of shelly hoof from sheep which had other hooves that were affected. It is likely that it is the *extent* of the degeneration of the horn that leads to clinical signs of shelly hoof. The three distinctive physical features of shelly hoof seen using TEM technology include a) irregular edges of the dorsal horn with micro-fissures that penetrate deeper into the laminar corum, b) separation ('un-zipping') and disintegration of cell membranes creating gaps between the cells, and c) poor keratinisation of the cells and their weak attachment to the cell membranes. These defects undoubtedly contribute to the degradation and 'flaky' appearance of the hoof that is characteristic of shelly hoof in sheep.

Future R&D resulting from this project *(Include any other non-tangible benefits and state any TCS actions)*

It is clear that the use of TEM in this pilot study has highlighted the physical properties of horn breakdown within a population of animals that is affected by shelly hoof. The hypotheses that previous nutritional status affects horn structure leading to shelly hoof, has only been suggested through current knowledge of the condition in other animal species, and was not proven scientifically. The scope for detailed nutritional and genetic interaction studies in this area is therefore considerable, particularly as it is possible that resistance to shelly hoof in harsh hill environments is an adaptation to low levels of nutrition, inferring greater efficiency of utilisation of nutrients.

As well as TEM technology, alongside this study we looked at the potential for Computer Tomography (CT) to be used in assessing 'whole hoof' integrity. This helped with anatomical positioning for the TEM work, and assisted our understanding considerably of hoof biology for this study. The initial result from the use of CT for assessing hooves is very encouraging. As the benefits of using CT means that animals do not need to be slaughtered to gain detailed anatomical information, its potential for use by sheep breeders for hoof quality assessments is promising, particularly as a proportion of elite sheep breeders are already routinely using CT for *in vivo* assessments of meat quality. This means that potentially, the information that is routinely generated for sheep performance and efficiency can be extended to characteristics affecting sheep welfare.

Specifically, it would be important to determine if lesions that cause discomfort and produce changes in locomotory behaviour can be detected and quantified both by visual observation and CT. This will allow us to assess if there are recognised physical characteristics of hooves that differ in their resistance to hoof disorders and use this information to select breeding stock only from sheep with sound hoof structure. This would need to be assessed in relation to the benefits over and above those that can be done by eye – i.e. through the use of phenotyping for shelly hoof, as has been undertaken as part of the scoring of shelly hoof for the genetic studies in this project.

Previous studies on bone properties using CT at SAC have shown that key physiological events (e.g. pregnancy and lactation) significantly affect bone quality (and also likely to affect tooth loss in ewes). This is a major barrier to extending the lifespan of breeding ewes. Knowledge of the extent to which changes in suspensory weight-bearing system and/or horn production at dermal/epidermal junctions are associated with changes in physiological state would facilitate future strategies for lameness control.

The results from the genetic studies show that whether or not sheep have shelly hoof is, in part, determined genetically. Further work needs to be undertaken to examine the extent of pleiotropy i.e the extent to which to which susceptibility to footrot is linked to that for shelly hoof. Such genetic studies to estimate the genetic correlations between the two hoof conditions needs to be undertaken, to determine the best approach to breed for resistance to both of these lameness causes. Similarly, the genetic associations among shelly hoof and other key performance traits such as growth rate and carcass quality also needs to be known if shelly hoof is to be incorporated into breeding programmes to improve lameness.

As well, the use of genome-wide association studies (GWAS) for both shelly hoof and footrot, potentially, would ultimately alleviate practitioners of the hoof-scoring burden. With the recent availability of the ovine SNP ship, the data generated during LK0668 together with the DNA collected on the same animals, we are in a unique position in the UK (and Europe) to undertake the first validations of this technology for 2 UK breeds to improve lameness levels in sheep and improve welfare through such breeding methods.

Industrial relevance and plans for future commercial exploitation

The knowledge of the genetic basis to poor horn structure leading to the expression of shelly hoof probably indicates that there are structural properties of the hoof that predispose them to the condition, which in the long term can be solved through genetic selection. This is relevant to sheep breeders who can use this information in their breeding decisions to select against sheep with this condition. Due to the hierarchical stratification of the UK sheep industry, such breeding decisions made by breeders will filter through to the commercial sheep sector leading to improved hoof health and ultimately, reduced lameness. The use of EBVs for shelly hoof could be integrated into sheep breeding programmes in its own right. However, it may be preferable to combine it with resistance to footrot, although further research on the genetic associations between footrot and shelly hoof is required before a more general EBV for lameness can be recommended.

The evidence reported here for suggested nutritional basis for shelly hoof could lead to the commercial development of nutritional supplements for improved hoof horn quality. The long-term health, welfare and productivity benefits of such supplementation would first need to be quantified.