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Original Article

A survey of prevalence, and investigation of predictors and staining patterns of the split upper eyelid defect in Hebridean sheep

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Abstract

Abstract: The split eyelid condition (SUED) is a congenital defect of the upper eyelid thought to be exclusive to multi-horned sheep. Eleven flocks with a high proportion of multi-horned Hebridean sheep were visited in 2011. Statistical analysis was performed generating Pearson's chi-squared analysis as well as (a) logistic regression (b) ordinal logistic regression and (c) linear regression models.

Four hundred and seventy-three purebred Hebridean sheep and one crossbred lamb were examined. Of all the multi-horned animals inspected in 2011, 9.7% of adults had evidence of SUED in one or more eyelids, with 17.6% of lambs presenting with one or more eyelid affected.

Having five or more horns was protective in the linear regression model on eye-level data ($p=0.045$). Forward-facing horns were consistently associated with a 'worst' eye score in the eye-level data, with an odds ratio as high as 9.4 when compared to a base of backward-facing horns ($p=0.002$).

Eyes positive for SUED were significantly more likely to be Rose Bengal stain positive in all four analysis including Multi-level mixed effect ordered logistic regression ($p<0.001$, OR 149.3).

A novel lesion was identified during the course of the study with 3.4% of lambs presenting with dermoid. SUED was also identified in a cross bred animal. Further work is needed to quantify the exact cost to animals with unilateral or bilateral SUED with subtle and production cost of SUED.

Keywords: Hebridean; Rose Bengal; SUED; sheep; polyceraty

Introduction

Rare breeds of British livestock are of inherent importance to the diversity of the UK livestock industry, with applications in conservation grazing, stewardship schemes and maintaining the genetic diversity of the livestock population. The importance of maintaining a diverse livestock population has previously been emphasised, enabling optimal utilisation of the pastoral system, maintaining access to a broad gene pool for further breed advancement in productivity or health characteristics, for advancement of human and animal health and for ensuring variation in the advent of novel disease emergence (Hall and Henderson, 1999, Notter, 1999, Groeneveld and others, 2010, Torres and others, 2010, Brown and others, 2014).

The Hebridean sheep is a hill sheep belonging to the Northern short-tailed group originating from north-western Europe. It is a primitive breed with a distinctive solid black fleece, and both ewes and rams are horned. The most populous horn number configuration is two-horned animals, however polycerate bloodlines exist. Polled animals are considered to be genetically four-horned and are not observed in two-horned-only matings. Hebrideans are used in conservation grazing projects, as hill ewes for crossbreeding and also often on smallholdings. Members of the HSS submit census and registration details to the society annually. In 2011, 274 multi-horned breeding ewes were recording in the register of the Hebridean Sheep Society (HSS) i.e. 7.4% of the breeding ewe population. In 2011, 153 multi-horned lambs were deemed eligible for pedigree registration, equating to 8.2% of the annual lamb crop. Multi-horned Hebrideans were reported from 47 flocks (29.1% of the total notifying flocks in 2011). They are currently listed with the Rare Breed Survival Trust (RBST) as a 'native breed'.

The split upper eyelid defect (SUED) is a congenital defect believed to be found exclusively in multi-horned varieties of sheep. SUED was first recorded in 1969 in Jacob sheep (Littlejohn, 1969), but is also present in polycerate lines of the Hebridean, Manx Loaghtan, Icelandic, Damara and Churro Navajo sheep with an isolated case reported in multi-horned goats in Spain (Herrera and others, 2007).

Previous work examining multi-horned breeds in the UK (including the Hebridean) suggested a SUED prevalence at 18.6%, although the authors did not specify the age profile for animals included in the survey (Henson, 1981). Only two Hebridean flocks were included in this work.

The Hebridean Sheep Society (HSS) define the grades of different severities of SUED (Table 1). The eyelid defect is a congenital defect which can be accurately scored from birth (Pemberton, 1981) and is routinely scored at shows and sales. Breeders are strongly advised not to breed from Category 3 or 4 males, or Category 4 females (Hebridean Handbook, 2015). Work in an extensively managed Manx Loaghtan flock on the Isle of Mann suggested that the SUED is not absolutely deleterious to the breed or productive life, with a high prevalence of SUED and survival to sexual maturity (Henson, 1981). No further work has been performed examining the clinical consequences of the condition, the effects on performance or the possible welfare implications of this congenital defect.

A more recent paper survey was distributed amongst HSS members who were asked to score their own flocks (Small, 2009). Two hundred and twenty-eight lambs and 142 Hebridean adults were recorded from flocks, although the number of flocks was not specified. This survey found 14.7% of the 2008 lamb crop to have unilateral or bilateral evidence of SUED.

The survey also examined whether other phenotypic characteristics were linked to increased risk of lambs having the most severe grades. The data suggested that there was no association with sex or horn number, but that animals with forward-facing horns were more likely to have the condition. No other phenotypic risk factors were considered. This paper survey was the most substantial review of SUEd prevalence in the Hebrideans since the 1980s but its major limitations were the number of independent recorders submitting data, the possibility for bias and the subjective nature of classifying eyelids.

The split upper eyelid defect has not been recorded in two-horned animals, and although accepted that SUEd is linked to the polycerate characteristic, a definitive model of inheritance has not been determined and no direct genomics work looking at SUEd inheritance has been performed (Henson, 1981; Small 2009; Hadfield and others, 2014).

Given the size of the population, the importance of avoiding selective breeding protocols for or against a phenotype without greater understanding of the mechanism of inheritance of the trait and any co-inherited genes (Pemberton, 1981), the potential for reducing the genetic diversity of a small population, and the importance of avoiding selecting for additional mutations has been previously stressed. Despite the protestations of the original researchers and warnings to preserve the genetic pool until further evidence was available, there has been a notable reduction in the relative proportion of multi-horned Hebridean sheep within the population (Kinsman, 2001). Anecdotally, Hebridean sheep were a predominantly multi-horned population, yet ‘multi-horns’ accounted for just 7.4% of the 2011 breeding ewe population (HSS, 2011)

The eye-level physiological changes associated with SUEd have not previously been

examined, although increased lacrimation and tear staining have been noted by keepers. The proposed mechanism of eye damage is exposure of the sclera and resultant increased risk to infectious eye disease and relative inability to clear debris from the eye. In other species (human and domesticated animals), eye health is evaluated using a variety of diagnostic tools including conjunctival staining (Fluorescein and Rose Bengal respectively). Sensitivity and specificity of these diagnostic tests have been evaluated (Lemp and others, 2011), and they can be used as indicators to evaluate gross damage to the surface of the eye. The effects of SUED on these parameters, however, has not been evaluated.

The project aims to quantify the prevalence of SUED in the Hebridean multi-horned population in 2011 and to identify key phenotypic risk factors for expression of the condition.

Materials and Methods

Flock Selection

To the authors' knowledge, there has never before been a comprehensive survey of the population of individual breed with physical inspection of phenotypes by an individual recorder examining SUED. Flocks with a high prevalence of multi-horned sheep can be identified from breed society records and were invited to participate. Eleven flocks were visited prior to dispersal of stock at annual sales and culling decisions.

Animals were physically examined by one of the authors (EG), and phenotypic data was recorded for all animals (e.g. unique identifying number (UK number or HSS tag), age in years, number of horns, direction of horns (forward-facing, vertical, backward-facing or other), presence of fused horns or scurs, eyelid score for right and left eyes in accordance

with HSS guidelines, 'worst' overall eyelid score (i.e. the poorest grading eye), presence of the topknot (an excess of fleece on the poll of the animal), and any additional lesions noted. For the lamb population, where known, the 'worst' eyelid score of their dam and sire were additionally recorded.

Additional information was recorded for animals with unilateral or bilateral SUED, including stain results (using Rose Bengal staining (Rosets Rose Bengal Ophthalmic Strip, Chauvin Pharmaceuticals Ltd, England)). Animals with SUED were examined using Rose Bengal because it detects subtle damage to the surface of the cornea and specifically loss of mucin coverage (Feenstra and Tseng 1992; Figure 2). The contra-lateral eyes of animals with SUED as well as eyes of a convenience sample of some normal, additional animals were stained.

Due to previous concern over risk for corneal ulceration, fluroscein was initially used, however it became apparent that the level of corneal damage was relatively subtle in comparison to corneal ulceration, and, as a consequence, Rose Bengal was preferred.

Statistical analysis

Data analysis was performed using Stata 12 (StataCorp 2014, USA). Two datasets were generated: (a) animal data, where individual animals were given a unique identification code and (b) eye data, where eyes were considered individually. Analysis of data at the eye level was necessary because eye score varied by animal between right and left eyes, therefore analysis at this level permitted further assessment of staining results.

Prior to data analysis, data cleaning and correction was performed, referring back to original copies of data collection sheets, checking for and correcting any errors within the dataset and classifying data. Some data were grouped when low numbers of animals within separate categories existed (e.g. when categorising age data, animals five years old and greater were merged; when categorising horn data, animals with greater than five horns were merged).

Following categorisation of data sets and in order to explore the data, Pearson's Chi Square was used to compare relative proportions within categorical phenotypes and clinical datasets (e.g. sex versus eye staining, etc.), with $p < 0.05$ considered statistically significant.

Null multilevel ordered logistic regression models were generated to investigate the clustering of 'worst' score at the flock level for individual animals as well as at the animal and flock level for individual eyes. There was no evidence of flock-level clustering in either dataset, suggesting that the population was homogenous with regards to penetration of the condition between flocks, despite likely variation in foundation animals or breeding policies. As expected, there was evidence of animal-level clustering in the eye-level dataset, so all multilevel modelling of eye data therefore included an animal-level random effect.

The categorisation of SUED by HSS is by linear, ordered categories with distinctions between the classes (Table 1) necessitating the use of ordered logistic regression models. Conversely, when categorising males, HSS suggests a binary presence (Categories three or four) or absence (Categories one or two) of SUED. Given this categorisation, logistic regression modelling was considered to be appropriate, using 'worst' score for each animal. Finally, the examiner (EG) observed variations in severity within categories, suggesting a

continuous spectrum of disease expression and hence the applicability of linear regression.

Therefore, given the potential variations in categorisation, multiple models were built and were considered in order to identify associations within the data and identify which types of analysis were the most appropriate. Three types of univariable models were therefore built: (a) logistic, (b) ordered and (c) multilevel ordered. All factors of interest in these models (p -value approaching 0.1) were then included in multilevel ordinal logistic regression models.

Ethical review

The project was accepted by the University of Cambridge Veterinary School's Ethical Review committee (CR18 1/7/2011).

Results

Animals examined

A total of 473 purebred Hebridean sheep and one crossbred lamb were presented for examination to one of the authors (EG), so in total, 946 Hebridean eyes from across 11 flocks were examined. This sample included 17 two-horned Hebridean sheep.

Flock breeding policies

Flocks varied in breeding policy, with some selecting heavily against SUED in accordance with HSS recommendations, and others opting to contradict these recommendations and continue to breed from ewes with Category 3 and 4 lesions. Additionally, ram selection varied between flocks, with some preferring to breed from multi-horned rams and others preferring to rotate rams or utilise two-horned rams. All of the flocks involved in the survey were open flocks (e.g. buying in rams, ewes or youngstock).

Flock demographics

Flocks ranged from 8-106 Hebridean multi-horned animals (mean: 43, standard deviation (SD): 26). Flocks included in the survey were spread across the UK and included flocks from lowland to marginal hill land. Flock function varied from conservation grazing (low input, low lamb output systems) to breeding of pedigree and registered replacements, to “genetic conservation” farms founding their flocks on diverse bloodlines.

Descriptive variables

Multi-horned animals with a range of horn numbers were examined. Polled animals are considered to be genetically multi-horned, so were also examined. Two-horned animals were not routinely examined due to the apparent absence of SUED in two-horned animals in the literature. The median number of horns in multi-horned animals was four (range 0-8). The author examined 176 multi-horned Hebridean 2011-born lambs, and the median number of horns examined in lambs was four (range 0-6).

Hebridean animals ranged from spring lambs ($n=178$) in the year of birth to 16-year-old sheep. The average known age of sheep examined was 1.6 years ($SD=0.09$, $n=444$).

Prevalence of SUED in whole multi-horned population data and lamb-specific data is summarised in Table 2 and Figure 1. Lamb-specific data are from animals born in 2011 and examined before culling and selection decisions were made.

When considering lamb population, there was no statistically significant difference between the incidence of lamb ‘worst’ score in males and females although there was a trend for males to have more SUED ($n=176$, Pearson’s Chi Square, $p=0.07$; Table 3).

When changing the definition of SUED by merging Categories 1 and 2 together and Categories 3 and 4 together, 34.7% of the lamb crop had evidence of SUED (i.e. formerly Categories 3 or 4) in one or more eyes.

Modelling Results

Maternal score

Maternal score was recorded as the overall ‘worst’ score of the maternal parent (where known); 145 Hebridean lambs had recorded maternal scores. Maternal score was not a significant predictor of ‘worst’ eye score in Chi Square analysis, but was positively associated with ‘worst’ lamb score ($p=0.10$ ewe score 4 vs. ewe score 3, OR: 5.24) in the univariable animal-level linear regression model (see Table 4a).

Having a multi-horned dam vs. a two-horned dam was not predictive of lamb ‘worst’ score in either the lamb- or eye-level analyses (Table 4a and 4b respectively).

Paternal score and mating

There were 91 lambs with two multi-horned parents, 54 animals with one multi-horned parent and 31 lambs where one or more parents were unknown. Ram ‘worst’ score was not a predictor of lamb ‘worst’ score in any of the analyses in either dataset ($n=153$ lambs, $n=306$ eyes where sire known). This may be a reflection of lack of power due to the dataset size, but may have also resulted from low numbers of sires with poorer scores. Having a multi-horned sire or two multi-horned parents were not predictive of ‘worst’ score in either dataset.

Horn number and characteristics

Horn number, horn direction, presence of scurs and presence of fused horns were recorded in all animals examined. Horn number was recorded for all 176 multi-horned animals.

At the animal level, horn number was not significant in any of the analyses, but, when considering eye-level data with flock as a random effect, having five or more horns appeared to be protective relative to four horns ($p < 0.05$ in both multilevel ordered logistic and linear regression models).

The presence of fused horns was also recorded (i.e. where distinguishable, horns that had not completely separated during development, $n=25$). At the animal and eye levels, fused horns were not significant in either Chi Square, multilevel logistic, multilevel ordered logistic or linear regression models.

Scurs (loose horn material) were also recorded in seven lambs. Presence of scurs was not associated with 'worst' score at either lamb or eye level in any of the models.

Horn direction was recorded in 174 lambs; deviations from vertical were considered to be backward-facing, forward-facing or other if not conforming to a given pattern (i.e. polled lambs have no horn direction). Given the desire to assess direction of horn pattern in multi-horned animals, two-horned animals were excluded from analysis.

When examining animal-level data from the lamb population, analysis of horn direction (where baseline was vertical) identified an association between forward-facing horns and 'worst' score in all models. For example, the logistic regression models showed a statistically significant association irrespective of base horn group - animals with forward-facing horns

had an OR of 3.2 (compared to backward-facing horns; $p=0.007$) and an OR of 5.5 (compared to animals with vertical-facing horns; $p=0.001$).

This phenomenon was replicated in ordered logistic regression, where animals with forward-facing horns had an OR of 2.8 (compared to backward-facing horns; $p=0.02$). Polled horn patterns were not a significant predictor in any of the models.

When considering the lamb population and eye-level data, this pattern was repeated in logistic regression models, with animals having forward-facing horns showing an OR 4.2 times greater than those with vertical-facing horns ($p=0.01$), and 9.4 times greater than those with backwards-facing horns ($p=0.002$). This was also reflected in the multilevel ordered logistic and linear regression models (Table 4a and 4b).

Having a polled horn pattern was not significant in any models using animal- or eye-level data.

This continuous significance across four models in eye level data is highly suggestive of a real association between SUEd and horn direction. This phenomenon is supportive of previous evidence presented from the farmer submission survey (Small, 2009) which suggested a link between horn direction and presence of SUEd.

Topknots

Twenty-four animals were recorded as having topknots across the whole population ($n=24/478$, 5.1%) including six lambs recorded as having topknots ($n=6/178$, 3.4%).

There was no significant difference between male and female prevalence of topknots in the lamb population and they were not associated with SUED in any of the analyses.

Rose Bengal staining

Rose bengal staining results are illustrated in Figure 3. Chi Square analysis of eye-level data showed a significant positive association between increasing eye score and increased staining ($p < 0.001$). Logistic regression also identified a statistically significant association (OR=3.91 $p < 0.001$), suggesting that, as eye score increased, the probability of positive staining increased. This effect was consistently significant in multilevel ordered logistic (OR=149.3 $p < 0.001$) and linear regression ($p < 0.001$).

Dermoid

Dermoids were recorded in six lambs and six adults (2.6% prevalence in multi-horned population; Figure 4). No animals had evidence of bilateral dermoids. Dermoids were attached to the nictitating membrane and along the upper eyelid, and were significantly associated with SUED in Chi Square analysis ($p < 0.001$), multilevel ordered logistic regression (OR 115.0, $p = 0.001$) and linear regression ($p < 0.001$).

SUED in a cross-bred animal

A cross-bred lamb was presented for examination in one of the flocks that used terminal sires across a proportion of their Hebridean ewes to produce commercial carcasses. The animal had been identified by the shepherd on routine inspection of the lamb crop, and had a Beltex (Belgian x Texel) sire and ½ Hebridean dam (i.e. the lamb was ¼ Hebridean). This black lamb, whose sex was not recorded, was polled and found to have unilateral SUED with a Category 4 split in its right eyelid. SUED has not previously been reported in a cross-bred

animal. Neither sire nor dam were available for examination.

SUED in a 2-horned animal

Henson (1981) previously reported that SUED was exclusive to multi-horned animals. Two-horned animals were not routinely examined as part of the survey. One two-horned ewe lamb was presented with a Category 3 eyelid score in her right eye and a Category 1 score in her left. Her sire was a four-horned ram with a ‘worst’ eyelid score of 1, and her dam had a ‘worst’ eyelid score of 3. The lamb was positive on Rose Bengal staining on the right eye and negative on the left. There was no evidence of the defect in any other two-horned animals examined ($n=17$).

Discussion

This is the largest survey to date on the UK-wide Hebridean population by a single examiner. Key findings include generation of prevalence data of SUED in multi-horned Hebridean sheep, the identification of associations of physiological characteristics (specifically horn direction) with SUED, the use of Rose Bengal staining to characterise the degree of damage to the surface of the cornea, the first description of dermoids in Hebridean sheep, the first description of SUED in a two-horned and a crossbred animal and the first estimate of topknot prevalence in the Hebridean population.

Of all the animals inspected in 2011, 9.7% of adults had evidence of SUED in one or more eyelids, with 17.6% of lambs presenting with one or more eyelid affected. This difference is likely to be a reflection of culling practices i.e. not retaining animals with SUED in the breeding flock. In another published study by Small (2009), 14.7% of the 2008 lamb crop were identified as having SUED in one or more eyelids. This may be reflective of either

underestimation by farmers submitting their data in 2009, annual variation or an increasing incidence between annual lamb crops. When considering the lamb population, 34.7% of lambs would be ineligible for registration based on SUED category alone if Category 3 and 4 eyelids were considered positive (i.e. as currently defined by HSS for ram lambs. This places substantial selection pressure on this single trait with potential consequences for the genetic diversity of the breed and highlights the challenge for those wishing to breed and register pedigree animals.

Initial modelling suggested that eye score was not clustered within flocks and therefore that the population was relatively homogenous with regards to eye score. This phenomenon is interesting as bloodlines, breeding and culling practices vary substantially between flocks, suggesting that these have minimal contribution to flock incidence of SUED in lambs.

Clustering demonstrated within animals when considering eye-level data was anticipated based on biology, however, there were extremes within animals noted in this dataset (e.g. animals with Category 1 scores in one eye and Category 4 in the contra-lateral eye).

The breed society's current policy on eligibility for pedigree registration is based on work suggesting a heritable component to expression of SUED (Henson, 1981). The sample size in this original work was small with minimal opportunity for statistical analysis. In the present work, multiple models were utilised to investigate the associations of SUED with several other variables. When considering the association of SUED with forward-facing horn pattern and corneal staining with Rose Bengal, significance in both logistic and ordered logistic models supported the observations of a scale of disease. However, the retention of significance in the logistic model (i.e. presence vs. absence of SUED, Category 3 and 4 being considered homologous) also supports the idea that Category 3 eyes are full expressions of

SUED. The current HSS registration policy enables ewes with Category 3 eyelids to be registered which may be undermining a lack of progress with regards to the prevalence of SUED. These results should be carefully considered by the breed society in the future with regards to registration of animals with Category 3 eyelids.

The presence of a trend but absence of significance in the association of ewe 'worst' score with SUED (Table 4a and 4b) in the remaining ewe models based on lamb-level and eye-level data may indicate an association, however both the ewe and ram analyses had low power due to the numbers of animals present, especially rams, with most having a 'worst' score of 2 or less due to breed selection process. This lack of inheritance data may also continue to undermine breeding decisions, but it is difficult to make concrete suggestions for change without definitive evidence of an association.

When considering phenotypes, animals defined as 'multi-horned' have here been shown to be associated with the presence of SUED in previous work (Henson, 1981). The number of horns and its association with SUED score have not previously been examined. Having five or more horns was protective in the linear regression model on eye-level data ($p=0.0045$, CI -0.58,-0.01). The absence of documented cases of SUED in the literature or within the breed society meant that this was not broadly examined within this project and a population-wide evaluation of two-horned animals has not been completed. However, the presence of a two-horned animal with evidence of SUED may warrant reinvestigation.

Animals with forward-facing horns are considered undesirable by breeders, predominantly because of the impact of the longer horns on the grazing ability of adults. Forward-facing horns were consistently associated with a 'worst' eye score in the lamb-level data and eye-

level data, with an odds ratio as high as 9.4 when compared to a base of backward-facing horns ($p=0.002$). Being polled did not significantly affect the outcome relative to the other horn categories. The relationship between horn direction and SUED expression may be complex. Whilst the biological relationship may not be immediately apparent, it is plausible that given the undesirability of the forward-facing horn trait, there may be pre-existing culling bias for forward-facing horns and concurrent absence of selection for SUED status, instead of a direct causal relationship. This may mimic the effect seen in the global dairy industry with intense selection pressure for milk yield and consequential negative impact on Holstein- Friesian fertility (Pryce et al., 1997)

The original survey work done in 1981 recorded 18.6% of sheep positive for SUED in one or more eyes, and a similar prevalence identified in this work suggests there has been no significant difference in disease expression despite 30 years of breeding decisions. With demand high for two-horned stock which avoids necessity to manage SUED, the demand for multi-horned stock can be limited in comparison to two-horned animals and in 2011 accounted for just 8.2% of lambs registered. Participants in the survey raised concerns about whether the attention drawn to SUED as a result of previous studies had also contributed to the decrease in relative percentage of multi-horned animals vs. two-horned animals. This lack of progress may be being undermined by the apparent lack of relationship with parental score and offspring worst score seen in the logistic regression models. The inability of these models to show a significant association potentially reflects the lack of power resulting from grouping these data into a binary outcome. The role of the X chromosome in relation to SUED has also been suggested in recent work (Hadfield and others, 2015), which warrants further investigation.

Staining

No previous work has been done looking at the consequences of SUED on the surface of the eye and ocular health. Rose Bengal is a water soluble stain used to assess the integrity of the pre-corneal tear film. Positive staining indicates a focal deficit in the mucin layer (Brooks and others, 2000). Damage indicated by positive Rose Bengal staining is superficial in comparison to Fluorescein. In this study, there was no evidence of corneal ulceration, with the exception of animals in the flock suffering from infectious conjunctivitis.

Rose Bengal staining retained significance in the linear regression model suggests that there is a spectrum of clinical disease which may actually be continuous rather than confined to the strict categories applied by the breed society. Furthermore, the significance in the logistic model where Categories 1 and 2 were merged and compared to 3 and 4 suggest that there is minimal difference between the two latter categories, and that Category 3 could be classified as SUED.

Damage may be due to increased corneal exposure in Category 3 and 4 eyes and entropion in Category 4 eyes. The absence of a flock effect in either animal-level or eye-level models suggests that there are no flock factors (i.e. subclinical endemic disease, pasture type, bloodlines) confounding the outcome of positive staining, and, therefore, that this is a real effect linked to SUED presence.

Additional lesions

A novel lesion was identified during the course of the study with 3.4% of lambs presenting with dermoids associated with the upper eyelid, conjunctiva, or nictitating membrane.

Dermoids have never previously been reported in the breed. The presence of demoids was

linked to increasing 'worst' score in eye-level data when only lamb data was analysed, and was significant using Pearson's Chi Square analysis as well as multilevel ordered logistic and linear regression. SUED-positive eyes were more likely to have a dermoid vs. animals with SUED-negative eyes (OR=115.0, $p<0.001$) in the multilevel ordered logistic regression. This may suggest a common defect or coinheritance, although the prevalence of dermoids is significantly lower than SUED in both the general population and the lamb population. Ocular dermoids have been described in other species including canines and bovines.

Further anomalies identified in this survey included evidence of SUED in a two-horned animal and in a cross-bred lamb. Both animals were in flocks with high proportions of multi-horned animals. Neither phenomena have been reported in the literature previously, which may support a complex mechanism of inheritance. Furthermore, SUED has been confirmed in other multi-horned breeds of sheep and also in multi-horned goats in Spain.

The low complication rate associated with SUED, the lack of fatalities and the continued penetrance of the trait in the breeding population indicates that, in contrast to other congenital abnormalities, the condition is not absolutely deleterious to the breed and the welfare implications to date not quantified. No breeders reported increased costs associated with management animals with SUED or complications other than an inability to sell them through pedigree markets.

Further work is needed to quantify the exact cost to animals with unilateral or bilateral SUED with subtle, less subjective indicators such as daily live weight gain, alongside a comprehensive survey of disease status on farm which will enable assessment welfare and production cost of SUED.

A genome wide association study from the USA has identified the key locus involved in the inheritance of polyceraty with relevant genes identified on ovine chromosome 2 (OAR 2) in Jacob, Navarro Churo and Damara sheep (Hadfield and others, 2015; Greyevenstein and others, 2016). Hadfield and others (2015) also proposed that the signal linked to SUED expression was linked to the ovine chromosome X (OAR X).

The recent advancement in the understanding of the gene loci involved in this acknowledged risk factor (polyceraty), and further work should also consider the role of horn direction in expression of the SUED, the protective role of horn fusion and possible interaction with dermoids. Whilst maternal eye score does appear to be a significant predictor for lamb eye score, the population examined here will be skewed by breeding selection i.e. with a lack of breeding ewes and rams with Category 4 eyes.

This study was comprehensive with a substantial number of flocks visited and a large proportion of the multi-horned population examined, but it highlights the challenge of achieving sufficient power when examining flocks for links to method of inheritance such as links to maternal or paternal score. Prudence for the relevant breed societies to investigate genetic-based work before adapting breeding policies for the future is suggested.

Conclusions

The split eyelid condition presents a significant obstacle to the expansion of the multi-horned Hebridean population. This survey documents the prevalence of SUED in the Hebridean population in 2011 and the annual incidence in lambs born in 2011 and documents the apparent lack of improvement in prevalence of the condition since 1981.

The mechanism of inheritance is not defined in this study, but links to phenotypic and potentially heritable characteristics were demonstrated. The agreement of a number of methods of analysis strengthens the reliability of the associations presented. Repeatable significance in the linear regression models suggests there is a spectrum of clinical disease which may actually be continuous rather than confined to the strict categories applied by the HSS. Furthermore, modelling suggests there may be minimal differences between Category 3 and 4 eyelid scores, and that the former should be classified as SUED. Further genetic studies are warranted, and since data collection, there have been substantial genetic advancements in understanding of the loci of the involved genes.

Conflict of Interest Statement

The primary author is a member of the HSS and a multi-horned Hebridean breeder.

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Table1: Description of SUED categories as defined by the Hebridean Sheep Society. (Category 1 and 2 courtesy of Dr David Williams, University of Cambridge; Category 3 courtesy of Cathy Cassie).

Category	Photo	Description	Eligible for Pedigree Registration?
1		Normal	Yes
2		Break in the pigment in upper eyelid	Yes
3		Break in the pigment in upper eyelid and triangular notching.	Yes (Females ONLY)
4		Break in the pigment in the upper eyelid, triangular notching and entropion.	No

Table 2: Eyelid score summary in animal- and eye-level data sets in whole Hebridean 2011 population and 2011 Hebridean lamb population.

Data	Who?	Category 4 SUED	Category 4 SUED both eyes
Animal ID	Whole population (worst)	67/454 (14.8%)	22/454 (4.8%)
Animal ID	Lambs (worst)	31/176 (17.6%)	13/176 (7.4%)
Eyes	Whole population	89/910 (9.7%)	
Eyes	Lambs	44/352 (12.4%)	

Footnote: Animals were excluded if they were crossbred or two-horned ($n=17$ and $n=1$, respectively). To appear in the “worst” population, animals had to have two eyelids recorded. When animals only had one eyelid recorded they could contribute to the eye-level dataset only. Two animals had unilateral scores only recorded, and therefore contributed to the eye-level dataset but not animal level worst score data.

Table 3: Sex and Split Eyelid Condition (SUED) in the 2011 Hebridean lamb population when considering ‘worst’ score per lamb as unmerged i.e. category 4 or merged data a binomial outcome (i.e. Categories 1 and 2 vs. 3 and 4)

Data	Who?	Sex	Category 4 SUED	Category 3 and 4 merged
Animal	Lambs (worst)	Male	22.2%	41.10%
Animal	Lambs (worst)	Female	12.7%	27.90%

Table 4a: Comparison between four analysis methods to illustrate consistent outcomes in coefficients, odds ratios and statistical significance suggesting importance of specific variables (animal-level data for the 2011 Hebridean lamb population when considering “worst” score as outcome of interest)

Lamb Data	Pearson's Chi Square	Logistic regression				Ordered logistic regression				Linear regression				
		Variable	<i>p</i>	Predictor	<i>p</i>	OR	CI 95%	Predictor	<i>p</i>	OR	CI 95%	Result	<i>p</i>	CI 95%
Horn direction	0.04	<i>Vertical horns</i>	Baseline	e	3.20	1.37-7.40	<i>Vertical horns</i>	e	2.06	0.98-4.32	<i>Vertical horns</i>	Baseline	-0.01-0.88	
														Forward-facing horns
		<i>Backward-facing horns</i>	Baseline	e	5.50	1.94-15.52	<i>Backward-facing horns</i>	Baseline	e	2.75	1.17-6.45	<i>Backward-facing horns</i>	Baseline	-0.01-0.88

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15 Table 4b: Comparison between four analysis methods to illustrate consistent outcomes in coefficients, odds ratios and statistical significance
 16 suggesting importance of specific variable (eye level data, lamb eye “score” as outcome of interest).

Eye Data	Pears ons chi suar ed	Multi-level mixed effect logistic regression i.e. Unique ID forced				Multi-level mixed effect ordered logistic regression i.e. Unique ID forced				Linear regression				
		Variable	<i>p</i>	Predictor	<i>p</i>	O R	CI 95%	Predictor	<i>p</i>	OR	CI 95%		<i>p</i>	CI 95%
Horn direction	0.005	<i>Lambs with vertical horns</i>	Baseline				<i>Lambs with vertical horns</i>	Baseline				Lambs with all patterns	Baseline	
		Forward	0.01	4.23	1.42-12.61	Forward	0.04	3.17	1.07-9.38	Forward	0.06	6	0.12-0.72	
Dermoid	<0.001	<i>Absence</i>	Baseline			<i>Absence</i>	Baseline					<i>Absence</i>	Baseline	
		Presence	NS			Presence	0.001	114.99	6.53-2022.90	Presence	<0.01	01	1.20-2.84	
Stain (RB)	<0.001	<i>Negative stain</i>	Baseline			<i>Negative stain</i>	Baseline					<i>Negative stain</i>	Baseline	
		Positive stain	<0.01	3.91	2.22-6.68	Positive stain	<0.01	149.3	16.0-1391.1	Positive	<0.01	01	1.23-2.00	

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Figure 1: Sex and Split Eyelid Condition (SUED) in the 2011 Hebridean lamb population when considering 'worst' score per lamb ($n=176$)

Figure 2: A category four eye positive for Rose Bengal staining.

Figure 3: Percentage of eyes positive of Rose Bengal staining based on HSS score ($n=122$)

Figure 4: A dermoid on the medial canthus of the eye of a lamb with a SUED score of 3. This eye has been stained with Rose Bengal and has stained positive.