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Breeding for breech flystrike resistance - Phase 3

Department of Primary Industries and Regional Development
GOVERNMENT OF WESTERN AUSTRALIA

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EXECUTIVE SUMMARY

This report reports on the outcome of Phase 2 of the Breech strike experiment to identify effective indicator traits that can be used to breed indirectly for breech strike resistance. Two phases have now been completed.

Phase 1 of the project was carried out from 2006 to 2009 and focused on the inheritance of breech strike and finding indicator traits for breech strike in a scenario where no preventative treatments such as mulesing, crutching or jetting were carried out on the flock. Struck animals were treated with short term insecticide. Dags were by far the most important indicator trait followed by urine stain, breech wrinkle and breech cover.

Phase 2 of the project was carried out from 2010 to 2014 and focused on the inheritance of breech strike and finding indicator traits for breech strike in a scenario where no preventative treatments such as mulesing or jetting were carried out on the flock, but where the hoggets were crutched at yearling age prior to the onset of the winter rainfall season that reflects industry practice. This was done to reduce the amount of dags that can accumulate during the winter season by removing the wool in the breech. Struck animals were treated with short term insecticide.

To date 7704 lambs were produced over a nine year period (2006 to 2014) from 244 sires that were mated to 4930 dams at the Mt Barker research station in Western Australia over both phases 1 and 2. This report relate mostly to data collected in Phase 2 of the project but to provide a more complete picture information of Phase 1 are also included where appropriate.

Flystrikes were recorded over two seasons from birth to weaner shearing (~4 months of age), and from 4 months of age up to hogget shearing at 16 months of age for each of the 4 years. The same recording procedures as in Phase 1 were followed.

Animals were scored for the important key indicator traits of dags, wrinkle, breech cover, wool colour, and urine stain at birth, marking, weaning, post-weaning, yearling, hogget age. The wool cover traits were scored on the face (face cover), breech (breech cover) and belly (Belly cover). Wrinkle was scored on the neck, body, breech, and tail. Additional traits, such as dag moisture content, faecal consistency score, and faecal worm egg counts were also recorded at different times of the year.

All production records on fleece traits, body weight and reproduction rates were recorded, and fibre diameter and wool quality traits were measured annually on all hoggets and the mature ewes. All hogget production and classing data have been submitted to Sheep Genetics Australia. Adult data will also be submitted when completed.

Average incidence of breech strike from birth to hogget shearing for all the animals born in 2010, 2011, 2012 and 2013 and that were crutched, were 4%, 9.5%, 9.5% and 9.1%, respectively. These rates were significantly lower than that achieved during phase 1 where the strike rates varied from 23% to 38% in uncrutched animals. Thus, crutching is a very effective method to reduce the risk of breech strike.

Extremely low rates of body strikes (<1%) and virtually no pizzle strikes were recorded during this phase.

Large differences in the incidence of breech strike from birth to hogget shearing (0 to 28%) were also detected between sire progeny groups in Phase 2, but less than in Phase 1.

Ewes that were struck in their first year of life have a high risk of being struck again in subsequent years throughout their life.

The ranking of sire progeny groups on their level of susceptibility or resistance for breech strike correlate strongly correlate with that in a subsequent year, even when the progeny were crutched (R^2 =0.77).

The heritability of early breech strike up to first weaner shearing was 0.21 ± 0.03 on all the data collected from 2006 to 2014. Although relatively low, it compares well with the heritability of faecal worm egg count and also milk production where huge changes have been made with selection and progeny testing. However, the heritability decreased to 0.11 ± 0.03 when only the data that were collected during Phase 2 from 2010 to 2014 were used. This is much lower than the heritability obtained in the first phase (0.51 ± 0.03), which implies that replacement animals must be selected only after they have being challenged by flies in an **UN-CRUTCHED** environment, in order to identify genetically resistant sheep accurately. Although this estimate is low, it compares favourably with the heritability of reproduction traits and resistant sires can be identified through progeny testing.

Dags in ram and ewe lambs explained most of the phenotypic variation in breech strike up to weaner shearing, i.e. 23.7% and 9.2%, respectively, in a winter rainfall environment. Urine stain (7.6%) and tail wrinkle (4.3%) explained an additional amount of the variation in breech strike up to weaner shearing.

Breech cover (3.2%), dags at yearling (2.3%) and dags at hogget age (3.5%) explained relatively little of the phenotypic variation in breech strike between weaner shearing and hogget shearing where the rams were crutched.

Breech wrinkle explained 84.2% of the phenotypic variation in breech strike in hogget ewes between weaner and hogget shearing in a scenario where the animals were crutched. This is similar to the Armidale results where dags have been removed through crutching. Urine stain explained 6.4% of the variation in breech strike between weaner and hogget shearing in ewes.

Dags recorded at post weaning, yearling or at hogget age, was the most important genetic indicator trait for breech strike. The most effective time to record dags for selection purposes was at yearling age, as predictions show that this will result in up to four times faster genetic response in breech strike than selecting directly on breech strike in a crutched environment. Dag moisture content at yearling age was also an important indicator trait but less effective than using dag score only.

The second most important indicator trait was tail wrinkle recorded at post hogget shearing. Selecting to reduce tail wrinkle will result in 3.5 faster predicted response in breech strike than selecting directly on breech strike in a crutched environment. Neck wrinkle at yearling, marking and post weaning, and body wrinkle at birth also qualified as indicator traits but would less effective than tail wrinkle.

The most important indicator for breech strike that was measured before weaning was body wrinkle at birth. This shows that wrinkle is a key risk factor in breech strike, that supports previous research.

Selection for breech strike resistance resulted in the resistant line diverging from the susceptible line. The control line was twice as likely to be struck than the selection line up to weaner shearing (1.9% vs 3.8%). The incidence of strikes from birth to hogget shearing in the control line was 16.5% vs 12.1% for the selection line. This relatively small difference can be ascribed to the fact that the animals did not receive adequate challenges during the experiment due to the mulesing (2006 and 2007) and crutching (2010 to 2013) that were carried out during the life of the experiment. In 2014 when the animals were again not crutched the incidence of breech strike in the resistant line was 15% vs 32% in the control line.

The five most susceptible sires' progeny were 3 times more likely to be struck than the five most resistant sires' progeny (40% vs 13%) in 2014. The two most resistant sires were homebred sires while the third most resistant sire was an industry sire. The most susceptible sire was from an industry flock. This shows that large genetic differences exist in industry flocks and that it is possible to identify extreme resistant and susceptible animals for breech strike. This finding suggests that the current progeny testing evaluation scheme should be expanded to progeny test sires for breech strike resistance where possible.

Moisture content, amount of wax and suint from the mid-side wool samples did not contribute to differences between sheep that were struck and not struck by flies.

Breech wool of a selection of the most resistant ewes had significantly more wool fat (16.2 vs 12.1%) than the most susceptible ewes.

The humidity of the wool in the breech of susceptible ewes was 2-5% higher, whereas the temperature near the skin under the wool was slightly (~2 ºC) cooler in susceptible ewes than in resistant ewes.

No significant differences were found between resistant and susceptible rams for humidity, but susceptible rams had a significantly ($P<0.05$) higher temperature in the breech than resistant rams.

Differences in microbial populations between resistant and susceptible sheep were investigated. No differences could be found in microbial diversity amongst hogget ewes and rams from both the Armidale and the Mt Barker flocks. However, mature rams appear to have a higher microbial diversity than hoggets.

No differences in Fungi and Yeast species were found between resistant and susceptible animals in both the Armidale and Mt Barker flocks.

A member of the family *Geodermatophilaceae* appears to have a positive association with breech strike in the Mt Barker flock. Susceptible animals had a significantly higher amount of this organism. No trend was found in the Armidale flock.

Blood immunoglobulins (IgE and IgA) were investigated as an alternative to dag scoring and faecal worm egg counting. A negative relationship was found between specific IgE and ASBV for worm egg count (*Teladorsagia*) but no relationship was found between specific IgE and dags where *Teladorsagia* was the major worm species.

TABLE OF CONTENTS

INTRODUCTION

Breech strike is a major animal health issue for Australian merino sheep. Surgical mulesing is a highly effective method of reducing the prevalence of breech strike and Merino sheep farmers rely heavily on this management tool. One strategy available to reduce the incidence of breech strike in un-mulesed sheep is through breeding. A review by James (2006) of Australian research on blowfly strike suggests that genetic improvement of breech strike resistance in sheep is possible.

Previous research has shown that a number of traits are correlated to the incidence of breech strike. These indicator traits are moisture content in the fleece, fleece rot, dermatophilosis, amount of dags, wool cover in the breech/bare skin area around anus and vulva, amount of wool wax, urine stain, wool colour to indicate suint content and wrinkles (Belschner, 1953; Dun and Eastoe, 1970; Raadsma and Rogan, 1987; Raadsma, 2000; Watts 1979: Watts *et al.* 1979; Watts and Merrit 1981; Scobie *et al.* 2002; Scobie *et al.* 2008). Greeff *et al* (2011) showed that dags was the most important predisposing factor for breech strike in a Mediterranean environment, followed by urine stain, neck wrinkle and breech cover when the animals were not crutch prior to hogget shearing. They also showed that breech strike from lambing to hogget shearing was a moderate to highly heritable trait ($h^2 = 0.51$) which should respond to selection. However, this heritability estimate is not representative of the general industry because it was determined in a scenario where no preventative treatments were carried out. This raised the questions as to whether the

1. indicator traits would be the same in an environment where crutching as per industry standard is applied, and
2. accuracy of selection (h^2) would be lower where animals have been crutched due to the lower expected incidence of breech strike.

The research flock Mt. Barker research station was chosen for the experiment as blowflies are a relatively predictable annual problem on this research station and therefore satisfies the need for a challenging environment. For scientific, industry demonstration and adoption purposes it was essential that the husbandry and economic factors of running large numbers of un-mulesed sheep be quantified.

Animal ethics approval

Animal ethics approval was obtained from the Animal Ethic Committee of the Department of Agriculture and Food WA AEC 3-11-14; AEC 3-10-18.

Aims of the project

1. Develop best practice breeding guidelines for the Australian sheep industry.
2. Determine the inheritance of breech strike to hogget shearing in a production system where animals are crutched and not mulesed.
3. Estimate the phenotypic and genetic correlations between breech strike in un-mulesed and crutched animals and potential indicator traits.
4. Provide genetic information (heritability and correlations) to 'Sheep Genetics' to develop breeding values for the key indicator traits to breed indirectly for breech strike resistance.
5. Elucidating the underlying causes of breech strike.

HYPOTHESIS

1. Sire progeny groups differ in their susceptibility to breech strike.
2. The heritability of breech strike is lower where animals have been crutched as compared to where no preventative treatments are applied
3. The genetic correlation between breech strike at weaning age and at hogget age, is lower in a production system where crutching is being carried out

PROJECT OBJECTIVES

1. To demonstrate to Merino sheep producers that there are sheep within the Merino population that are resistant to breech strike, even when animals are crutched.
2. To demonstrate that the known indicator traits for breech strike are still important in a scenario where animals are crutched.
3. To demonstrate that breeding for breech strike resistance is feasible.

GENETIC PARAMETERS OF BREECH STRIKE AND INDICATOR TRAITS

BACKGROUND

Phase 2 of the experiment started in 2010 and was carried out on the AWI breech strike flock that was established in 2006 at Mt Barker Research station of the Department of Agriculture and Food Western Australia. In 2008 the Rylington Merino flock was added to this flock to

1. add value to the existing experiment by including the historical data
2. increase the number of ewes mated in order to obtain more accurate genetic parameters for the breech strike traits and
3. progeny test outside industry sires for breech strike resistance
4. Include sheep in the experiment that vary greatly in their susceptibility to develop dags.

Since the inclusion of the Rylington flock the total population consisted of approximately 900 breeding ewes that were mated annually to 22 rams in single sire mating groups. The animals were measured for a wide range of different production and potentially indicator traits for breech strike.

METHODOLOGY

Selection of rams

A wide variety of rams were originally selected from WA research flocks and from industry flocks from across Australia that could contribute to this experimental flock to generate as much variation as possible between progeny groups to determine the most important indicator traits for breech strike. Table 1 shows a list of the industry rams used. For details on the ram contributions please see the previous final report EC940. These animals were very representative of the WA Merino population. Where possible all potential rams were identified and selected on their performance using the Dual Purpose Plus index of Sheep Genetics.

Table 1. Sources of rams that were used at establishing the Breech strike flock at Mt Barker

Stud
• Calcookara (Cojack) • Centre Plus • Cherry Tree Estate • Cranmore Park • Rylington Merino • Toland Poll • Yeendalong Farm • GSARI (control) • Wallinar • Margan • Centre Plus WA • Calcookara (Garreth) • Majuba • Merinotech • CSIRO (links across sites)

The control rams were obtained from the Katanning research station and which were the progeny of rams sourced over 15 years (1982 to 1998) from Cranmore Park, Merinotech (Webb, Honey, Corke, Young), Barloo, Lewisdale, Hagley, Bungadale, Glenbyrne, Quailerup, Ejanding, Mianelup, Colvin, Condeena and Woolkabin. These studs were and still are major ram suppliers to industry flocks.

In 2009 the flock was split into two lines by pooling the most resistant ewes into a resistant line and pooling the most susceptible ewes in a control line. The pooled lines were subdivided into 6 mating groups per line. The Rylington Flock was kept separate and used to progeny test industry sires for resistance to breech strike to generate more relevant data. The most resistant and productive rams on the Dual Purpose Plus index of Sheep Genetics were identified and matched with rams that had the same DP+ index value, but that were susceptible to breech strike. This selection practice was used to ensure that no differences in production were unknowingly introduced by the selection process. Two or three sires were selected and used as link sires across years.

Selection of ewes

Mature ewes were sourced from the Department of Agriculture WA's Research flocks at Katanning, Mt Barker and Badgingarra. These flocks regularly sourced rams from Cranmore Park.

Sixty ewe lambs were sourced from the following WA flocks in 2005

- Billandri
- Cherry Tree Estate
- J Coole & Co
- Felspar Pty Ltd
- GSARI
- C D, D N & S H Herbert
- Kilandra Pastoral Co
- Majuba
- I & D Robertson
- W M & V A Webb

Mating

Suitable rams were identified from within the flock as described above. However, in 2009 three industry rams (Merinotech, Centreplus and Thompson), and in 2014 six industry sires (Anderson, Coole, Merinotech, AMS, Ella Matta, Centre Plus) were introduced to increase the genetic linkage with industry flocks and to progeny test industry sires for breech strike resistance.

Mating was carried out over a 5 week period starting in mid-February. Within each line 40 to 50 ewes were single sire mated to the selected rams. To prevent inbreeding in the flock, a cyclical mating system was followed. Mature ewes stayed in the family group they were allocated to or were born in, while rams were selected from within each line and mated to the following family group other than the birth group that they were born in. All replacement ewes were allocated to the family group in which they were born.

Inseminations

The ewes allocated to the industry sires, were inseminated approximately 2 to 3 weeks after the start of the mating season. This was done to ensure that the lambs are born at the same time as those from natural mating.

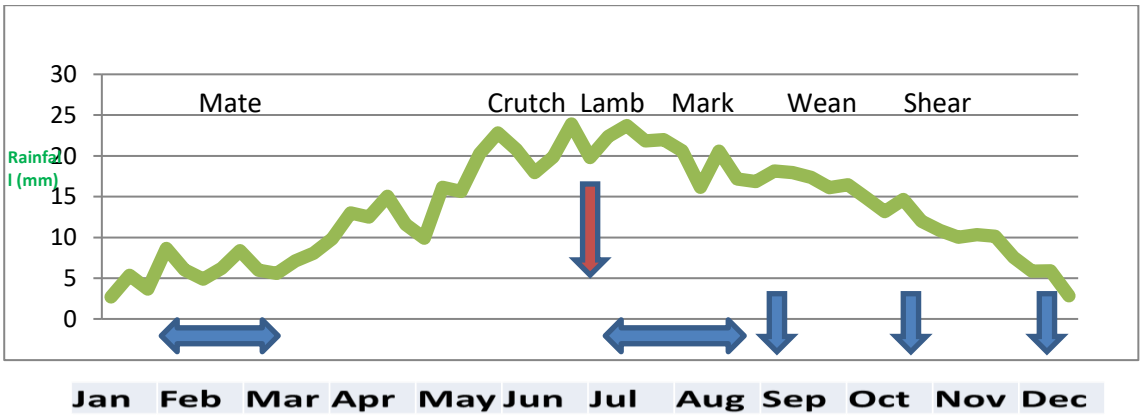
Lambing
Prior to lambing the ewes were drafted into their sire mating groups and placed on 2 hectare lambing plots. At birth, lambs were weighed, tagged and their sex and type of birth recorded. Full sire and dam pedigrees were recorded.

Treatments
All ram lambs were left entire. When the lambs were marked at approximately 3 weeks of age the tails were also docked according to industry practice. After marking, the different sire lambing groups were combined and managed as one ewe and lamb flock until the lambs were weaned at about four months of age. At this age the ram and ewe lambs were separated and run and managed in two separate flocks.

Shearing
All animals were shorn in December every year. The lambs were post weaning, while the ewe and ram hoggets were shorn in the following December. General ewe shearing was also conducted in December.

Crutching
For the four drops born in 2010 to 2013, a preventative crutching was carried out at approximately yearling age in July just prior to the onset of the winter rainfall season.
In all phases the mature ewes were crutched pre-lambing before they went on the lambing plots as it would have been unethical to have ewes with extreme dags while suckling lambs.

Management
The annual long-term rainfall from 1950 to 2015 and the management program that was followed, is shown below.



Mating took place in February to March and ewes lambed in July and August. After lambing, all the progeny, from both the natural mating and the inseminations, were pooled at marking into one large management group and managed together until weaning. At weaning the lambs were split into males and females and managed separately until hogget shearing.
No preventative chemical treatments against breech strike were applied, but animals were crutched. Animals were monitored every 1 to 3 days depending on the predicted rate of blowfly challenge. Sheep that were positively identified as being struck were treated immediately. The wool around the strike area was clipped away and the area treated with short acting Extinosad® according to the manufacturer's recommendations. Treated animals were spray marked for easy identification and then returned to the flock. Their subsequent health and recovery was continuously monitored.

Fly challenge

The fly challenge was monitored during the year using fly traps for one day of the week from September to May inclusive with a lower rate of monitoring during the winter months. This information combined with the local weather station data was used to predict the occurrence of flies.

Measurements and Observations

All animals were scored as per the Sheep Genetics, 'Visual Sheep Scores" Booklet (published by AWI and MLA). Additional traits that do not appear in the Visual Sheep Score booklet were scored and the methods of scoring are explained below. Scoring at birth (B) was followed by scoring at marking (M), weaning (W), post-weaner shearing (P), yearling (Y), hogget (H) and post hogget shearing (pH). These prefixes were used with the traits below to indicate when the traits were recorded. Where traits were recorded at different dates in as specific age phase, then the prefix was followed by a number. For example, if animals were weighed three times during the weaning age (W) phase, then the body weight (WT) at the third weighing in this phase would be described as W3WT.

Indicator Traits

Wool Coverage (COV)

- Face - (Visual Sheep Score Booklet - FACE)
- Breech - Visual Sheep Score Booklet - BR)
- Crutch & udder - (Visual booklet score – C) Belly – (BE scored:1 = bare and 5 = high coverage).

Wrinkles (WR)

- Neck - (Visual booklet score - NK)
- Side - (Visual booklet score - BD)
- Tail/rump - (1 for wrinkles around the base of the tail and 5 for a heavily wrinkled tail (TA)
- Breech - (Visual booklet score - BR)

Wool

- Colour - (Visual booklet score - COL)
- Character (crimp definition) - (Visual booklet score - CHAR)
- Dust penetration - (Visual booklet score - DUST)
- Wax (1 for dry fleece and 5 for heavily waxed fleece - WAX)
- Staple structure - (Visual booklet score - SSTR)
- Staple weathering – (Visual Booklet score – WEATHER)
- Black wool (Visual booklet score - BLK)
- White shoulder (scored 1 with no sign of white wool bleach on shoulder to 5 for a high level of white wool bleach marks on the shoulder - SHLDR)

Fleece characteristics

- Presence of dermatophilosis (1 for no dermo and 5 for heavy dermo - DERMO)
- Fleece Rot (NSW Agriculture (2003) Agfacts A3.3.41) Scoring sheep for fleece rot (FLROT)

Other

- Dag Score (Visual Booklet score - DAG)
- Dag Moisture/wetness (1 very dry pellet to 5 fluid faeces - DAGM)
- Urine Stain (for ewes) (1 no urine stain to 5 high urine stain - US)
- Urine Stain moisture (1 dry stain to 5 wet stain)
- Faecal moisture (consistency score) (FMOIST)

- A fluff score (FLUF) was given to sheep based on the amount of soft hair-like fibres on the bare non-wool growing skin area around the anus. It was assumed that this may show a larger tendency to mould or shedding fibres.
- A pluck score (PLUC) was given after weaner shearing and prior to hogget shearing. This score involves scoring the ease with which a bundle of fibre could be pulled out with relative ease through a plucking action using the thumb and fore finger. Sheep were scored in the belly under the mid-side site, which is approximately in the middle between the front and back legs. The wool adjacent to the edge between the fleece and the belly (BE) wool were plucked and scored from 1 to 5. A high score was given when a bigger bundle of fibres could be pulled out with relative ease. A low score was given when a few fibres could be pulled out.

Additional measurements (cm) at marking

Tail length in cm (TALE)
Tail score (TAILSC) which as a subjective score of the length of the tail relative to the cannon bone)
Tail width in cm (TAWDTH)
Bare area around the anus and vulva were also recorded.

- Bare skin area length under anus (ANBALE – measured in cm)
- Bare skin area width across anus (ANBAWD - measured in cm)
- Fluff - amount of soft hair on bare area around anus

Wool production traits

Greasy fleece weight (GFW)
Clean Yield of greasy wool (YLD)
Clean fleece weight (CFW)
Fibre diameter (FD)
Coefficient of variation of fibre diameter (FDCV)
Staple strength (SS)
Staple length (SL)
Fibre curvature (CURV)
Standard deviation of fibre curvature (SDCURV)
Proportion of fibres below 15 micron (F15)
Proportion of fibres above 30 micron (F30)
Fibre diameter Coarse edge (CE)
Calculated Resistance to compression (RtoC)
Bulk

2. Conformational trait
   Horn score (1 = poll and 5 = full horns)

Reproduction traits

Number of ewes joined (NEJ)
Number of lambs born dead and alive (NLB)
Number of lambs weaned (NLW)
Scrotal circumference (SC)

Disease traits

Flystrike, date of the strike, location/site on the body and severity of the strike were recorded.

Only breech strike from birth to weaning (EBRSTRWEAN) and breech strike from birth to hogget shearing (BRSTRHOG) were used in this study as the incidence of flystrike at the pizzle, poll and on the body was very low. In this report BRSTRTOTAL indicates the total number of breech strikes from birth to hogget shearing. The number of strikes were used in this analysis where 1 denotes zero strike and 2 indicates an animal was struck one, and a value of 3 indicated an animal was struck twice, etc. Thus, a mean value of 1.12 for a group means that 12% of the group was struck. This was done to satisfy the statistical program package's requirements.

Individual faecal worm egg counts (FEC) were regularly monitoring to ensure adequate challenge. To ensure an adequate challenge, a mob had to reach an average of 500 eggs per grams based on 10 random samples collected from the mob, before the whole mob was sampled. Faecal moisture content (FMOIST) of the dung pellets was scored on a 1 (hard pellets) to 5 (fluid faeces).

Deaths and most likely causes.

Body weight traits

Body weights (WT)
Condition score (CS).
Eye muscle depth (EMD)
Subcutaneous fat depth (FAT)

Body weights were recorded at birth, marking, weaning, post weaning, yearling, hogget, and post hogget shearing. All mature sheep were weighed after shearing, prior to mating, after completion of mating, at weaning of their lambs, and at the annual classing.
The abbreviations for all the traits recorded on the sheep are given Appendix 5.

Blood and Skin Samples

Blood samples were collected from all of the annual progeny drops, their buffy coats collected, and which were stored at -70°C for DNA analysis. The plasma comment was also extracted, frozen and stored for immunoglobulin assays.
Skin samples with wool attached were collected from the 2012, 2013, 2014 and 2015 drops into the appropriated storage conditions for microbacterial profile studies of the skin and wool using 16S RNA technologies.

Number of records

Two hundred and forty four sires were mated to 4930 ewes from 2006 to 2014. This resulted in a total of 7705 lambs born as follows in the different years

Year of birth	**Number of animals born per year**
2005	660 lambs purchased from industry flocks (data recorded only from weaning to hogget shearing)
2006	222
2007	647
2008	994
2009	1234
2010	994
2011	950
2012	879
2013	815
2014	970
Total	**7705**

Pedigree records that were recorded previously on lambs born from 1998 in the Rylington Merino flock were also included to increase the number of sire groups in this study.

Statistical analysis

Percentage of variation explained by indicator traits

The data were analysed with ASREML (Gilmour et al. 2007).

Generalised mixed model analyses were carried out on the data. No transformations were made to the breech strike data in the initial analysis. The breech strike data from birth to weaner shearing consisted of all the data collected on the animals born from 2006 to 2014, whereas the breech strike data from weaner shearing until hogget shearing consisted only of the data collected from 2010 to 2013 as this was the period where the animals were crutched prior to the winter rainfall season.

Sex (males and females) and year (2006 to 2014) and their interactions were initially fitted as fixed effects in the base model. The covariates were centred to eliminate possible collinearity between traits and to provide meaningful interpretations of the estimates. Missing values were replaced with the average value for the group. The raw breech strike trait at weaning, and from weaning to shearing at hogget age, was regressed against the indicator traits shown in Table 2 in a linear model. Each potential indicator trait was added in turn as a random covariate to the base model to identify which one explained the most variation. That trait was then added to the model if it was significant ($P<0.05$) when tested with the likelihood ratio test with one degree of freedom using a Chi- square distribution. The process was repeated until no remaining traits were significant. All the significant traits were then confirmed in the accumulated model to be significant. Non-significant traits were dropped from the final model.

As sex and year and their interactions were statistically significant ($P<0.001$), each year's data was subsequently analysed separately for males and females to determine whether the indicator traits that explain most of the variation in breech strike were the same across years for each sex. The covariates were again centred, and missing values were replaced with the average value for the group. The raw breech strike trait at weaning, and from weaning to shearing at hogget age, was regressed against the indicator traits in Table 5 in a linear model. Significant traits for each data set were again identified by the forward selection process described above.

As breech strike is a categorical trait, a Box-Cox power transformation procedure was also carried out separately on each dataset collected in each year for each sex to remove the relationship between the mean and the variance. The slope *b* of the logarithm of the absolute value of the residuals plotted against the logarithm of the predicted value was used as Gilmour *et al.* (2009) indicated that y^{1-b} might have less of a mean-variance relationship, where *y* is breech strike. The slopes between datasets differed markedly because of the large differences in incidence of breech strike between the different years and groups. Breech strike was therefore log transformed to standardise the transformation to make interpretations comparable across all datasets. The results showed that the distribution of the proportion of variance explained by the most important traits was virtually identical between using various Box-Cox transformations or a standard log transformation of breech strike. Assessment of the residuals plotted against the predicted values show the slopes to be virtually zero. Consequently, we were satisfied that a log transformed approach was acceptable and the results from the Box-Cox analyses are therefore not shown.

A series of analyses were carried out to determine whether log transforming the breech strike data will improve the amount of variation explained. The results between transformed and untransformed breech strike traits were in general very similar with a maximum difference of only up to a 2 % of variation explained by the different traits between the two types of statistical analyses. This gave us confidence that

the estimates of the proportion of variation explained by the most important indicator traits using the untransformed breech strike traits separately for each year and sex, and across years for each sex, give a reasonable representation of the importance of the different traits.

Finally, the most important factors for total number of breech strikes from birth to hogget shearing were fitted as normal covariates to estimate their regression coefficients in order to use them as predictors for breech strike, separately for males and females.

Genetic analysis

The genetic analysis was carried out by fitting an animal model to the data using ASREML (Gilmour et al. 2007). The data were first analysed to identify the significant fixed factors in this dataset. Year of birth (2010 to 2014), sex of lamb (male, female), age of dam (2 to 7 years), birth status (singles versus multiples) were fitted with their interactions. Non-significant fixed factors and interactions were removed in the final model that was used to estimate the genetic parameters. The pooled breech strike data from birth to hogget shearing that were collected from 2006 to 2014 were also analysed as this data is similar to the data collected from 2010 to 2014 and therefore will provide more robust genetic parameter estimates for this breech trait than using only the data from 2010 to 2014.

ASREML was used to estimate the variance components of breech strike. Although Breech strike is a discrete trait it was first analysed as an untransformed trait after which it was log transformed. The model fitted year of birth, birth type, age of the dam and sex of the animals and their interactions as fixed factors and animal genetic effects, maternal genetic effects and permanent maternal environmental effects were fitted as random factors. Non-significant fixed factors were dropped from the model. The random factors were then tested for significance with a Loglikelihood ratio test. Heritability estimates were calculated by dividing the additive animal genetic variance by the phenotypic variance. Very small differences were found between the genetic parameters estimated from the transformed or untransformed data. Thus, the untransformed breech strike values were used in subsequent analysis.

Bivariate genetic analyses were then carried out to estimate the genetic and phenotypic correlations between total breech strikes from birth the hogget shearing and the indicator traits. Again, only the significant environmental factors were fitted as well as the significant maternal genetic and permanent maternal environmental effects in order to remove these factors that could bias the variance components. The correlations were estimated as described in ASREML (Gilmour et al. 2007)

RESULTS AND DISCUSSION

The means of the different traits and the fixed environmental factors that affected the traits significantly ($P<0.05$) are shown in the Appendix 1 and 2 from birth to weaner shearing and from weaners shearing until hogget shearing, respectively. Year of birth and sex of the lambs were significant ($P<0.05$) in most cases. Changing seasonal conditions are mostly responsible for the effect of year. As the lambs were separated in female and males group at weaning, the sex differences post weaning are confounded with environment. This would also explain the significant year *sex interactions.

Age of the dam affected a number of traits significantly ($P<0.05$). It is well known that age of the dam has an effect on body weight and also on condition score, as well as fleece weight and fibre traits. However, it also affected dags, dagM, urine stain and wrinkle traits significantly ($P<0.01$) at different ages. At closer inspection, however, it appears that there were no clear relationships between age of the dam with most of the traits, except that in most of these significant cases, hogget ewes progeny's performance were lower than that of mature animals especially for body weight, body condition, wrinkle score and wool traits. This agrees with previous results and other published research.

Incidence of breech strike

Figures 1 shows the long-term rainfall, the weekly rainfall and the weekly incidence of breech strike for 2011, 2012, 2013 and 2014 seasons.

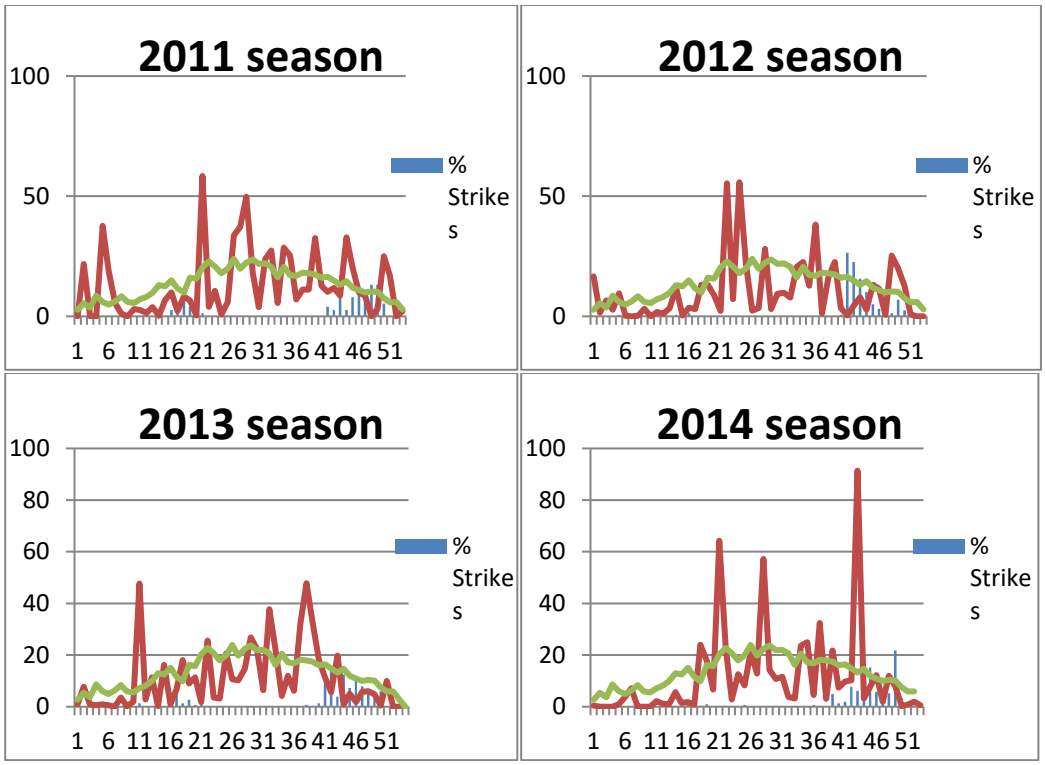


Figure 1. Long term rainfall, weekly rainfall and the incidence of breech strike for 2011, 2012, 2013 and 2014 seasons.

The long-term rainfall trend reaches a peak in July and then slowly declines. The weekly rainfall varied considerably during the year, but the breech strikes generally occurred from week 40 up to week 50 when shearing took place. A very small proportion of animals were also struck during weeks 16 to 20. These strikes were included in the total strikes from post weaning to hogget shearing.

Figure 2 shows the incidence of breech strike for different sire progeny groups from 2006 to 2014. During phase 1 when no preventative treatments were applied, a relatively high level of breech strike of 27.5%, 23.3%, 39.0% and 33.5% were recorded in the lambs born in 2006, 2007, 2008 and 2009, respectively. A much lower incidence of breech strike of 3.9%, 9.5%, 9.5% and 9.1% was found for animals that were born in 2010, 2011, 2012 and 2013, respectively. These animals were crutched at yearling age prior to the onset of the winter rains. The animals that were born in 2014 and that were not crutched, had a relative high incidence 28.5% which agrees with the trend found during phase 1. It is clear that the incidence of breech strike was much lower during phase 2 when the animals were crutched compared to the other years when the animals were not crutched.

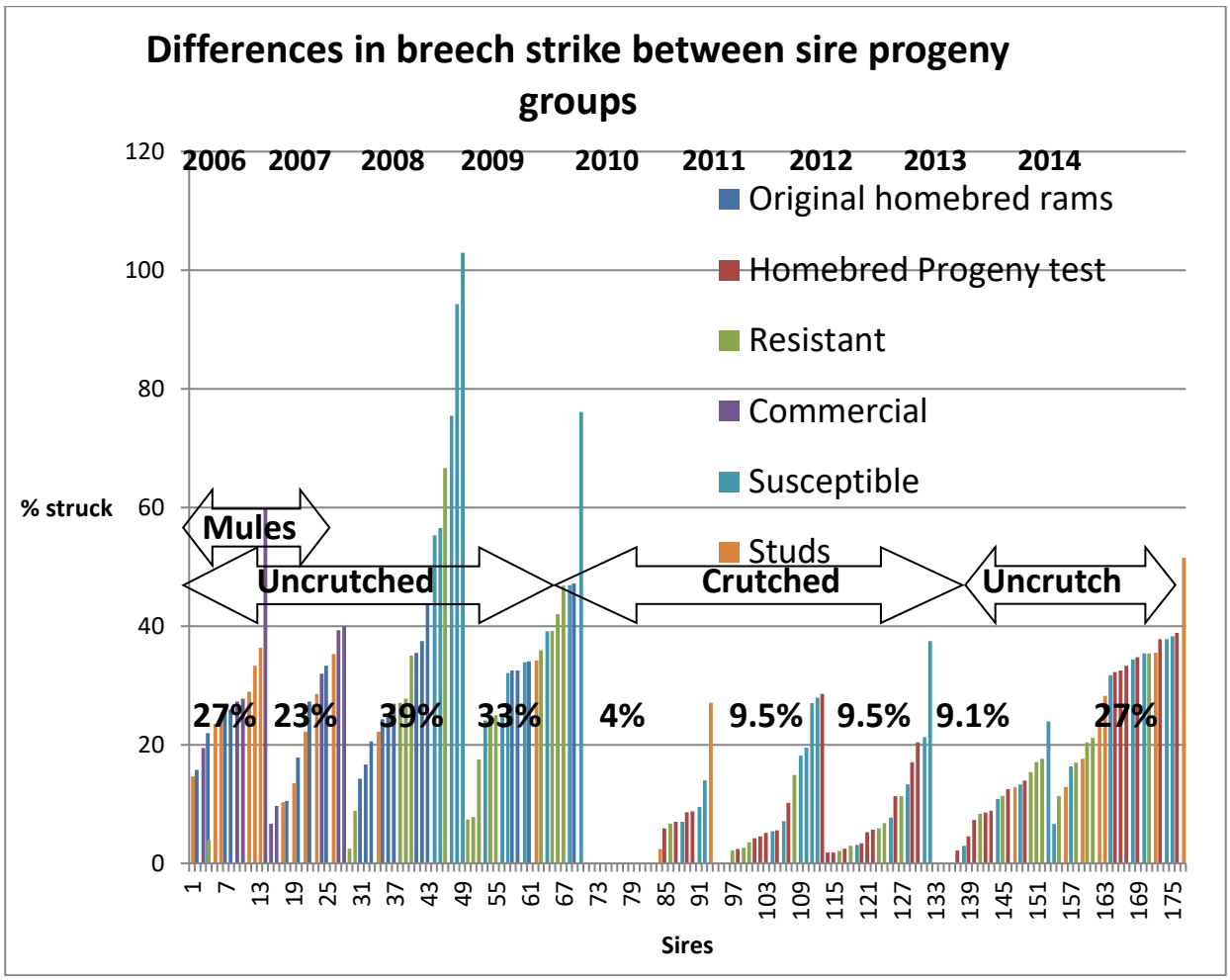


Figure 2. Incidence of total breech strike from birth to hogget shearing from 2006 to 2014.

Breech strike – threshold trait

Figure 3 shows a diagram of the effect of a threshold trait such as breech strike on the accuracy of selection where the incidence of breech strike is 30%. In this case approximately 19% of the animals would have been struck once, 9% struck twice and 2% struck three times. As the trait is clearly visible on the animals that have been struck, an accurate phenotypic description of this trait is obtained. However, for 70% of the animals that were not struck, it is not possible to differentiate between the most resistant (left side of normal distribution) and those close to the first threshold. It is thus clear that as the incidence of breech strike declines, more and more of the animals will not be struck, which will continue to reduce the accuracy of selection. It is thus imperative that a reasonable proportion of animals should be struck to obtain reliable estimate. The optimum percentage struck in a flock for a trait such as flystrike is 50%.

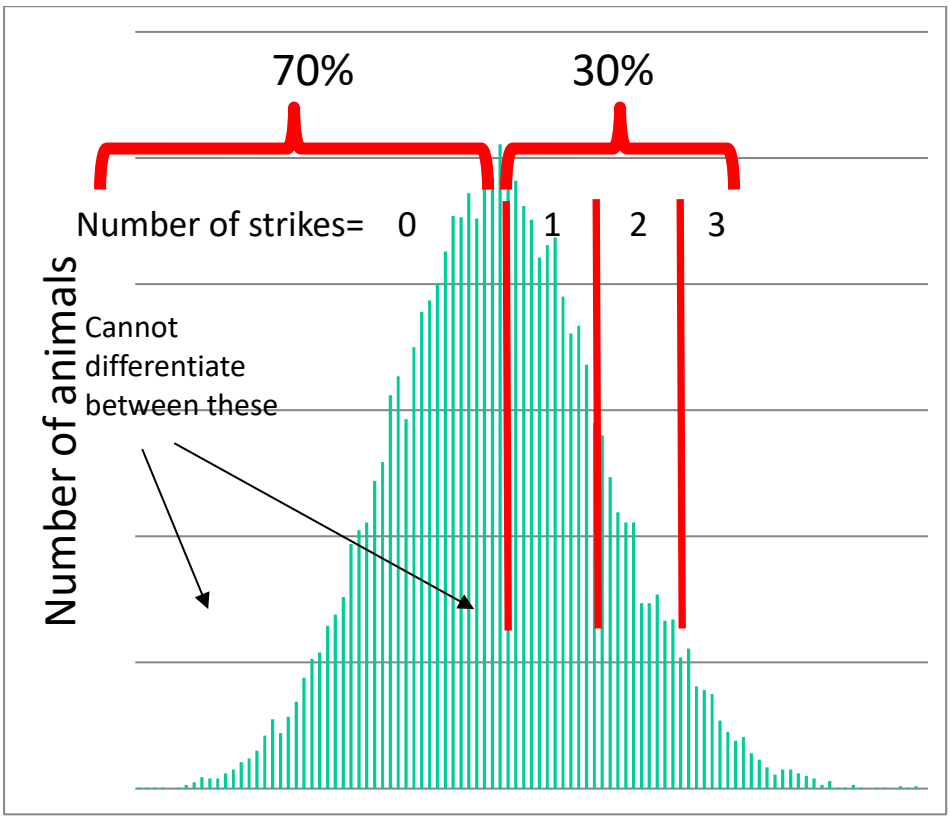


Figure 3, Diagram showing a threshold trait with an underlying distribution

Breech strike - birth to weaner shearing

Table 2a lists the trait averages for all the traits that were recorded from birth to early post weaning on the resistant and control lines of the Breech strike flock that were born during phase 1 and 2, and Table 2b show the trait average for all the traits during phase 2 from 2010 to 2014, only. No significant differences were found between the lines for any trait in Tables 2a and 2b. No significant differences were found between singles and twins for breech strike up to weaner shearing.

Table 2a. Trait averages (±SD) of the traits recorded and measured from birth to early post weaning for the resistant and control line lambs that were born from 2006 to 2014 during phase 1 and phase 2 in the Breech strike flock.

	Resistant			Control		
Trait	**n**	**Mean**	**SD**	**n**	**Mean**	**SD**
BIRTHWT	3850	4.6	0.87	3761	4.3	0.81
BBDWR	3335	2.9	1.05	3260	2.8	1.04
MWT	546	14.5	3.24	412	13.5	3.31
MNKWR	3395	2.2	0.96	2874	2.2	0.91
MBDWR	3008	1.8	0.96	2874	1.7	0.89
MTAWR	3007	1.9	1.00	2874	1.8	0.88
MBRWR	3008	1.5	0.92	2874	1.4	0.73
MBCOV	3394	3.5	0.84	2874	3.3	0.81
MBFLUF	2012	3.5	0.71	2087	3.5	0.75
MCCOV	2012	3.2	0.72	2087	3.2	0.65
MLEGSB	2012	2.6	0.27	2087	2.7	0.31
MFACE	2012	2.5	0.34	2087	2.6	0.37
MCOL	2454	1.9	0.55	2457	1.9	0.47
DERMO	919	0.3	0.66	853	0.3	0.66
MTALESC	3339	3.2	1.39	3324	3.2	1.38
MDAG	3796	1.2	0.54	3341	1.2	0.47
MDAGDM	1908	1.6	1.01	1511	1.8	1.11
MURINE	3740	1.1	0.48	3252	1.1	0.43
ANBAWD	2399	4.6	1.84	2087	4.2	1.12
ANBALE	2399	4.7	1.41	2087	4.9	1.38
MTAWDTH	2399	4.6	0.86	2084	4.7	0.99
MTABAWD	2399	4.0	0.75	2084	4.1	0.96
MTALE	3720	25.4	3.85	3302	25.1	3.86
MSpinelength	1398	74.3	5.94	1254	72.4	5.85
MTABALE	2390	10.9	1.81	2081	10.7	1.69
W1FEC	3432	1333	2843	3206	514	691
WFMOIST	3385	2.2	0.88	3600	2.3	0.90
W2WT	1664	27.0	5.13	1246	26.5	5.15
W2CS	1118	3.1	0.50	837	3.3	0.31
W2DAG	2132	1.2	0.44	1730	1.2	0.40
W2DAGSDM	973	1.8	1.04	775	1.9	1.05
W2URINE	1497	1.2	0.47	883	1.2	0.51
W2FACE	2833	2.4	0.50	2921	2.5	0.51
W2DERMO	1991	1.0	0.05	2079	1.0	0.05
W2FLROT	2431	1.0	0.23	2446	1.0	0.16

W2SHLDR	2430	1.3	0.56	2446	1.2	0.47
W2COL	2431	2.5	0.50	2446	2.5	0.44
W2CHAR	1991	3.0	0.74	2079	3.0	0.70
W2DUST	1991	1.7	0.54	2079	1.6	0.54
W2WAX	1991	2.1	0.45	2079	2.1	0.39
W2SSTRC	1990	2.1	0.39	2079	2.3	0.46
W2NKWR	3361	1.5	0.68	3313	1.5	0.63
W2BDWR	3361	1.2	0.51	3313	1.2	0.46
W2TAWR	3361	1.4	0.65	3313	1.4	0.57
W2BRWR	3361	1.3	0.62	3313	1.2	0.52
W2LEGSF	2430	2.3	0.42	2446	2.4	0.42
W2LEGSB	2431	2.6	0.43	2446	2.7	0.45
W2BCOV	2833	3.0	0.58	2921	3.1	0.57
W2CCOV	2431	3.0	0.40	2446	3.1	0.44
W2BECOV	2431	3.0	0.42	2446	3.1	0.45
W2TOES	923	1.5	0.53	834	1.5	0.54
W3WT	3741	27.0	5.77	3733	27.5	5.14
W3CS	3227	2.7	0.47	3355	2.8	0.41
E1WT	2085	26.0	6.08	1899	26.6	5.42
E1FACE	2509	2.3	0.56	2664	2.3	0.64
E1CS	1586	2.8	0.42	1394	2.8	0.41
E1WCOL	1932	2.4	0.59	1979	2.4	0.56
E1NKWR	2107	2.1	0.55	2189	2.1	0.58
E1BDWR	2107	1.3	0.41	2189	1.3	0.43
E1TALE	478	9.0	1.12	463	8.9	1.19
E1TAWDTH	478	7.8	0.95	463	7.7	1.01
E1TAWR	2012	1.5	0.42	1980	1.5	0.38
E1BRWR	2107	1.1	0.28	2189	1.1	0.27
E1LEGSF	2107	2.4	0.44	2189	2.5	0.50
E1LEGSB	2107	2.7	0.45	2189	2.8	0.51
E1BCOV	2107	3.1	0.65	2189	3.1	0.64
E1BFLUF	1932	2.9	0.52	1980	2.9	0.52
E1CCOV	2109	2.9	0.42	2194	3.0	0.44
E1BECOV	2306	2.9	0.53	2405	3.0	0.68
E1TOES	1030	2.1	0.26	1058	2.1	0.26
E1SC	722	17.6	3.53	759	19.7	3.81
E1DAG	1447	1.4	0.64	1489	1.1	0.35
E1DAGDM	873	1.8	0.94	601	1.4	0.80
E1URINE	496	1.1	0.24	1487	1.0	0.15
EBRSTRWEAN (%)	3877	1.1	0.31	3828	1.0	0.21
E2WT	2093	27.9	5.95	2297	29.6	5.12
E2CS	1453	2.7	0.34	1410	2.8	0.30
E2FACE	399	2.2	0.44	465	2.4	0.37
E2NKWR	399	1.7	0.58	465	1.8	0.63
E2BDWR	399	1.1	0.27	465	1.1	0.28

E2TAWR	399	1.2	0.31	465	1.2	0.33
E2BRWR	399	1.0	0.16	465	1.0	0.16
E2BCOV	399	2.7	0.43	465	2.8	0.42
E2URINE	398	1.0	0.05	463	1.0	0.08
E2DAG	1550	1.4	0.63	1284	1.1	0.33
E2DAGDM	409	1.5	1.01	210	1.1	0.39
E3WT	1860	29.1	5.85	1828	30.1	5.53
E3CS	1865	2.6	0.34	1839	2.7	0.31
E3DAG	1510	1.4	0.69	1692	1.2	0.53
E3DAGDM	736	1.5	0.80	499	1.4	0.80

Table 2b. Trait averages (±SD) of the traits recorded and measured from birth to early post weaning for the resistant and control line lambs that were born during phase 2 from 2010 to 2014 in the Breech strike flock.

Trait	Resistant			Control		
	n	Mean	SD	n	Mean	SD
BIRTHWT	1149	4.7	0.85	1062	4.6	0.91
BBDWR	1148	2.9	1.04	1061	2.9	1.02
MWT	301	14.5	3.20	242	14.6	3.30
MNKWR	914	2.3	1.14	900	2.2	1.00
MBDWR	914	1.9	1.14	900	1.7	0.95
MTAWR	914	2.1	1.27	900	1.9	1.09
MBRWR	914	1.8	1.19	900	1.6	1.01
MBCOV	914	3.1	0.77	900	3.0	0.68
MBFLUF	423	3.2	0.65	400	3.2	0.56
MCCOV	423	2.9	0.32	400	2.9	0.28
MLEGSB	423	2.5	0.19	400	2.5	0.15
MFACE	423	2.4	0.22	400	2.5	0.16
MCOL	611	2.3	0.33	653	2.3	0.31
DERMO	1150	0.2	0.53	1066	0.3	0.71
MTALESC	1131	3.7	0.61	1051	3.8	0.65
MDAG	1148	1.1	0.37	1066	1.2	0.50
MDAGDM	625	1.3	0.84	553	1.6	1.07
MURINE	1147	1.1	0.50	1066	1.1	0.50
ANBAWD	423	3.8	0.75	400	3.6	0.66
ANBALE	423	4.6	1.37	400	4.5	1.31
MTAWDTH	423	4.7	0.83	400	4.9	0.84
MTABAWD	423	4.0	0.68	400	4.2	0.71
MTALE	1134	24.5	3.50	1051	24.7	3.73
MSpinelength	727	74.2	5.48	666	74.4	6.41
MTABALE	422	10.3	1.53	396	9.9	1.30
W1FEC	1099	1684	3773	1020	1312	3225
WFMOIST	1081	2.0	0.86	1013	2.3	0.95
W2WT	720	27.6	4.87	745	26.9	5.27
W2CS	422	3.3	0.31	501	3.3	0.31
W2DAG	725	1.1	0.24	747	1.1	0.30
W2DAGDM	355	1.2	0.55	320	1.4	0.85
W2URINE	537	1.2	0.54	494	1.2	0.49
W2FACE	840	2.4	0.45	818	2.4	0.45
W2DERMO	418	1.0	0.00	399	1.0	0.00
W2FLROT	604	1.0	0.22	652	1.0	0.29
W2SHLDR	604	1.3	0.64	652	1.5	0.77
W2COL	604	2.5	0.50	652	2.7	0.60
W2CHAR	418	2.3	0.39	399	2.4	0.41
W2DUST	418	1.8	0.46	399	1.8	0.53
W2WAX	418	1.9	0.44	399	2.0	0.47
W2SSTRC	418	1.9	0.35	399	2.0	0.33

W2NKWR	1126	1.8	0.76	1056	1.7	0.69
W2BDWR	1126	1.4	0.63	1056	1.3	0.55
W2TAWR	1126	1.6	0.78	1056	1.6	0.69
W2BRWR	1126	1.5	0.75	1056	1.4	0.68
W2LEGSF	604	2.1	0.24	652	2.2	0.25
W2LEGSB	604	2.4	0.19	652	2.5	0.19
W2BCOV	840	2.9	0.61	818	2.9	0.59
W2CCOV	604	2.8	0.33	652	2.9	0.34
W2BECOV	604	2.8	0.34	652	2.8	0.35
W2TOES	420	1.4	0.50	502	1.5	0.56
W3WT	1123	28.9	5.50	1050	29.2	5.56
W3CS	840	3.0	0.43	815	3.0	0.42
E1WT	383	29.1	7.03	407	29.0	6.56
E1FACE	653	2.1	0.66	556	2.1	0.65
E1CS	185	3.4	0.20	252	3.2	0.29
E1WCOL	417	2.7	0.49	390	2.6	0.50
E1NKWR	417	1.9	0.51	390	1.9	0.48
E1BDWR	417	1.2	0.31	390	1.2	0.29
E1TALE	233	9.0	1.15	245	9.0	1.09
E1TAWDTH	233	7.8	0.94	245	7.8	0.97
E1TAWR	417	1.4	0.41	390	1.5	0.42
E1BRWR	417	1.1	0.22	390	1.1	0.25
E1LEGSF	417	2.2	0.29	390	2.3	0.28
E1LEGSB	417	2.5	0.20	390	2.6	0.21
E1BCOV	417	2.8	0.38	390	2.9	0.39
E1BFLUF	417	2.8	0.44	390	2.8	0.43
E1CCOV	418	2.8	0.38	391	2.9	0.36
E1BECOV	536	2.8	0.68	470	2.8	0.58
E1TOES	233	2.0	0.20	245	2.0	0.22
E1SC	198	19.2	3.46	185	18.7	3.57
E1DAG	233	1.1	0.42	244	1.1	0.35
E1DAGDM	233	1.2	0.65	244	1.2	0.63
E1URINE	233	1.0	0.16	245	1.0	0.11
EBRSTRWEAN (%)	1150	1.9	1.43	1066	3.8	1.95
E2WT	413	31.7	5.73	498	31.3	5.71
E2CS	413	2.8	0.37	498	2.8	0.34
E2FACE	233	2.2	0.42	166	2.2	0.46
E2NKWR	233	1.8	0.62	166	1.6	0.50
E2BDWR	233	1.2	0.28	166	1.1	0.25
E2TAWR	233	1.3	0.33	166	1.2	0.27
E2BRWR	233	1.1	0.17	166	1.0	0.13
E2BCOV	233	2.7	0.47	166	2.8	0.38
E2URINE	233	1.0	0.00	165	1.0	0.08
E2DAG	413	1.1	0.36	498	1.1	0.40
E2DAGDM	50	1.4	0.66	71	1.5	0.73

E3WT	413	32.4	5.60	495	31.8	5.49
E3CS	413	2.9	0.31	495	2.8	0.31
E3DAG	412	1.2	0.54	495	1.2	0.67
E3DAGDM	204	1.1	0.63	272	1.2	0.68

Factors explaining the variation in breech strike

Table 3 shows the traits that explains significant (P<0.05) amounts of variation in early breech strike up to weaner shearing for male and female lambs born from 2006 to 2014. A large number of traits made significant contributions to breech strike up to weaner shearing. However, only those traits that explain more than 1% of the variation are shown.

Table 3. Percentage of variation that the most important traits explain in early breech strike up to weaner shearing for male and female lambs born at Mt Barker from 2006 to 2014.

Source	Males	b ± SE	Females	b ± SE
Number of animals	3837		3866	
W2DAG	23.65	0.136 ± 0.016	3.53	0.057 ± 0.019
W3DAGS	2.45	0.044 ± 0.009	9.16	0.089 ± 0.011
W2TAWR			4.33	0.063 ± 0.012
W3URINE			7.64	0.083 ± 0.010
Unexplained variance	73.90		75.33	
Variance	0.079		0.088	

Table 3 shows that dags was a significant contributor to breech strike especially in ram lambs. Urine stain also made a significant contribution of 7.64% in females. However, it was not always possible to score urine stain accurately where dags were present. In females, tail wrinkle explained an extra 4.3% of the total variation in breech strike but it was not significant in (P>0.05) males. However, a large proportion of variation remains unexplained in both males and females.

Genetic parameters

Table 4 shows the total variation (V_p), the heritability (h^2_g), maternal heritability (h^2_m) and the permanent maternal environmental component of the total variation (h^2_{pe}) and the phenotypic (r_p) and genetic correlations (r_g) between early breech strike up to weaning and the potential indicator traits for breech strike on the lambs born from 2006 to 2014. No maternal heritability were estimated where the maternal genetic variance was not significant as tested by a loglikelihood ratio test. (P>0.05).

Table 4. Heritability estimates (± SE) of all the potential indicator traits and the genetic and phenotypic correlations (± SE) between breech strike until weaner shearing (EBRSTRWEAN) and the potential indicator traits collected from 2006 to 2014 (EBRSTRWEAN [2]include breech strike data collected from 2010 to 2014).

Trait	Vp	h^2_g	SE	h^2_m	SE	h^2_{pe}	SE	r_g	SE	r_p	SE
EBRSTRWEAN	0.07	0.21	0.03								
EBRSTRWEAN^2	0.03	0.11	0.02								
BBDWR	1.03	0.24	0.03	0.10	0.02	0.08	0.02	0.34	0.09	0.09	0.02
BIRTHCOAT	1.39	0.57	0.03			0.07	0.01	-0.11	0.07	-0.01	0.01
MANBALE	1.10	0.27	0.04			0.06	0.02	-0.27	0.10	-0.02	0.02
MANBAWD	0.39	0.34	0.04			0.03	0.02	-0.21	0.09	-0.01	0.02
MBCOV	0.28	0.29	0.03					0.08	0.08	0.02	0.01
MBDWR	0.44	0.39	0.03			0.07	0.02	0.17	0.08	0.09	0.02
MBFLUF	0.19	0.40	0.04					0.15	0.09	0.03	0.02
MBRWR	0.34	0.19	0.03	0.04	0.02	0.03	0.02	0.15	0.10	0.06	0.01
MCCOV	0.10	0.30	0.04					-0.03	0.10	0.00	0.02
MCOL	0.10	0.30	0.03			0.05	0.02	0.06	0.09	0.00	0.02
MDAG	0.24	0.27	0.03					0.39	0.08	0.10	0.01
MDAGDM	0.42	0.40	0.05					0.02	0.10	0.03	0.02
MFACE	0.11	0.46	0.04			0.00	0.00	0.26	0.08	0.06	0.02
MHAIR	0.19	0.47	0.05			0.02	0.02	-0.16	0.09	-0.04	0.02
MNKWR	0.52	0.44	0.03			0.07	0.01	0.20	0.08	0.08	0.01
MTABALE	1.92	0.47	0.04			0.00	0.00	0.11	0.08	-0.01	0.02
MTABAWD	0.37	0.41	0.04					0.21	0.09	0.04	0.02
MTALE	9.25	0.38	0.03			0.06	0.01	0.07	0.08	0.03	0.01
MTALESC	0.32	0.31	0.03			0.05	0.01	-0.07	0.10	0.00	0.01
MTAWDTH	0.38	0.38	0.04			0.06	0.02	0.25	0.09	0.05	0.02
MTAWR	0.35	0.40	0.03					0.14	0.08	0.08	0.02
MURINE	0.19	0.13	0.02			0.00	0.00	0.42	0.10	0.09	0.01
W1FEC	1.5E6	0.25	0.05					0.02	0.12	0.01	0.02
Log(W1FEC+10)	0.72	0.35	0.02					0.02	0.07	0.01	0.02
W2BCOV	0.15	0.24	0.03					0.01	0.09	0.00	0.01
W2BDWR	0.13	0.26	0.02					0.05	0.09	0.06	0.01
W2BECOV	0.06	0.28	0.03					-0.01	0.09	0.00	0.02
W2BRWR	0.15	0.22	0.02					0.10	0.09	0.07	0.01
W2CCOV	0.07	0.36	0.03					-0.02	0.09	0.00	0.02
W2CHAR	0.17	0.19	0.04			0.04	0.02	-0.03	0.12	0.00	0.02
W2COL	0.17	0.25	0.03	0.00	0.00	0.01	0.02	-0.09	0.10	0.03	0.02
W2CS	0.06	0.16	0.05	0.06	0.03	0.00	0.00	-0.15	0.19	0.00	0.03
W2DAG	0.16	0.58	0.05					0.72	0.06	0.21	0.02
W2DAGDM	0.21	0.36	0.07					0.23	0.12	0.05	0.02
W2DUST	0.10	0.26	0.04					-0.18	0.10	-0.01	0.02
W2FACE	0.21	0.52	0.03			0.00	0.01	0.18	0.07	0.05	0.02
W2NKWR	0.24	0.28	0.03			0.03	0.01	0.10	0.08	0.08	0.01
W2SSTRC	0.12	0.28	0.04			0.01	0.02	-0.09	0.10	-0.01	0.02
W2TAWR	0.16	0.27	0.02					0.11	0.09	0.10	0.01

W2URINE	0.18	0.28	0.05			0.02	0.03	0.24	0.10	0.09	0.02
W2WAX	0.14	0.38	0.04					-0.20	0.09	0.00	0.02
WFMOIST	0.60	0.30	0.03					-0.21	0.07	-0.10	0.01
E1BCOV	0.11	0.30	0.04					0.18	0.10	0.05	0.02
E1BDWR	0.13	0.48	0.04			0.05	0.02	0.18	0.08	0.09	0.02
E1BECOV	0.24	0.25	0.03					0.03	0.10	0.02	0.02
E1BFLUF	0.14	0.35	0.04					0.23	0.09	0.05	0.02
E1BRWR	0.06	0.41	0.04					0.28	0.09	0.07	0.02
E1CCOV	0.15	0.58	0.04	0.02	0.02	0.00	0.00	0.03	0.08	0.03	0.02
E1DAG	0.25	0.33	0.05					0.35	0.11	0.15	0.02
E1DAGDM	0.29	0.03	0.03					-0.05	0.29	0.03	0.02
E1FACE	0.23	0.36	0.03					0.12	0.08	0.02	0.02
E1NKWR	0.25	0.51	0.04					0.26	0.08	0.09	0.02
E1TAWDTH	0.77	0.22	0.08			0.02	0.07	-0.01	0.22	0.12	0.04
E1TAWR	0.14	0.47	0.04					0.31	0.09	0.08	0.02
E1URINE	0.03	0.71	0.06					0.42	0.09	0.02	0.03
E1WAX	0.08	0.21	0.10					-0.10	0.21	0.02	0.03
E1WCOL	0.15	0.38	0.04					0.13	0.09	0.04	0.02
E2BCOV	0.10	0.30	0.12			0.06	0.07	0.59	0.19	0.09	0.04
E2BDWR	0.08	0.52	0.17	0.06	0.10	0.15	0.09	0.30	0.16	0.19	0.04
E2BRWR	0.03	0.41	0.13			0.27	0.07	0.21	0.17	0.23	0.05
E2DAG	0.24	0.31	0.05					0.49	0.11	0.16	0.02
E2DAGDM	0.65	0.00	0.00					0.00	0.00	0.03	0.03
E2FACE	0.18	0.61	0.12					0.35	0.13	0.20	0.04
E2NKWR	0.34	0.54	0.12					0.28	0.14	0.14	0.04
E2TAWR	0.10	0.47	0.13			0.09	0.08	0.35	0.15	0.20	0.04
E3DAG	0.35	0.23	0.04			0.02	0.03	0.67	0.10	0.13	0.02
E3DAGDM	0.39	0.01	0.03					0.04	0.59	0.03	0.03

Heritability estimate of early breech strike and potential indicator traits

Early breech wrinkle (EBRSTRWEAN) had a low heritability of 0.21 (±0.03), which is most likely due to the low incidence of breech strike at this age. However, the heritability of breech strike by using only the breech strike data collected on the animals that were born from 2010 to 2014, was only 0.11 (±0.02). This low estimate is partly due to the low incidence of breech strike during this time relative to the previous period, as well as to the large reduction in the number of animals in this cohort. Therefore, using less data and with a low incidence will result in a significantly reduced accuracy of selection.

Except for E1DAGDM, E2DAGDM and E3DAGDM, all the other traits were heritable to varying extent. Urine stain at marking (MURINE) had the lowest heritability (h^2=0.13 ± 0.02) while early urine stain post weaning (E1URINE) had the highest heritability (0.71 ± 0.06). This indicates that all the traits will respond under selection.

Phenotypic relationship between breech strike and the indicator traits

The phenotypic correlations between early breech strike and the indicator traits were generally low. The highest phenotypic correlations were found for W2DAG r_p=0.21 ± 0.02), W2DAGSS (r_p=0.20 ± 0.01,) E2BRWR (r_p = 0.23 ± 0.05), E2FACE (r_p = 0.20 ± 0.04) and E2TAWR (r_p = 0.20 ± 0.04).

Genetic relationship between breech strike and the indicator traits

The dag traits MDAG (r_g=0.39 ± 0.08), W2DAGSS (r_g=0.72 ± 0.06), W2DAGSS (r_g=0.75 ± 0.05), E1DAG (r_g=0.35 ± 0.1) E3DAG (r_g=0.67 ± 0.10) and E2DAG (r_g=0.49 ± 0.11) had generally the strongest genetic relationship with early breech strike. The wool cover traits MANBALE (r_g= -0.27 ± 0.10), MANBAWD (r_g= -0.21 ± 0.09), E2BCOV (r_g=0.59 ± 0.19), E2FACE (r_g=0.35 ± 0.13) and the wrinkle traits E2TAWR (r_g=0.35 ± 0.15), E2NKWR (r_g=0.28 ± 0.14), E1BRWR (r_g=0.28 ± 0.09) was also found to have a low genetic relationship with early breech strike. This confirms previous results that dag is the most important predisposing trait for early breech strike in un-crutched sheep.

Breech strike – Weaner shearing to hogget shearing

Table 5 shows the trait averages for all the traits that were recorded from post weaning until hogget shearing on the resistant and control lines of the Breech strike flock during Phase 2 from 2010 to 2014. No significant differences were found between the lines for any trait during this period. No significant differences were found between singles and twins for breech strike up to hogget age.

Table 5. Trait averages (±SD) of the traits that were recorded from post weaning until hogget shearing on the resistant and control lines of the Breech strike flock during Phase 2 from 2010 to 2014.

Trait	Resistant			Control		
	n	Mean	SD	n	Mean	SD
P1WT	414	34.7	7.06	413	36.0	6.21
P1CS	415	3.1	0.72	413	3.2	0.64
P1DAG	296	1.6	0.98	332	1.8	1.05
P1DAGDM	110	1.5	1.05	151	1.5	0.96
P1URINE	185	1.0	0.10	247	1.0	0.13
P2WT	816	35.4	6.22	781	36.2	6.13
P2CS	817	2.9	0.51	781	3.0	0.52
P2DAG	818	1.4	0.73	787	1.5	0.80
P2DAGDM	234	1.6	0.83	311	1.6	0.97
P2URINE	184	1.1	0.20	241	1.0	0.15
P3WT	582	36.6	6.03	610	36.7	5.81
P3CS	582	3.0	0.32	617	2.9	0.34
P3DAG	402	1.4	0.68	376	1.7	0.89
P3DAGDM	123	1.6	0.78	175	1.8	1.01
P3URINE	226	1.0	0.16	236	1.0	0.17
P3FACE	175	2.5	0.39	139	2.7	0.46
P4DERMO	811	1.0	0.02	780	1.0	0.04
P4FLROT	811	1.1	0.18	780	1.0	0.17
P4SHLDR	811	1.2	0.31	780	1.2	0.31
P4COL	811	2.6	0.38	780	2.6	0.47
P4CHAR	175	2.4	0.38	139	2.6	0.35
P4DUST	175	2.1	0.23	139	2.2	0.25
P4WAX	175	2.2	0.26	139	2.3	0.28
P4SSTRC	175	2.0	0.30	139	2.1	0.34
P4NKWR	811	1.3	0.40	780	1.3	0.38
P4BDWR	811	1.0	0.18	780	1.0	0.09
P4TAWR	811	1.0	0.11	780	1.0	0.08
P4BRWR	811	1.0	0.03	780	1.0	0.00
P4LEGSF	175	2.3	0.27	139	2.4	0.29
P4LEGSB	175	2.6	0.24	139	2.7	0.25
P4BCOV	811	2.9	0.45	780	3.0	0.40
P4CCOV	811	3.1	0.37	780	3.1	0.35
P4BECOV	175	2.9	0.27	139	3.0	0.24
P4DAG	812	1.6	0.82	781	1.9	0.93
P4DAGDM	418	2.9	0.85	506	3.1	0.85

P4URINE	583	1.4	0.55	588	1.3	0.53
P4URINE	166	2.8	0.65	148	2.8	0.68
P4TOES	631	1.5	0.71	539	1.5	0.73
Y1WT	810	45.0	9.37	773	45.3	9.87
Y1CS	721	3.2	0.36	717	3.2	0.33
Y1DAG	810	2.0	0.93	775	2.2	1.02
Y1DAGDM	529	2.7	1.20	581	2.6	1.22
Y1URINE	521	1.2	0.42	429	1.2	0.45
Y2WT	177	56.1	9.24	239	54.5	8.74
Y2CS	180	3.1	0.23	240	3.0	0.22
Y2TAWR	635	1.5	0.41	637	1.5	0.41
Y2BRWR	635	1.2	0.30	637	1.2	0.31
Y2BCOV	635	3.2	0.68	637	3.1	0.64
Y2DAG	180	1.9	0.84	240	1.8	0.80
Y2DAGDM	119	3.2	0.44	160	3.1	0.50
Y2URINE	180	1.0	0.15	240	1.0	0.23
Y3WT	630	48.2	7.63	535	48.2	8.41
Y3CS	630	3.2	0.30	535	3.2	0.31
Y3DAG	630	1.8	0.99	535	2.1	1.08
Y3DAGDM	370	2.7	1.14	373	2.7	1.14
Y3URINE	174	1.0	0.15	137	1.1	0.34
H1WT	720	57.7	8.67	662	57.2	9.16
H1CS	720	3.4	0.27	662	3.3	0.28
H1DAG	453	1.7	0.68	398	1.9	0.80
H1DAGDM	291	1.9	0.76	304	2.0	0.81
H1URINE	223	1.0	0.17	233	1.0	0.16
H2WT	398	59.0	8.40	368	58.7	8.58
H2CS	283	3.4	0.34	240	3.4	0.36
H2DAG	396	1.4	0.56	367	1.6	0.64
H2DAGDM	193	2.0	0.69	239	2.1	0.68
H2URINE	222	1.0	0.19	232	1.0	0.14
H3WT	176	62.1	11.04	238	60.2	10.96
H3CS	179	3.2	0.23	240	3.1	0.24
H3FACE	354	2.2	0.46	373	2.2	0.49
H3DERMO	806	1.0	0.00	773	1.0	0.00
H3FLROT	806	1.1	0.28	773	1.0	0.21
H3SHLDR	806	1.1	0.29	773	1.1	0.28
H3COL	806	2.8	0.56	773	2.9	0.58
H3CHAR	806	2.9	0.58	773	3.1	0.57
H3DUST	806	1.6	0.44	773	1.6	0.46
H3WAX	806	2.8	0.64	773	2.9	0.62
H3SSTRC	806	2.7	0.69	773	2.7	0.69
H3WEATH	631	1.8	0.43	636	1.8	0.47
H3NKWR	354	1.8	0.89	373	2.1	1.01
H3BDWR	353	1.4	0.59	373	1.5	0.72

H3TAWR	354	2.1	0.96	373	2.5	0.98
H3BRWR	354	1.7	0.93	373	2.0	1.01
H3LEGSF	354	1.9	0.61	373	1.8	0.65
H3LEGSB	354	2.4	0.57	373	2.3	0.63
H3BCOV	354	2.4	0.77	373	2.5	0.80
H3BFLUF	175	2.4	0.45	137	2.5	0.37
H3CCOV	354	2.9	0.91	373	3.0	0.97
H3BECOV	175	2.7	0.27	137	2.7	0.27
H3DAG	804	1.6	0.66	773	1.8	0.72
H3DAGDM	477	2.6	0.62	547	2.6	0.59
H3URINE	806	1.0	0.20	773	1.1	0.26
H3TOES	584	2.2	0.81	541	2.3	0.77
H3BLK	416	1.0	0.20	422	1.1	0.74
H3SPOT	418	1.0	0.44	420	1.0	0.39
H4FD	804	18.8	1.34	760	19.0	1.56
H4FDSD	804	3.8	0.46	760	3.9	0.47
H4FDCV	804	20.5	2.48	760	20.4	2.44
H4FDCE	799	0.8	0.65	755	0.8	0.63
H4FFC	804	99.5	0.88	760	99.3	1.06
H4FD30	735	0.6	0.90	691	0.7	1.09
H4FDSF	804	18.3	1.24	760	18.4	1.43
H4FD15	804	15.6	8.86	760	15.2	9.20
H4CURV	804	95.6	10.60	760	92.5	11.98
H4CURVE	804	56.0	5.91	760	55.2	6.65
H4YLD	804	70.2	3.80	760	71.3	3.95
H4FEM	804	6.5	0.74	760	6.5	0.76
H4CEM	804	6.9	0.88	760	7.0	0.87
H4SL	804	93.0	11.57	759	95.3	11.63
H4SS	804	29.6	6.97	759	29.5	7.12
H4pRtoC	633	5.4	0.76	625	5.3	0.82
H4BULK	171	6.2	0.66	134	6.0	0.80
H4GFW	805	4.2	0.65	758	4.3	0.73
H4CFW	801	3.0	0.49	757	3.0	0.55
H4GFW_	576	3.9	0.63	595	3.9	0.70
H7WT	623	56.3	9.44	528	55.6	10.51
H7CS	624	3.3	0.28	528	3.3	0.33
H7FACE	627	2.2	0.34	532	2.3	0.32
H7HORN	280	3.3	1.66	292	3.4	1.62
H7SHLDR	490	1.3	0.47	407	1.2	0.40
H7COL	806	2.8	0.57	767	2.9	0.63
H7NKWR	627	1.9	0.60	532	2.0	0.65
H7BDWR	627	1.2	0.37	532	1.2	0.37
H7TAWR	627	1.3	0.40	532	1.4	0.45
H7BRWR	627	1.1	0.20	532	1.1	0.22
H7LEGSF	627	2.1	0.22	532	2.1	0.23

H7LEGSB	627	2.5	0.21	532	2.5	0.21
H7BCOV	627	2.4	0.42	532	2.5	0.41
H7BFLUF	284	2.3	0.40	241	2.4	0.41
H7CCOV	627	2.5	0.34	532	2.6	0.33
H7BECOV	627	2.5	0.27	532	2.6	0.28
H7TOES	627	2.9	0.54	532	2.9	0.53
H7SC	309	30.5	2.56	263	29.9	2.70
H8FECST	854	366	447	865	343	452
H8FECNT	552	215	352	605	203	327
H8FEC	660	320	453	659	292	481
H8FMOIS	691	3.3	0.67	619	3.3	0.67
pH9WT	627	57.7	8.23	623	56.8	9.08
pH9CS	513	3.4	0.46	395	3.2	0.51
pH9EMD	800	26.3	3.13	761	25.7	3.52
pH9FAT	798	3.3	0.92	758	3.2	1.04
H10Wate	228	19.2	2.57	160	18.7	2.22
H10Wax_	228	24.4	7.58	160	20.5	6.26
H10Suin	228	8.4	3.90	160	8.5	4.53
H10Dust	228	4.2	2.71	159	3.6	2.55
H10Dust	228	2.7	1.57	159	2.4	1.53
H13TALE	174	8.3	1.13	135	8.6	1.01
H13TAWD	174	9.8	1.07	135	10.0	1.07
H13JAW	174	1.0	0.30	135	1.0	0.00
HBRSTRHOG(%)[1]	1150	8.3	2.86	1066	9.6	3.01
HBRSTRTOTAL (%)[2]	1150	12.1	3.44	1066	16.5	4.05

[1]HBRSTRHOG – Total breech strike from weaning to hogget shearing
[2]HBRSTRTOTAL – Total breech strike from birth to hogget shearing

Factors explaining the variation in breech strike from weaner up to hogget shearing

Table 6a (weaner to hogget shearing) and 6b (birth to hogget shearing) shows the traits that explain significant amounts of variation in breech strike. The animals were crutched at yearling age to reduce the development of dags.

Table 6a. Factors explaining the variation in breech strike from weaner shearing to hogget shearing in animals that were crutched at yearling age.

Source	Males	b-coefficient	Females	b-coefficient
Number of animals	1808		1859	
P3BRWR			84.23	1.28 ± 0.166**
P4BCOV	3.22	0.04 ± 0.016**		
Y2DAG	2.24	0.03 ± 0.013**		
Y2URINE			6.36	0.36 ± 0.058**
H7BDWR			1.09	0.15 ± 0.025**
H1DAG			2.83	0.24 ± 0.016**
H2DAG			1.61	-0.18 ± 0.019**
H3DAG	3.46	0.04 ± 0.007**		
H3CCOV	2.27	-0.03 ± 0.008**		
Unexplained variance	88.82		3.88	
Variance	0.044		0.076	

** P<0.01

Table 6a shows that breech wrinkle scored after crutching at yearling age explained 84% of the variation in breech strike in ewes but nothing in males. Urine stain explained a further 6.4% of the variation in breech strike in ewes. However, Table 6b shows that when the pooled breech strike trait from birth to weaning was used, Breech wrinkle measured in autumn was again the most important traits. Thus, it appears that breech wrinkles may hold moisture longer and prevent the drying of the breech wool in ewes. However, breech wrinkle at marking was a significant factor in rams. But in rams, dags measured at different times was the most important trait contributing to total breech strike from birth to hogget shearing.

Table 6b. Factors explaining the variation in breech strike from birth to hogget shearing in animals that were crutched at yearling age.

Source	Males	b-coefficient	Females	b-coefficient
W2DAG	14.40	0.11 ± 0.04**		
P3BRWR			85.56	0.13 ± 0.02**
P3FACE	5.32	0.07 ± 0.02**		
MBRWR	6.61	0.08 ± 0.03**		
HDAG	0.73	0.02 ± 0.01*	4.64	0.23 ± 0.02**
HDAGDM	1.53	0.04 ± 0.01**		
Unexplained variance (%)	71.41		9.81	
Variance	0.09		2.00	

Genetic parameters

Heritability estimates of late breech strike and potential indicator traits

Table 7 shows the heritably estimates of breech strike between weaning and hogget shearing, and the phenotypic and genetic correlations between breech strike and potential indicator traits.

Table 7. Heritability estimates (± SE) of all the potential indicator traits on crutched sheep born from 2010 to 2013 and the genetic and phenotypic correlations (± SE) between breech strike between weaner shearing and hogget shearing (BRSTRHOG).

Trait	Vp	h^2	SE	r_g	SE	r_p	SE
BRSTRHOG (wean-hogget)	0.07	0.09	0.02				
P1DAG	0.91	0.23	0.06	0.60	0.17	0.08	0.03
P2DAG	0.47	0.22	0.04	0.75	0.10	0.13	0.02
P2DAGDM	0.57	0.08	0.05	0.30	0.28	0.07	0.03
P2URINE	0.03	0.00	0.03	1.69	5.75	0.04	0.03
P3DAG	0.48	0.42	0.07	0.65	0.12	0.07	0.02
P3FACE	0.16	0.66	0.12	0.40	0.18	0.04	0.04
P3URINE	0.02	0.13	0.06	0.57	0.22	0.06	0.03
P4BCOV	0.08	0.24	0.04	0.30	0.14	0.05	0.02
P4BDWR	0.02	0.03	0.02	0.21	0.24	0.09	0.02
P4BECOV	0.06	0.57	0.12	0.03	0.21	0.03	0.04
P4CCOV	0.09	0.30	0.04	0.18	0.13	0.05	0.02
P4CHAR	0.13	0.40	0.12	-0.09	0.24	0.07	0.04
P4DAG	0.47	0.31	0.05	1.38	0.41	0.16	0.02
P4DUST	0.05	0.13	0.08	0.06	0.34	-0.05	0.04
P4NKWR	0.14	0.40	0.05	0.20	0.14	0.09	0.02
P4SSTRC	0.10	0.24	0.10	0.45	0.29	0.00	0.04
P4TAWR	0.01	0.05	0.02	0.46	0.21	0.08	0.02
P4URINE	0.23	0.20	0.04	0.03	0.17	0.05	0.02
P4URINEDM	0.43	0.27	0.08	0.72	0.17	0.14	0.03
P4WAX	0.06	0.23	0.09	-0.49	0.26	-0.01	0.04
YDAG	0.67	0.46	0.05	0.68	0.10	0.12	0.02
YDAGDM	0.73	0.30	0.06	0.63	0.12	0.11	0.02
Y1DAG	0.79	0.51	0.05	0.68	0.10	0.12	0.02
Y1DAGDM	0.86	0.23	0.05	0.71	0.12	0.11	0.02
Y1URINE	0.13	0.18	0.04	0.05	0.18	0.03	0.02
Y2BRWR	0.08	0.16	0.04	0.47	0.16	0.09	0.02
Y2DAG	0.46	0.09	0.19	3.96	4.75	0.24	0.04
Y2TAWR	0.14	0.24	0.05	0.30	0.16	0.09	0.02
Y2URINE	0.02	0.10	0.06	0.36	0.32	0.18	0.04
Y2WAX	0.37	0.10	0.06	0.24	0.32	0.01	0.05
Y3DAG	0.55	0.49	0.06	0.65	0.11	0.16	0.02
Y3DAGDM	0.49	0.19	0.05	0.72	0.14	0.16	0.02
Y3URINE	0.08	0.07	0.05	-0.18	0.40	0.02	0.04

H1DAG	0.44	0.50	0.08	0.70	0.11	0.23	0.02
H1DAGDM	0.61	0.15	0.07	0.59	0.20	0.14	0.03
H2DAG	0.28	0.35	0.06	0.68	0.12	-0.06	0.02
H2DAGDM	0.32	0.06	0.05	0.66	0.32	0.04	0.03
H2URINE	0.04	0.10	0.06	0.19	0.27	-0.04	0.03
H3BCOV	0.76	0.15	0.04	0.28	0.19	0.08	0.02
H3BDWR	0.23	0.23	0.06	0.32	0.19	0.10	0.03
H3BECOV	0.08	0.47	0.11	0.24	0.20	0.10	0.04
H3BEPLUC	0.24	0.06	0.05	-1.20	0.41	0.04	0.04
H3BFLUF	0.16	0.46	0.10	0.32	0.20	0.17	0.04
H3BRWR	0.43	0.16	0.05	0.56	0.20	0.15	0.03
H3CCOV	0.64	0.18	0.05	0.20	0.20	0.02	0.03
H3CHAR	0.27	0.33	0.04	0.18	0.14	0.04	0.02
H3COL	0.26	0.28	0.05	0.18	0.14	0.02	0.02
H3DAG	0.41	0.37	0.04	0.68	0.09	0.09	0.02
H3DAGDM	0.30	0.05	0.03	1.00	0.18	0.09	0.02
H3DUST	0.06	0.13	0.03	0.03	0.18	0.02	0.02
H3FLROT	0.05	0.10	0.03	-0.27	0.18	0.01	0.02
H3NKWR	0.32	0.35	0.06	0.22	0.16	0.10	0.03
H3SHLDR	0.07	0.15	0.03	-0.06	0.17	0.07	0.02
H3SSTRC	0.31	0.17	0.03	0.28	0.15	0.02	0.02
H3TAWR	0.30	0.31	0.06	0.42	0.18	0.08	0.03
H3URINE	0.06	0.13	0.03	0.37	0.16	0.11	0.02
H3WAX	0.22	0.22	0.04	0.17	0.15	0.06	0.02
H3WEATH	0.19	0.07	0.03	0.15	0.21	0.04	0.02
H4BULK	0.60	0.75	0.12	-0.01	0.19	-0.11	0.04
H4CEM	0.90	0.65	0.04	-0.04	0.12	0.01	0.02
H4CURV	108.77	0.72	0.04	-0.11	0.12	-0.03	0.02
H4CURVESD	32.63	0.72	0.05	-0.08	0.12	-0.02	0.02
H4FD	1.85	0.60	0.05	0.13	0.12	0.02	0.02
H4FD15	61.71	0.37	0.05	-0.13	0.14	-0.01	0.02
H4FD30	1.36	0.57	0.05	0.10	0.13	0.01	0.02
H4FDCE	0.54	0.51	0.05	-0.08	0.13	0.01	0.02
H4FDCV	5.48	0.39	0.05	-0.10	0.13	0.00	0.02
H4FDSF	1.66	0.69	0.05	0.10	0.12	0.02	0.02
H4FEM	0.54	0.59	0.05	0.02	0.13	0.01	0.02
H4FFC	1.29	0.58	0.05	-0.10	0.13	-0.02	0.02
H4pRtoC	0.55	0.46	0.06	0.10	0.14	0.03	0.02
H4SL	106.7	0.52	0.05	0.12	0.12	0.00	0.02
H4SS	29.34	0.18	0.04	-0.05	0.16	-0.02	0.02
H4YLD	14.57	0.67	0.04	0.05	0.12	0.02	0.02
H7BDWR	0.10	0.28	0.05	0.36	0.15	0.11	0.02
H7NKWR	0.25	0.41	0.05	0.29	0.14	0.10	0.02
H7WT	28.10	0.59	0.06	-0.05	0.13	-0.07	0.02
H8FEC	133100	0.31	0.05	-0.09	0.15	0.01	0.02

H8FMOIST	0.29	0.11	0.03	0.24	0.18	0.07	0.02
H9EMD	6.80	0.27	0.04	-0.10	0.15	-0.12	0.02
H9FAT	0.55	0.18	0.03	-0.25	0.16	-0.10	0.02
H13TALE	1.50	0.82	0.13	-0.28	0.18	0.00	0.04
H13TAWDTH	1.03	0.26	0.09	0.90	0.22	0.03	0.04

Breech strike between weaner and hogget shearing (BRSTRHOG) had a relatively low heritability of 0.09 (± 0.02). This is much lower than the heritability estimates of 0.51 found during the first phase of the project where the sheep were not crutched. Thus, it appears that crutching reduces the incidence of breech strike significantly and that impacted on the accuracy of selection. This implies that it will be difficult to make genetic progress in selecting for breech resistance under a production system where crutching is carried out.

It is not practical for breeders to select animals for breech strike resistance under normal farming conditions because of the relatively large proportion of unstruck animals in a flock. However, they are able to identify the struck animals which are also the most susceptible animals. Culling struck animals will reduce the incidence of breech strike at later ages. This result again stresses the importance of finding effective indirect selection criteria that breeders can use to select indirectly for increased resistance without the need to challenge animals. In this context, selecting breeding animals for reduced wrinkle and dag scores, and culling animals for urine stain will contribute to reduce the incidence of breech strike in sheep flocks.

Most of the other conformation and production traits were heritable but some traits were not heritable at certain times. They were P2DAGDM, P2URINE, P4BDWR, P4TAWR, H2DAGDM, H3BEPLUC and H3DAGDM. However, YDAG and YDAGDM at yearling age, which is the first scores for these traits at the onset of the winter season, were moderately heritable. This is therefore the most important dag trait to score.

Phenotypic relationship between breech strike and indicator traits

The phenotypic correlations between breech strike and the indicator traits were generally very low. The highest phenotypic correlations were found for the DAG traits at post weaning (P4DAG: r_p=0.16 ± 0.02), yearling (Y2DAG: r_p=0.24 ± 0.04) and hogget age (H1DAG: r_p=0.23 ± 0.02),

Genetic relationship between breech strike and indicator traits

A number of traits had a genetic correlation higher than 1 with breech strike. This is probably due to the low incidence of breech strike which resulted in very sparse data for these traits. These traits were therefore ignored. A large number of the genetic correlations also had high standard errors which indicate that the genetic correlations are not reliable. The Dag traits, P1DAG (r_g =0.60 ± 0.17), P2DAG (r_g =0.75 ± 0.10), P3DAG (r_g =0.65 ± 0.12), P4DAG (r_g =0.41 ± 0.16), YDAG (r_g =0.68 ± 0.10), YDAGDM (r_g =0.63 ± 0.12), Y1DAG (r_g =0.68 ± 0.10), Y1DAGDM (r_g =0.71 ± 0.12), Y3DAG (r_g =0.0.65 ± 0.11), Y3DAGDM (r_g =0.72 ± 0.14), H1DAG (r_g =0.70 ± 0.11), H1DAGDM (r_g =0.59 ± 0.20), H2DAG (r_g =0.68 ± 0.12), H2DAGDM (r_g =0.66 ± 0.32), H3DAG (r_g =0.68 ± 0.09) and H3DAGDM (r_g =1.00 ± 0.18) consistently had the highest relationship with breech strike. From yearling age dag moisture also became important. At hogget age, which coincided with the presence of *Lucilia cuprina*, dag moisture was virtually the same trait as breech strike. Thus, although this flock was crutched, this indicates how important moisture content is for breech strike. With wrinkles and breech cover as additional indicator traits, breech strike can be reduced significantly by selecting animals for low values of these traits,

The wrinkle traits were also important especially when scored after crutching at yearling age, i.e. Y2BRWR (r_g =0.47 ± 0.16) and Y2TAWR (r_g =0.24 ± 0.05) had the strongest relationship with breech strike. When the animals were scored at hogget age, the relationship was much lower which indicates that wrinkle traits scored after crutching at yearling age, is the most reliable wrinkle trait for breech strike.

One trait that stood out was tail length (H13TALE) measured after hogget shearing. It was highly heritable (h^2 = 0.82 ± 0.13). It is unclear as to why this is the case because the animals' tails were docked to a standard industry protocol. However, the width of the tail (H13TAWDTH) measured at this time had a strong correlation (r_g =0.90 ± 0.22) with breech strike. This may be related to the excess skin around the docked tail.

Breech strike – birth to hogget shearing on crutched sheep (2010 to 2013)

Table 8a (traits scored from birth to weaner shearing) and 8b (traits scored from weaner shearing to hogget shearing) shows the genetic parameters of the indicator traits and their genetic and phenotypic correlations with total breech strike from birth to hogget shearing (BRSTRTOTAL) on data collected from 2010 to 2013 on sheep that were crutched at yearling age.

Table 8a. Heritability estimates (± SE) of all the potential indicator traits recorded from birth to weaning on crutched sheep born from 2010 to 2013 and the genetic and phenotypic correlations (± SE) between breech strike from birth to hogget shearing (BRSTRTOTAL).

Trait	Vp	h^2	SE	r_g	SE	r_p	SE
EBRSTRWEAN	0.03	0.11	0.02	0.26	0.41	0.004	0.02
Log(EBRSTRWEAN)	0.01	0.01	0.01	0.30	0.39	0.003	0.02
BRSTRTOTAL	0.09	0.12	0.02				
ANBALE	1.03	0.47	0.08	-0.28	0.13	-0.02	0.02
ANBAWD	0.28	0.65	0.08	-0.26	0.11	-0.03	0.02
BBDWR	0.79	0.33	0.05	0.34	0.12	0.07	0.02
BIRTHCOAT	1.33	0.65	0.04	-0.29	0.10	-0.04	0.02
MHAIR	0.22	0.56	0.08	-0.21	0.13	-0.06	0.02
MBCOV	0.13	0.43	0.05	0.20	0.12	0.03	0.02
MBDWR	0.16	0.54	0.05	0.36	0.11	0.11	0.02
MBFLUF	0.15	0.54	0.07	0.27	0.12	0.04	0.02
MBRWR	0.06	0.37	0.05	0.37	0.12	0.11	0.02
MCCOV	0.08	0.32	0.06	0.22	0.14	0.03	0.02
MCOL	0.07	0.41	0.05	0.37	0.11	0.04	0.02
MDAG	0.21	0.33	0.05	0.09	0.13	0.03	0.02
MDAGDM	0.34	0.23	0.07	0.05	0.18	0.06	0.03
MFACE	0.04	0.28	0.07	-0.03	0.15	0.06	0.02
MLEGSB	0.03	0.02	0.02	-0.35	0.38	0.01	0.02
MNKWR	0.27	0.57	0.05	0.42	0.11	0.11	0.02
MTABALE	1.73	0.58	0.09	-0.03	0.13	0.00	0.02
MTABAWD	0.37	0.76	0.08	0.01	0.12	0.01	0.02
MTALE	8.12	0.51	0.05	0.00	0.11	0.00	0.02
MTALESC	1.47	0.07	0.03	-0.03	0.19	0.08	0.02
MTAWDTH	0.43	0.79	0.09	0.04	0.12	0.03	0.02

MTAWR	0.15	0.56	0.06	0.38	0.11	0.11	0.02
MURINE	0.14	0.20	0.04	0.40	0.13	0.16	0.02
W2BCOV	0.17	0.29	0.03	0.23	0.12	0.05	0.02
W2BDWR	0.12	0.33	0.04	0.17	0.12	0.10	0.02
W2BECOV	0.07	0.35	0.05	0.21	0.13	0.07	0.02
W2BRWR	0.13	0.31	0.04	0.25	0.12	0.10	0.02
W2CCOV	0.07	0.44	0.05	0.19	0.12	0.06	0.02
W2CHAR	0.17	0.53	0.09	0.09	0.14	-0.01	0.02
W2COL	0.19	0.29	0.05	0.03	0.14	0.02	0.02
W2CS	0.05	0.13	0.05	-0.04	0.21	0.01	0.02
W2DAG	0.09	0.15	0.04	0.53	0.17	0.02	0.02
W2DAGDM	0.41	0.20	0.09	0.14	0.23	0.08	0.04
W2DAGS	0.12	0.31	0.04	0.60	0.10	0.12	0.02
W2DAGSDM	0.62	0.28	0.19	0.17	0.29	0.07	0.07
W2DUST	0.13	0.69	0.08	-0.12	0.13	-0.02	0.02
W2FACE	0.19	0.45	0.05	0.13	0.12	0.08	0.02
W2LEGSB	0.03	0.20	0.04	0.35	0.14	0.04	0.02
W2LEGSF	0.05	0.31	0.05	0.38	0.13	0.07	0.02
W2NKWR	0.26	0.34	0.04	0.24	0.12	0.12	0.02
W2SSTRC	0.13	0.36	0.09	0.11	0.15	0.01	0.02
W2TAWR	0.15	0.33	0.04	0.24	0.12	0.11	0.02
W2URINE	0.17	0.26	0.05	0.50	0.13	0.17	0.02
W2URINES	0.27	0.37	0.10	0.51	0.16	0.22	0.03
W2WAX	0.15	0.77	0.08	-0.04	0.12	-0.01	0.02
W2WT	8.99	0.30	0.09	-0.22	0.19	-0.02	0.03
W3CS	0.07	0.35	0.05	-0.07	0.13	-0.04	0.02
W3WT	12.92	0.53	0.06	-0.11	0.12	-0.07	0.02
E1BCOV	0.07	0.39	0.07	0.34	0.13	0.08	0.02
E1BDWR	0.08	0.35	0.07	0.53	0.13	0.12	0.02
E1BECOV	0.34	0.11	0.04	0.34	0.17	0.03	0.02
E1BFLUF	0.10	0.40	0.08	0.40	0.13	0.07	0.02
E1BRWR	0.05	0.35	0.07	0.42	0.13	0.10	0.02
E1CCOV	0.10	0.49	0.09	0.36	0.13	0.07	0.02
E1CS	0.05	0.32	0.12	-0.23	0.23	-0.09	0.04
E1DAG	0.10	0.02	0.04	0.82	1.03	0.02	0.03
E1DAGDM	0.31	0.04	0.04	0.60	0.42	0.01	0.03
E1LEGSB	0.05	0.28	0.06	0.39	0.15	0.05	0.02
E1LEGSF	0.08	0.37	0.08	0.36	0.15	0.05	0.02
E1NKWR	0.22	0.40	0.07	0.46	0.13	0.10	0.02
E1TALE	0.76	0.14	0.08	0.30	0.25	0.03	0.03
E1TAWDTH	0.76	0.23	0.08	0.20	0.21	0.07	0.03
E1TAWR	0.15	0.39	0.07	0.44	0.13	0.10	0.02
E1URINE	0.03	0.49	0.11	0.52	0.14	0.14	0.03
E1WCOL	0.19	0.48	0.07	0.30	0.13	0.06	0.02
E1WT	13.92	0.65	0.08	-0.24	0.13	-0.09	0.03

E2BCOV	0.09	0.16	0.08	0.61	0.24	0.11	0.03
E2BDWR	0.06	0.57	0.16	0.45	0.19	0.17	0.04
E2BRWR	0.02	0.45	0.13	0.47	0.19	0.24	0.04
E2CS	0.08	0.35	0.07	-0.30	0.15	-0.10	0.02
E2DAG	0.13	0.08	0.04	0.48	0.22	0.12	0.02
E2DAGDM	0.25	-0.13	0.10			0.04	0.06
E2FACE	0.18	0.60	0.12	0.23	0.17	0.11	0.03
E2NKWR	0.34	0.53	0.11	0.33	0.17	0.14	0.03
E2TAWR	0.09	0.46	0.12	0.49	0.17	0.20	0.03
E2WT	11.74	0.53	0.09	-0.22	0.15	-0.06	0.03
E3CS	0.08	0.31	0.06	-0.32	0.15	-0.07	0.02
E3DAG	0.29	0.07	0.03	0.80	0.21	0.09	0.02
E3DAGDM	0.44	0.02	0.04	0.70	0.68	0.02	0.03
E3WT	13.18	0.52	0.09	-0.26	0.15	-0.06	0.03

Table 8b. Heritability estimates (± SE) of all the potential indicator traits recorded from post weaning to hogget age on crutched sheep born from 2010 to 2013, and the genetic and phenotypic correlations (± SE) between breech strike from birth to hogget shearing (BRSTRTOTAL).

Trait	Vp	h^2	SE	r_g	SE	r_p	SE
BRSTRTOTAL	0.09	0.12	0.02				
P1DAG	0.91	0.23	0.06	0.67	0.15	0.11	0.03
P2DAG	0.47	0.22	0.03	0.65	0.11	0.14	0.02
P2DAGDM	0.57	0.07	0.05	0.34	0.29	0.07	0.03
P2URINE*	0.03	0.01	0.03				
P3DAG	0.48	0.40	0.07	0.59	0.11	0.08	0.02
P3DAGDM	0.65	0.02	0.05	0.00	0.00	0.03	0.04
P3FACE	0.16	0.64	0.12	0.43	0.15	0.11	0.04
P3URINE	0.02	0.12	0.06	0.58	0.23	0.10	0.03
P4BCOV	0.08	0.23	0.04	0.35	0.12	0.05	0.02
P4BDWR	0.02	0.03	0.02	0.37	0.21	0.08	0.02
P4BECOV	0.06	0.55	0.12	0.15	0.17	0.06	0.03
P4BEPLUC	0.03	0.00	0.02	0.00	0.00	0.01	0.03
P4BRWR	0.00	0.05	0.02	0.00	0.00	0.59	0.02
P4CCOV	0.09	0.29	0.04	0.27	0.11	0.05	0.02
P4CHAR	0.13	0.40	0.12	-0.01	0.19	0.05	0.03
P4DAG	0.50	0.35	0.05	0.65	0.09	0.13	0.02
P4DUST	0.05	0.13	0.08	-0.04	0.28	-0.02	0.03
P4NKWR	0.14	0.39	0.05	0.23	0.12	0.08	0.02
P4SSTRC	0.10	0.24	0.09	0.24	0.23	0.07	0.03
P4TAWR	0.01	0.04	0.02	0.61	0.18	0.08	0.02
P4URINE	0.23	0.20	0.04	-0.01	0.14	0.04	0.02
P4URINEDM	0.42	0.26	0.08	0.66	0.16	0.15	0.03
P4WAX	0.06	0.21	0.09	-0.23	0.23	0.01	0.03
YDAG	0.67	0.45	0.04	0.59	0.09	0.13	0.02
YDAGDM	0.74	0.33	0.06	0.58	0.10	0.11	0.02
YURINE	0.08	0.06	0.05	1.32	0.77	0.10	0.02
Y1DAG	1.03	0.50	0.04	0.52	0.09	0.12	0.02
Y1DAGDM	0.94	0.30	0.05	0.55	0.10	0.10	0.02
Y1URINE	0.23	0.11	0.03	0.07	0.17	0.04	0.02
Y1URINEDM	0.76	0.11	0.12	0.31	0.46	0.01	0.08
Y2BDWR*	0.15	0.08	0.03				
Y2BRWR	0.06	0.13	0.03	0.49	0.14	0.11	0.02
Y2CHAR	0.63	0.23	0.10	-0.41	0.26	-0.02	0.05
Y2COL	0.82	0.34	0.12	-0.38	0.20	-0.02	0.05
Y2DAG	0.49	0.25	0.17	1.31	0.69	0.17	0.04
Y2DERMO	0.03	0.00	0.00	0.00	0.00	0.00	0.01
Y2DUST	0.56	0.16	0.07	0.34	0.26	0.01	0.05
Y2FLROT	0.28	0.34	0.12	-0.34	0.20	-0.01	0.05
Y2NKWR	0.37	0.23	0.08	0.66	0.18	0.03	0.05
Y2SHLDR	0.29	0.08	0.06	0.39	0.35	0.01	0.05

Y2SSTRC	0.64	0.14	0.08	-0.62	0.30	-0.02	0.05
Y2TAWR	0.10	0.19	0.04	0.35	0.13	0.10	0.02
Y2URINE	0.02	0.10	0.06	0.28	0.32	0.17	0.04
Y2WAX	0.37	0.10	0.06	0.24	0.32	0.01	0.05
Y3DAG	0.78	0.49	0.05	0.39	0.10	0.11	0.02
Y3DAGDM	0.87	0.36	0.05	0.29	0.12	0.08	0.02
Y3URINE	0.11	0.18	0.08	-0.14	0.22	0.01	0.03
H1DAG	0.72	0.38	0.05	0.42	0.11	0.08	0.02
H1DAGDM	0.92	0.31	0.06	0.35	0.14	0.09	0.02
H2DAG	0.27	0.35	0.06	0.58	0.11	-0.01	0.02
H2DAGDM	0.32	0.04	0.04	0.92	0.49	0.06	0.03
H2URINE	0.04	0.10	0.06	0.11	0.25	0.06	0.03
H3BCOV	0.76	0.15	0.04	0.28	0.17	0.06	0.02
H3BDWR	0.23	0.24	0.06	0.18	0.16	0.09	0.02
H3BECOV	0.08	0.47	0.11	0.20	0.17	0.03	0.03
H3BEPLUC*	0.24	0.03	0.05				
H3BFLUF	0.15	0.46	0.10	0.26	0.17	0.06	0.03
H3BRWR	0.43	0.16	0.05	0.38	0.19	0.11	0.02
H3CCOV	0.64	0.18	0.05	0.22	0.18	0.02	0.02
H3CHAR	0.26	0.33	0.04	0.22	0.12	0.04	0.02
H3COL	0.26	0.28	0.05	0.12	0.13	0.02	0.02
H3DAG	0.41	0.37	0.04	0.68	0.09	0.09	0.02
H3DAGDM	0.30	0.04	0.02	0.92	0.21	0.10	0.02
H3DUST	0.06	0.13	0.03	0.09	0.15	0.03	0.02
H3FLROT	0.05	0.10	0.03	-0.25	0.16	0.01	0.02
H3NKWR	0.32	0.35	0.06	0.16	0.14	0.08	0.02
H3SHLDR	0.07	0.16	0.03	-0.05	0.14	0.05	0.02
H3SSTRC	0.31	0.17	0.03	0.30	0.13	0.01	0.02
H3TAWR	0.30	0.31	0.06	0.32	0.16	0.06	0.02
H3URINE	0.06	0.13	0.03	0.32	0.15	0.12	0.02
H3WAX	0.22	0.22	0.04	0.14	0.13	0.05	0.02
H3WEATH	0.19	0.07	0.03	0.10	0.19	0.03	0.02
H4BULK	0.60	0.74	0.12	-0.12	0.16	-0.08	0.03
H4CEM	0.90	0.59	0.04	-0.04	0.10	0.02	0.02
H4CURV	108.0	0.70	0.04	-0.11	0.10	-0.02	0.02
H4CURVESD	33.49	0.74	0.03	-0.11	0.09	-0.02	0.02
H4FD	1.85	0.58	0.04	0.02	0.10	-0.01	0.02
H4FD15	64.1	0.36	0.04	0.01	0.11	0.02	0.02
H4FD30	1.28	0.55	0.04	0.04	0.11	0.00	0.02
H4FDCE	0.56	0.48	0.04	-0.09	0.11	0.01	0.02
H4FDCV	5.45	0.38	0.04	-0.05	0.11	0.03	0.02
H4FDSF	1.66	0.65	0.04	0.01	0.10	0.00	0.02
H4FEM	0.51	0.53	0.04	0.03	0.10	0.03	0.02
H4FFC	1.22	0.57	0.04	-0.04	0.10	0.00	0.02
H4pRtoC	0.58	0.39	0.05	0.09	0.12	0.01	0.02

H4SL	103	0.51	0.04	0.10	0.10	-0.01	0.02
H4SS	29.0	0.17	0.03	0.05	0.13	-0.01	0.02
H4YLD	14.4	0.63	0.04	0.08	0.10	0.02	0.02
H7BDWR	0.09	0.28	0.05	0.30	0.13	0.11	0.02
H7NKWR	0.25	0.41	0.05	0.28	0.12	0.10	0.02
H7WT	28.0	0.58	0.06	-0.07	0.11	-0.05	0.02
H8FEC	1.9E6	0.20	0.05	0.08	0.16	0.01	0.02
H8FMOIST	0.35	0.11	0.03	0.13	0.15	0.05	0.02
pH9HWT	29.7	0.60	0.07	-0.05	0.12	-0.09	0.02
pH9CS	0.17	0.34	0.07	-0.32	0.13	-0.15	0.02
pH9EMD	6.88	0.26	0.04	-0.26	0.12	-0.10	0.02
pH9FAT	0.54	0.21	0.03	-0.27	0.13	-0.09	0.02
H13TALE	1.49	0.80	0.13	-0.15	0.16	-0.03	0.03
H13TAWDTH	1.02	0.22	0.09	0.66	0.23	0.02	0.03

* Majority of scores were one

Most of the other conformation and production traits were heritable but some traits were not heritable at certain times. They were PDAGDM, P2DAGDM, P2URINE, P3DAGDM, P4BDWR, P4BRWR, P4TAWR, YURINE, Y2SHLDR, H2DAGDM, H3BEPLUC and H3DAGDM. But DAGDM at yearling age was moderately heritable.

Phenotypic relationship between breech strike (birth to hogget age) and the indicator traits

The phenotypic correlations between breech strike and the indicator traits were generally very low. The highest correlations were found for E3BRWR (r_p =0.24 ± 0.04), MURINE r_p =0.22 ± 0.04), and E2TAWR (r_p =0.24 ± 0.04).

The highest phenotypic correlations after weaner shearing was found for P4BRWR (r_p=0.59 ± 0.02), but only a few sheep had a score of 2 for this trait. Y2DAG (r_p =0.17 ± 0.04) and Y2URINE (r_p =0.17 ± 0.04) had low phenotypic relationship with breech strike.

Genetic relationship between breech strike (birth to hogget age) and the indicator traits

A number of traits had a genetic correlation higher than 1 with breech strike. This is probably due to the low incidence of these traits, their discrete nature and their skewed distribution. Even a transformation did not solve the problem and these traits were therefore ignored. A large number of correlations between traits also had high standard errors which indicate that the genetic correlations are not reliable.

The Dag traits, PDAG (r_g =0.61 ± 0.12), P1DAG (r_g =0.67 ± 0.15), P2DAG (r_g =0.65 ± 0.11), P3DAG (r_g =0.59 ± 0.11), P4DAG (r_g =0.65 ± 0.09), YDAG (r_g =0.59 ± 0.09), YDAGDM (r_g =0.58 ± 0.10), Y1DAG (r_g =0.52 ± 0.09), Y1DAGDM (r_g =0.55 ± 0.10), Y3DAG (r_g =0.39 ± 0.10), Y3DAGDM (r_g =0.29 ± 0.12), HDAG (r_g =0.58 ± 0.11), HDAGDM (r_g =0.50 ± 0.20), H1DAG (r_g =0.42 ± 0.11), H1DAGDM (r_g =0.35 ± 0.14), H2DAG (r_g =0.58 ± 0.11), H2DAGDM (r_g =0.92 ± 0.46), H3DAG (r_g =0.68 ± 0.09) and H3DAGDM (r_g =0.92 ± 0.21) consistently had the higher relationship with breech strike. From yearling age dag moisture became also important. At hogget age which coincided with the presence of *Lucilia cuprina*, dag moisture was virtually the same trait as breech strike. Thus, although this flock was crutched, this indicates how important moisture content is for breech strike. With wrinkles and breech cover as additional indicator traits, breech strike can be reduced significantly by selecting animals for low values of these traits.

The wrinkle traits were also important especially scored after crutching at yearling age as Y2BRWR (r_g =0.49 ± 0.14), Y2NKWR (r_g =0.66 ± 0.18), and Y2TAWR (r_g =0.35 ± 0.13) had the strongest relationship with breech strike. When the animals were scored at hogget age, the relationship was much lower which indicates that wrinkle traits scored after crutching at yearling age, is the most reliable wrinkle trait for breech strike.

One trait that stood out was tail length measured after hogget shearing. It was highly heritable (h^2 = 0.80 ± 0.13). It is unclear as to why this is the case because the animals' tails were docked to a standard industry protocol. However, the width of the tail measured at this time had a strong correlation (r_g =0.66 ± 0.23) with breech strike. This may be related to the excess skin around the docked tail.

Effective indicator traits for selection when animals are crutched

Table 9 shows the heritability and the genetic correlation as well as the predicted correlated response in breech strike by selecting on the indicator trait relative to selecting directly on breech strike resistance under a crutching regime.

In general, the dag traits (dags and dag moisture) were the most important indicator traits for breech strike followed by tail wrinkle, urine, and the neck and body wrinkle at different ages. The best time to record dags was at yearling age. It is predicted that using this trait can result in a 2.7 to 4.1 times improvement in breech strike resistance compared to selecting directly for breech strike resistance in a crutching regime. Tail wrinkle scored after hogget shearing will result in 3.5 times faster gain than selecting directly on breech strike in crutched sheep. These results show that using indirect selection criteria can result in significantly faster genetic changes in breech strike resistance than direct selection on the trait itself.

Table 9. Heritability ($h^{2)}$, genetic correlation (r_g) as well as the predicted correlated response relative to the direct response for the most important indicator traits for breech strike resistance under a crutching regime.

Indicator trait	h^2	r_g	CR/R[A]
Dags at yearling	0.50	0.52	2.7 - 4.1
Tail wrinkle at post hogget shearing	0.22	0.66	3.5
Dag moisture at yearling	0.30	0.55	1.8 - 3.2
Dags at post weaning	0.35	0.65	1.0 - 3.0
Dags at hogget	0.48	0.58	2.0 - 3.0
Urine moisture of the urine stain at post weaning	0.26	0.66	2.5
Neck wrinkle at yearling	0.23	0.66	2.1
Dag moisture at hogget	0.31	0.35	1.1 - 2.1
Body wrinkle at birth	0.33	0.34	1.6
Neck wrinkle at marking	0.57	0.42	1.6
Urine stain at weaning	0.37	0.51	1.0 - 1.5
Face cover post weaning	0.64	0.43	1.3
Neck wrinkle post weaning	0.53	0.33	1.3
Urine stain at yearling	0.06	1.32	1.0 -1.1
Breech wrinkle hogget	0.16	0.38	1.1
Tail wrinkle pre hogget shearing	0.31	0.32	1.1
Breech cover hogget	0.15	0.28	1.1
Tail wrinkle at marking	0.56	0.38	1.1
Body wrinkle at marking	0.54	0.36	1.0
Dags at weaning	0.31	0.60	1.0
Urine moisture at yearling	0.11	0.31	1.0
Neck wrinkle hogget	0.41	0.28	1.0
Tail wrinkle post weaning	0.39	0.44	1.0

[A] The range in the CR/R column indicates the range of relative response that was predicted by the genetic parameters for the traits at the time when they were recorded. This indicates that the outcome will depend on the season and the time of the recording of the trait.

Phenotypic and genetic trends of breech strike

The Breech strike flock experiment was designed to identify indicator traits that were genetically correlated with breech strike in order to select indirectly for improved resistance. The experiment as not designed to show whether selection for breech strike resistance would be effective as only two drops (lambs born in 2008 and 2009) experienced an appropriate challenge. In 2006 and 2007 half of each sire's progeny were mulesed, while the sheep that were born in 2010, 2011, 2012 and 2013 were crutched at yearling age. Both these practices reduced the incidence of breech strike, which resulted in a dramatic drop in the heritability and thus accuracy of selection of breech strike during this phase. The effect of selection on the 2008 and 2009 drops that did receive an adequate challenge, could not be appropriately assessed as their progeny were crutched in 2010 and in 2011, respectively. If genetic progress was the focus of the experiment then no mulesing and no crutching should have been carried out over the entire period of the experiment.

The flock was modified in 2008 with the realization that there are larger differences between sire groups than between the original three lines (resistant, industry and control). A resistant and a control line were thus established. Figures 4 and 5 show the phenotypic and genetic trends of breech strike changes in the two lines based on the average of the mating groups to which they were originally allocated to at mating. As indicated before the sheep were not crutched at yearling age in 2006 to 2009 and also in 2014 but were crutched from 2010 to 2013.

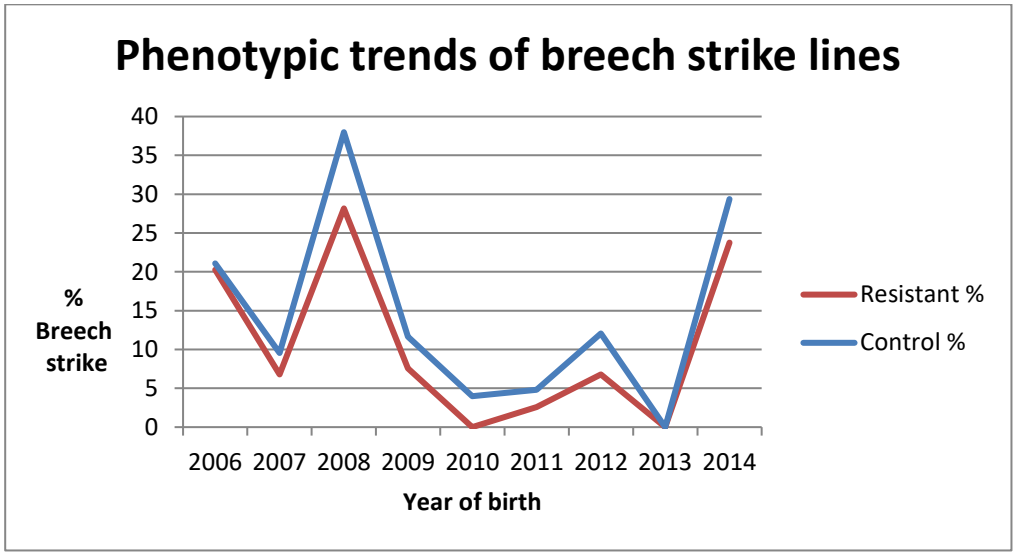


Figure 4. Phenotypic trend of breech strike experienced from birth to hogget shearing in the resistant and selection lines

It is important to note that the trends in Figure 4 are only for those animals in the breech strike lines and do not include the Rylington Merino animals which have also been included in Figure 2.

It is clear that breech strike fluctuated widely from year to year. A high fly challenge was experienced in the 2008 and in 2014 born groups. Although large differences were found between sire groups AFTER THEY HAVE BEEN PROGENY TESTED, it was not reflected in the two lines when the sheep were allocated, PRIOR to mating, based on their PREDICTED breeding values. Thus, accuracy of selection prior to progeny testing was low as confirmed by the low heritability estimate of 0.12. There were more variations within lines than between lines in breech strike. This does not mean that prediction will in general be ineffective. It will only be less effective where own performance data on individual animals a crutched environment, which obviously will result in a low incidence of breech strike, are used. Where progeny tested data are available, this will increase the accuracy of selection, which will increase even more when no crutching is carried out.

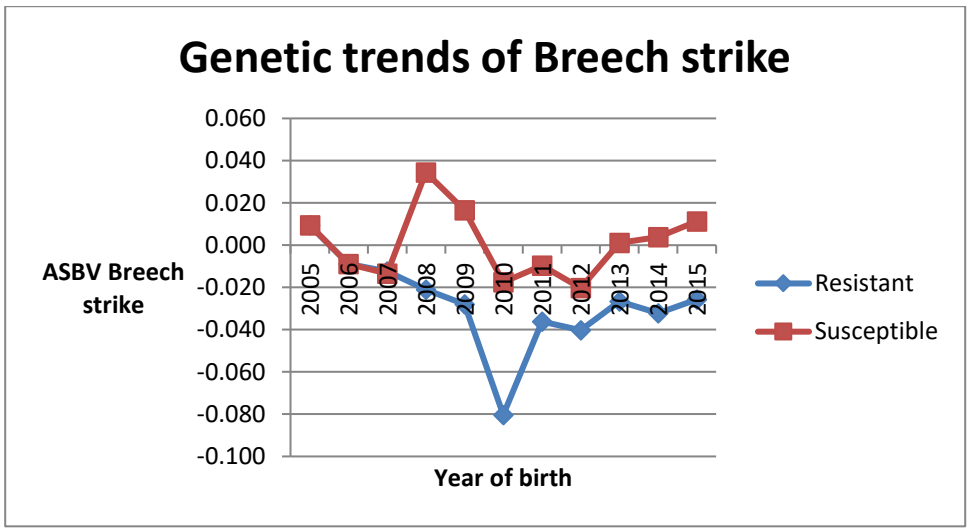


Figure 5. Genetic trend of breech strike experienced from birth to hogget shearing in the resistant and selection lines.

Figure 5 shows the genetic changes in total breech strike from birth to hogget shearing. The two lines were set up in 2008. A large divergence was achieved as the animal's breech strike information was available from previous years. Subsequently the lines did not diverge much, especially during the time when the groups were crutched. The 2015 born sheep have not been challenged which implies that their average ASBV of breech strike does not include any real breech strike information on themselves.

This graph shows how difficult it is to differentiate accurately between resistant and susceptible individuals for breech strike based on restricted information. It depends very much on the level of challenge that the animals have experienced. If the challenge is low, say less than 20%, then 80% of the population have not been struck. The 20% struck animals contribute information to the breeding value of the 80% unstruck sheep. For a discrete trait such as breech strike the optimum strike rate is 50% but such a high level of strikes was never experienced in this flock. This impacted on the accuracy of selection as is reflected in the differences in heritability estimates between the phase when the sheep were not crutched (sheep born from 2006 to 2009) and not crutched (sheep born from 2010 to 2013).

It is also important to note that although the original allocations did not result in a large divergence between the lines, it is now possible by using the historical data to re-allocate the existing animals to a resistant and a susceptible line much more accurately.

Selection has resulted in the resistant line diverging from the control line. The control line was twice as likely to be struck than the selection line up to weaner shearing (1.9% vs 3.8%). The incidence of strikes from birth to hogget shearing in the control line was 16.5% vs 12.1% for the selection line. This relatively small difference can be ascribed to the fact that the animals did not receive an adequate challenge due to the crutching that was carried out. In 2014 when the animals were again not crutched the incidence of breech strike in the resistant line was 15% vs 32% in the control line.

However, much more importantly is that the five most susceptible sires' progeny were 3 times more likely to be struck than the progeny of the five most resistant sires (40% vs 13%) when a reasonable challenge was experienced in 2014. This makes it possible to identify extreme animals for experimental purposes much more accurately. This again shows that to generate replacement sheep, especially for the resistant line, that sheep should be challenged to determine their level of resistant to breech strike accurately.

Genetic changes in dags and breech wrinkle

Figures 6 show the average ASBV per year for breech wrinkle and in dags from 2006 to 2014 for the resistant and control line.

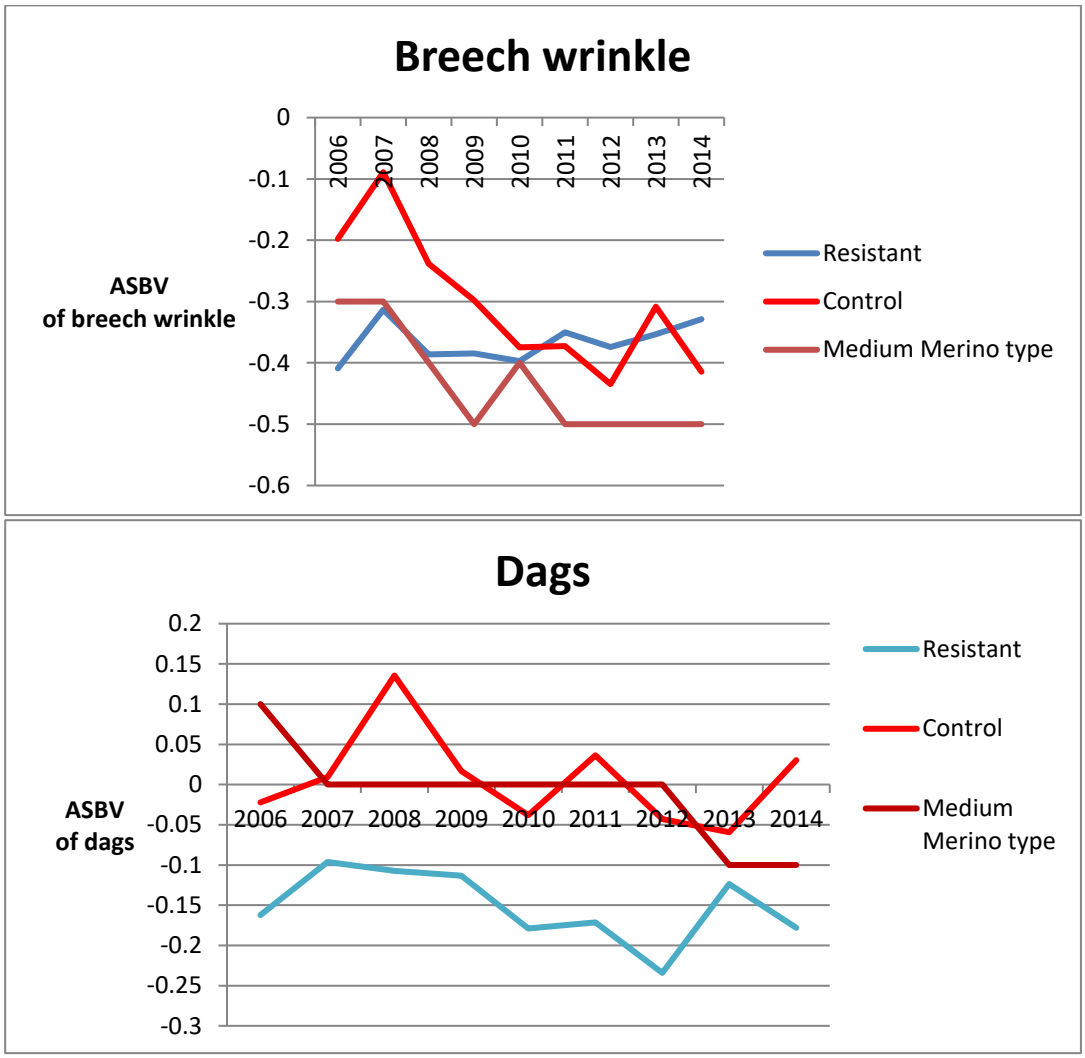


Figure 6, Genetic changes in breech wrinkle and dags of the resistant and control lines from 2006 to 2014, compared to that of the Medium Merino type from industry flocks.

The control line initially had a higher breech wrinkle value as the industry flock while it compared well with that of the resistant line. However, the control line declined considerable from 2006 to 2010 to the same level as that of the resistant line. This strong correlated response in wrinkle in the control line is quite surprising as wrinkle was completely ignored in the selection process. All animals in the resistant line were selected on breech strike resistance only. Performance was however considered, as only resistant ewes that had a Dual Purpose Plus index of higher than 120 qualified for selection. The lower performing ewes were culled. In the case of rams, only the most resistant rams for breech strike and that had a high DP+ index were considered for selection. Once these were identified, they were then matched with control animals solely on performance to prevent creating any biases in performance between the two lines. Breech strike or its breeding value was ignored in the control group as explained under material and methods. This dramatic decline in the wrinkle score in the control line could also explain the relatively small difference between the control and selection line in breech strike. However, it also indicates that other factors contribute to differences in breech strike between animals.

There was also a declining trend in late dags in both the lines, in spite of the fact that no selection against dags were carried out. However, as dags was the most important indicator trait for breech strike, this declining trend in the resistant line appears to be a correlated response. The dags in the control were very similar to that of the industry.

Figure 7. shows the average breech wrinkle scores for males and females at yearling age.

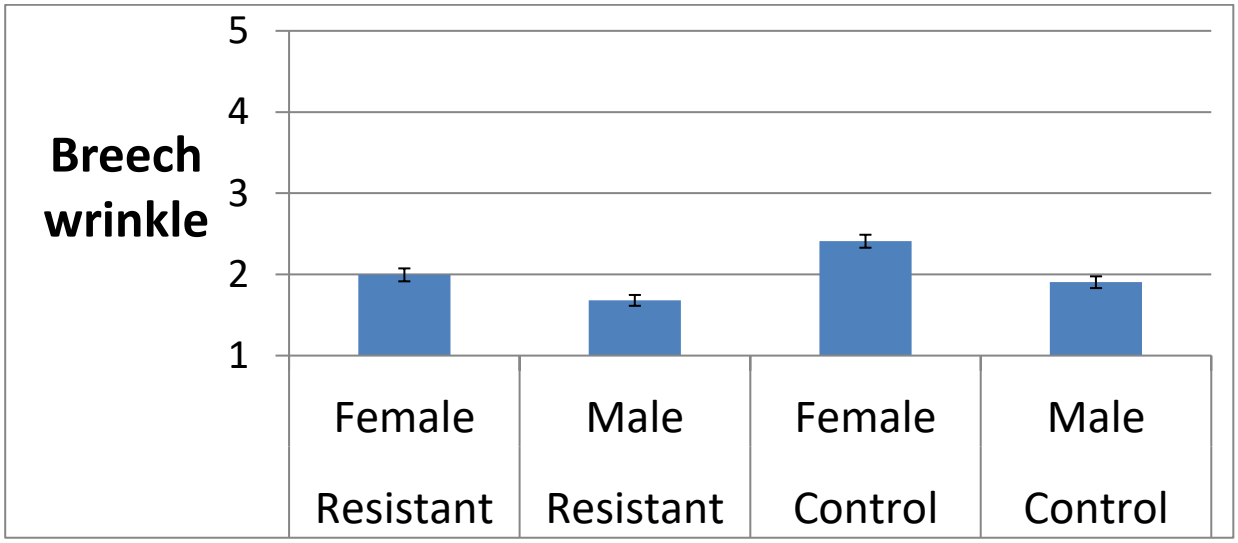


Figure 7. Average breech wrinkle scores of males and females at yearling age for the resistant and control lines.

It is clear that the animals in this flock are phenotypically relatively plain with an average breech wrinkle score of less than 2.5. The resistant line was significantly plainer than the control line, but the actual difference was very small.

Genetic changes in production traits in the breech strike flock.

All the production data that were collected on the breech strike sheep have been forwarded to Sheep Genetics for inclusion in the national database. The Australian Sheep Breeding Values (ASBV) were used in the selection of rams and ewes as described in material and methods.

Figure 8 shows the genetic changes in clean fleece weight, yearling weight and fibre diameter for the resistant and selection lines against that of the Medium Merino type which is typical of the WA type of Merino from where the sheep in this experiment were sourced. It is clear that there was a significant improvement in all three of these important production traits over time. This can be ascribed to the effectiveness of using the Dual purpose plus selection index performance values to identify genetically superior animals for breeding.

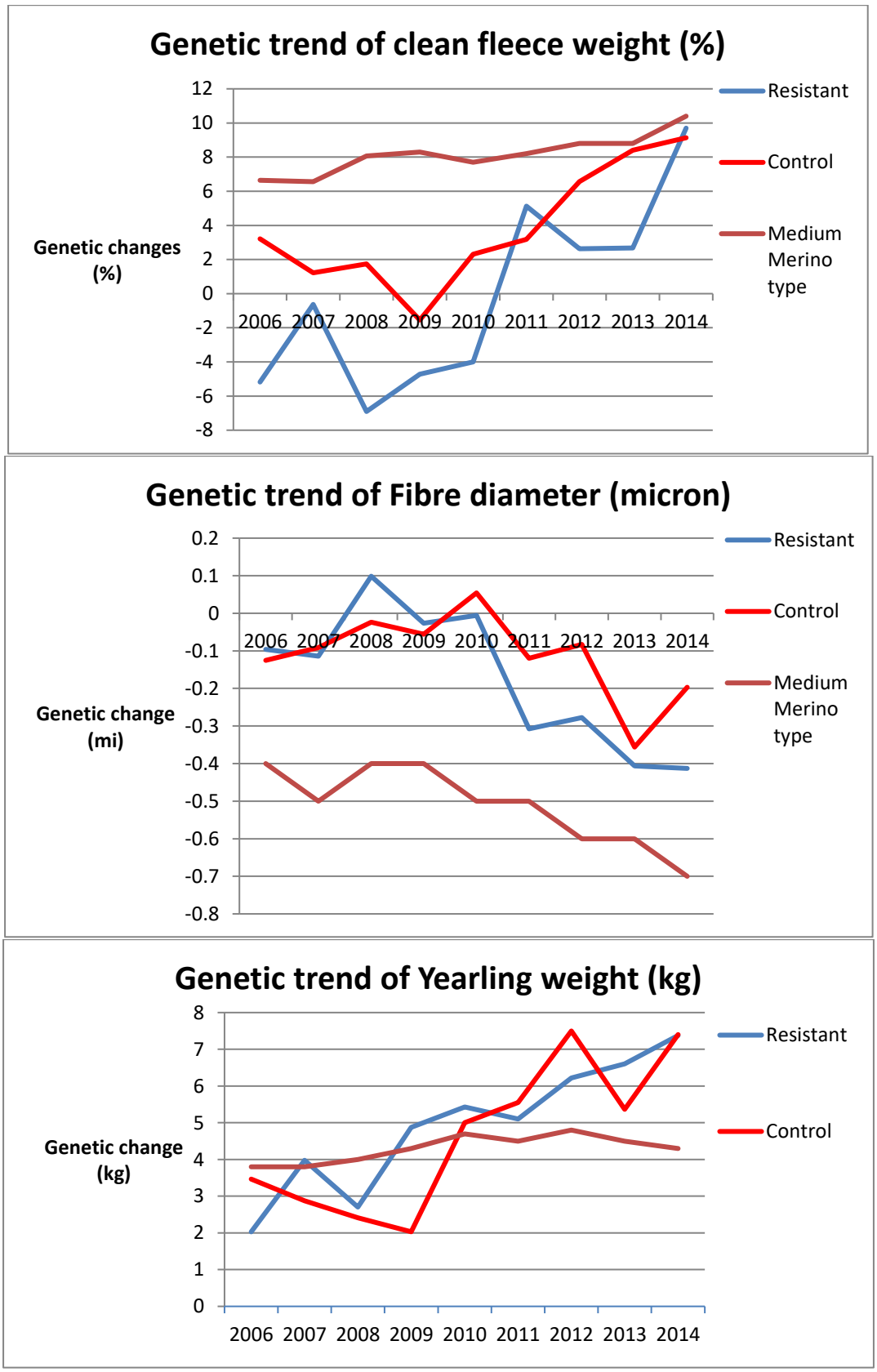


Figure 8. Genetic changes in clean fleece weight, fibre diameter and yearling weight of the resistant and selection lines from 2006 to 2008.

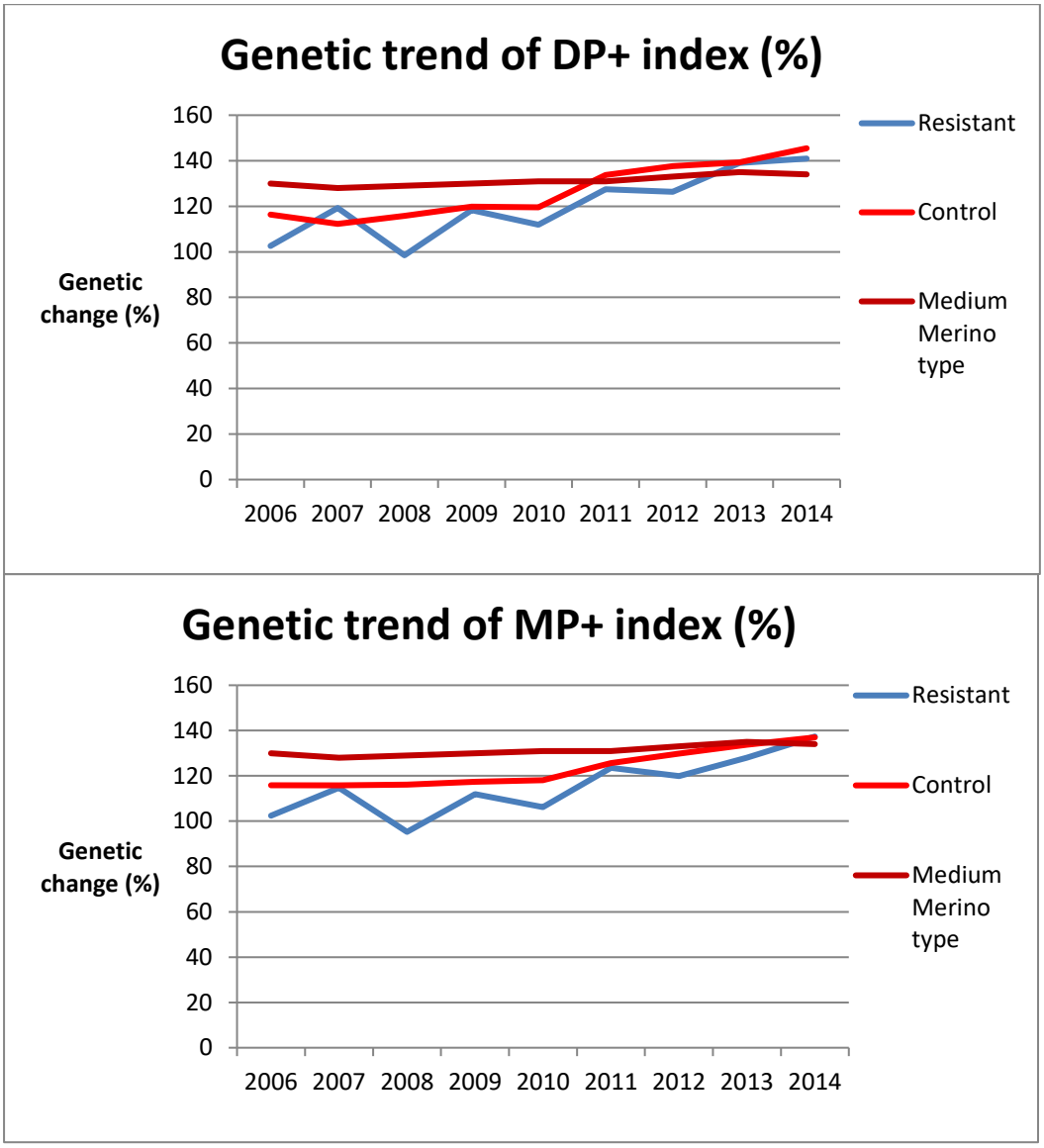
The following graphs in Figures 9 show the genetic changes in the Mt Barker flock obtained from Sheep Genetics for the most important production traits from 2005 up to 2014 in comparison with that of the Australian Merino industry. When interpreting these graphs, it is important to note that the WA sheep industry from where the sheep for this experiment were mostly sourced, is generally perceived to have bigger sheep than the eastern states. The higher body weights of the lines in this trial support that view. This may also be a contributing reason as to why the average fibre diameter of the sheep in this experiment was about 0.3 micron broader than that of the wider sheep industry flocks.

The most striking trend is the strong increase in the DP+ and MP+ indexes of the Breech strike flock over the 9 years. This was driven by an improvement in body weight, fibre diameter and fleece weight from 2010 to 2014 in both lines. This could also have been due to the lower incidence of breech strike due to crutching which resulted in a higher accuracy of selection and thus in an increased genetic response in fleece weight. It is clear that in 2014 there were no differences in fleece weight between these lines and that of the industry flocks that participate in the Sheep Genetics performance evaluation scheme.

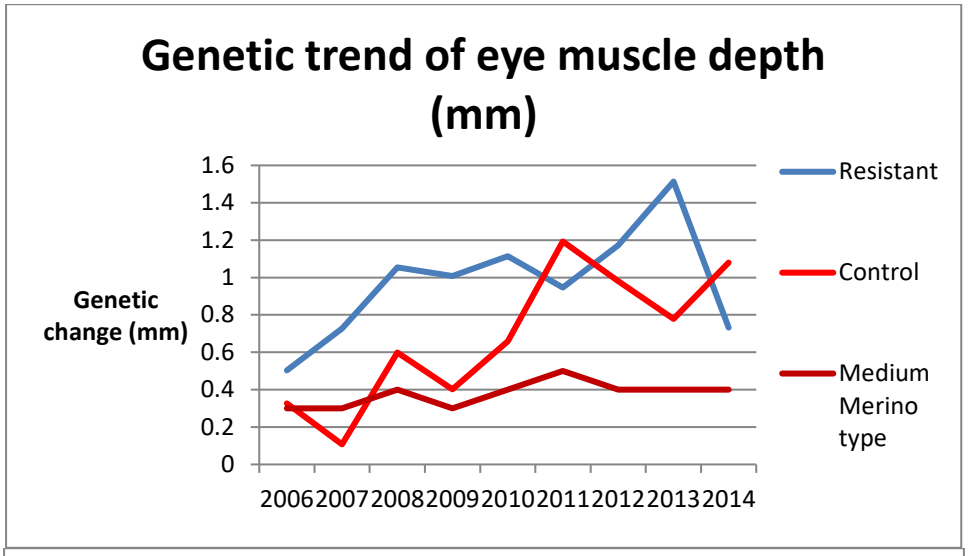
In 2014 the Breech strike flock was about 4kg heavier with 0.3mm more fat, 0.8mm more muscle and produced the same amount of wool that was 2-3N/Ktex sounder and that was 1 micron stronger than the ram breeding industry flocks. But in spite of the higher fibre diameter of these research flocks, their DP+ index was about 10% index points higher and had virtually the similar average MP+ index value of industry flocks. The research flocks were also significantly more resistant to worms as indicated by their lower faecal worm egg counts compared to industry flocks.

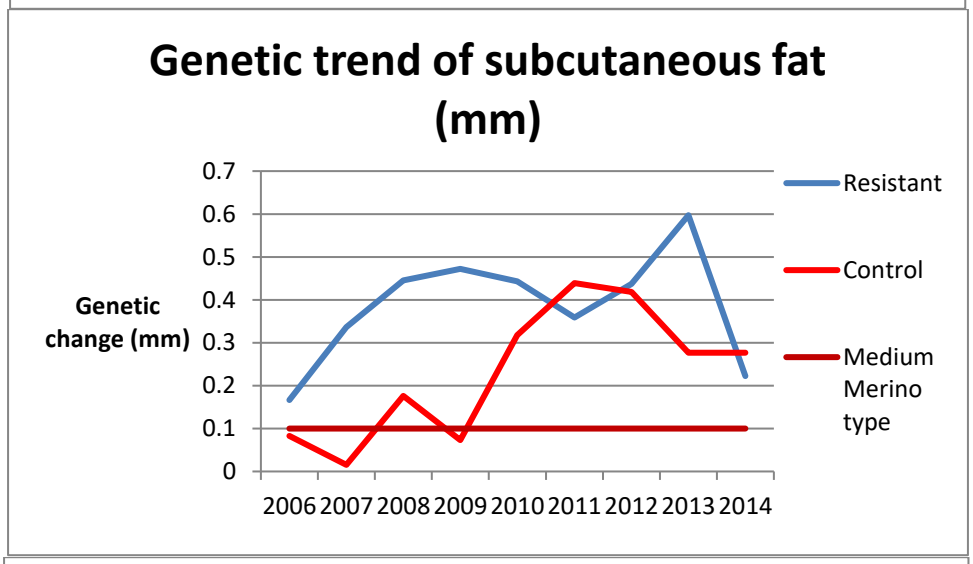
With regard to the number of lambs weaned, the resistant and control lines fluctuated around the industry mean except in 2013 and 2014 when they weaned about 2% more lambs than the industry flocks.

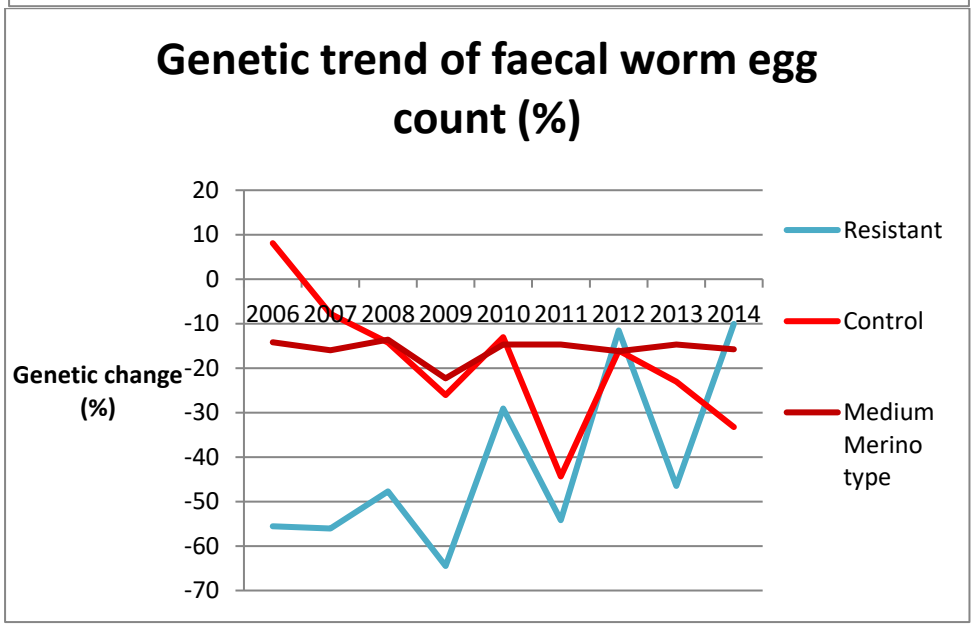
Thus, these lines have undergone significant genetic changed during the last 10 years from when they were sourced from industry flocks. Today this flock can be considered to be comparable to the better performance bred studs across the country and will certainly be above average compared to the wider commercial industry flocks.



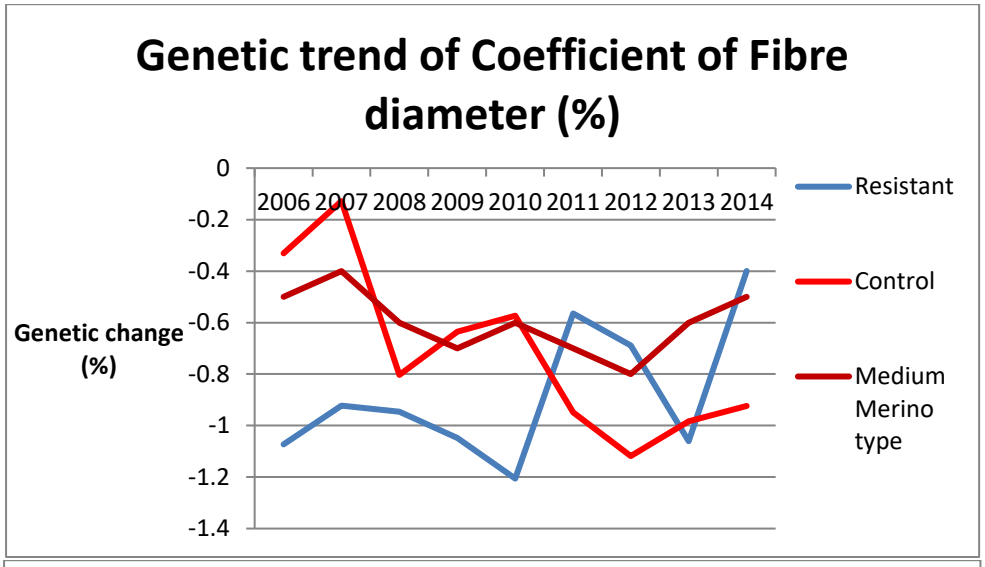
Figures 9a and b. Genetic trend of production traits between the Breech strike flocks and industry flocks.

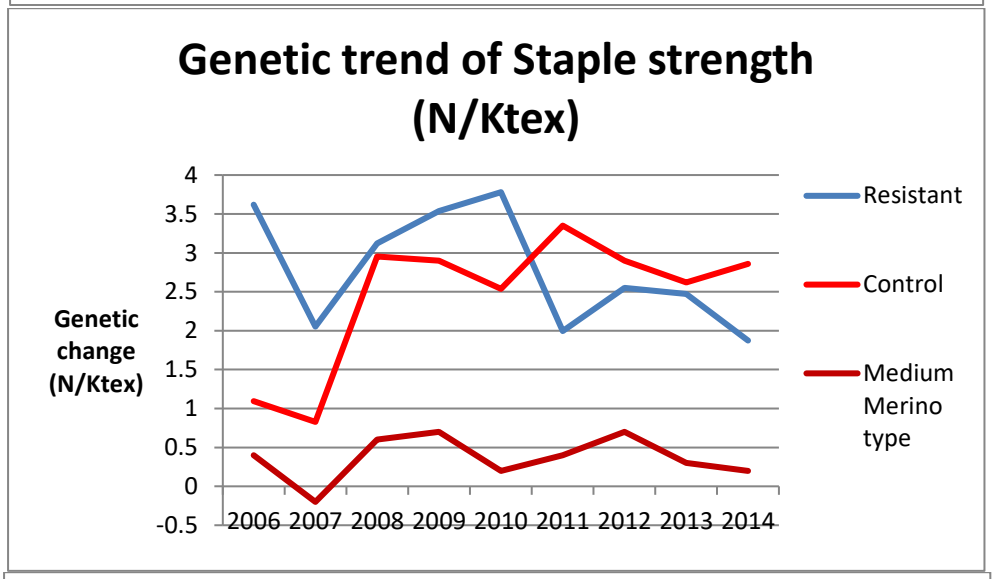


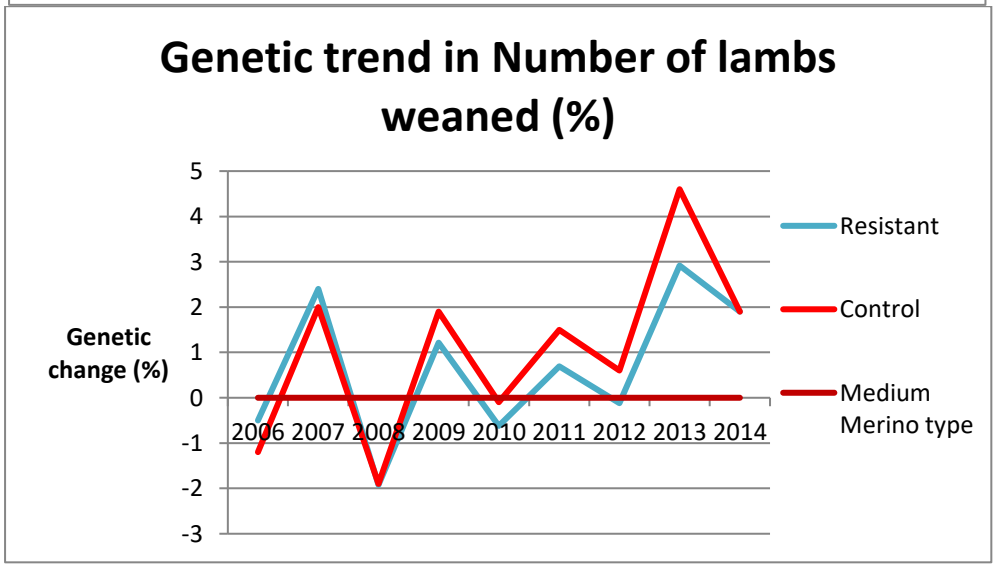




Figures 9c, d and e. Genetic trend of production traits between the Breech strike flocks and industry flocks.







Figures 9f, g and h. Genetic trend of production traits between the Breech strike flock and industry flocks.

Repeatability of breech strike

Research on the Breech strike flock showed that sheep that were identified as highly susceptible at hogget age were more likely to be struck repeatedly in subsequent years. Table 10 shows the incidence of breech strike in the two most susceptible and two most resistant sire progeny groups that were born in 2008. They were challenged with flies during their lifetime.

The hoggets were not crutched prior to the fly season and no preventative treatments were applied. The rams were discarded after hogget shearing and all the ewes were kept as replacements. These ewes were mated annually and were crutched before lambing, but no other preventative treatments were applied. These ewes were regularly sampled for wool odour studies, and also used to train the sniffer dogs in odour recognition (Greeff et al. 2013).

Table 10. Incidence of breech strike of the two most resistant and two most susceptible sire progeny groups born in 2008.

		Resistant		Susceptible
Trait	N	%	n	%
Hogget*	85	5.7	66	98.6
3 year	32	0.0	37	54.2
4 year	31	0.0	33	10.7
5 year	27	0.0	30	16.5

*Hoggets were not crutched prior to fly season but were crutched at later ages

The reduction in numbers of ewes is due to the natural death rates in this group. It is clear that a very small number (5.7%) of the resistant group were struck prior to hogget age while nearly every ewe (98.6%) in the susceptible group was struck over the same period. In subsequent years when the breeding ewes were crutched according to industry practice, none of the resistant ewes were struck up to five years of age, whereas a relatively large proportion of the susceptible ewes continued to be struck in every year. The relative low rates of strikes in year 4 (10.7%) and year 5 (16.5) were due to a naturally low strike rate in these two years. The incidence of breech strike in the total flock in this group's fourth year (2011) was very low as only 1.5% (13 struck amongst 888 mature ewes available) were struck. Similarly, the flock's average struck rate was 6% (54 struck amongst 903 mature ewes available) in 2012 when this group was in their fifth year. This indicates that these ewes in Table 6 had much higher strike rates than their contemporaries.

Progeny testing

Progeny testing is a more accurate method to obtain reliable levels of resistance to breech strike. Figure 10 show the relationship of breech strike of the sire progeny groups of the 2012 born lambs over two seasons, i.e. progeny group strike rates during the 2014 season against that recorded during the 2013 season in crutched sheep.

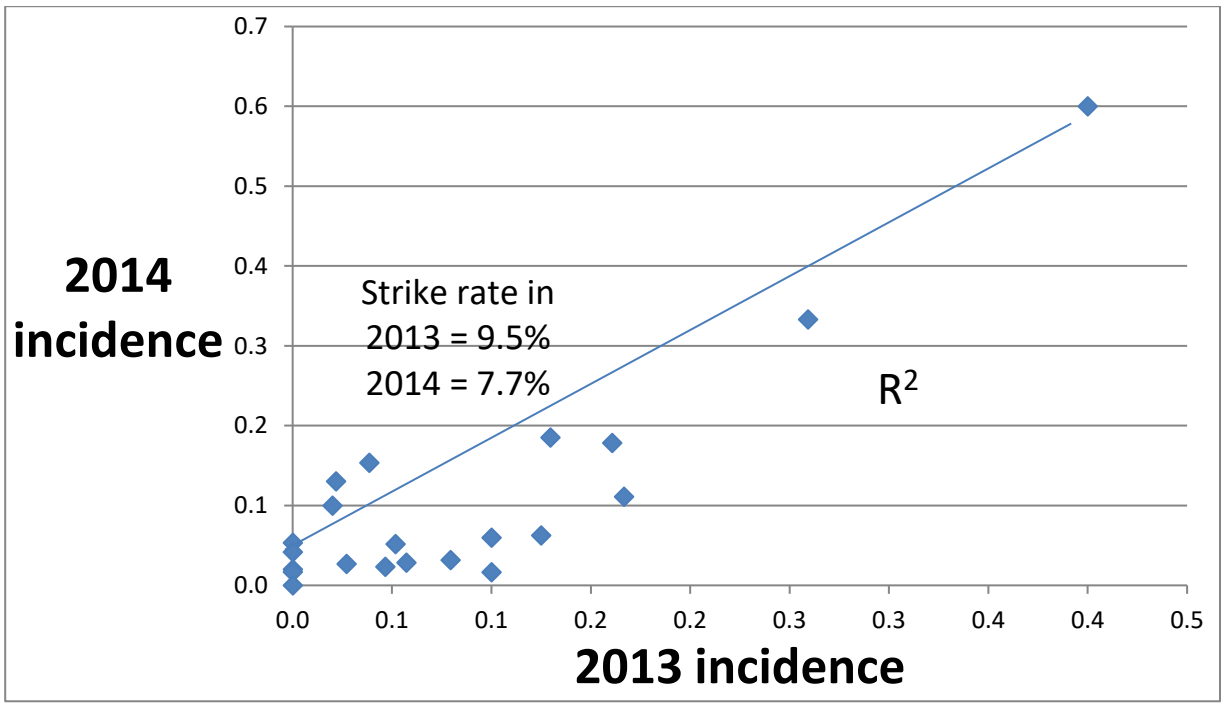


Figure 10, Average breech strike incidence of the 2012 born sire progeny groups in 2014 against their incidence of breech strike in 2013.

A strong relationship was found between the ranking of sire progeny groups in 2013 and in 2014. This shows that the trait is repeatable and supports the results obtained with the extreme ewes in Table 10. This implies that progeny testing will be able to differentiate between sires even when the incidence of breech strike is around 10%.

It is important to realise that this estimate does not refer to the repeatability of the trait on an individual animal, but to the average of the sire progeny group. The two extreme outliers appear to impact heavily on the trend. However, if one should remove the most extreme point then the R-square value decreased from 0.77 to 0.73. Removing the second most extreme point decreased the R-square value to 0.51. However, there is no scientific reason or justification to remove these two extreme points. This positive trend needs to be confirmed in different years under conditions where the animals experiencing a high challenge.

With the option to retrospectively being able to identify genetically resistant and susceptible animals and progeny group more accurately than any other flock for breech strike, makes this flock an extremely valuable resource for future investigations to determine what attract flies to specific susceptible sheep. Therefore, these flocks should be maintained until the attractants in susceptible sheep to breech strike, have been identified.

References

Greeff JC, Biggs A, Grewar W, Crumblin P, Karlsson LJE, Schlink AC and Smith J (2013) Dogs can differentiate between odours from sheep that are resistant or susceptible to breech strike. Proceedings of the 20th Conference of the Association for the Advancement of Animal Breeding and Genetics. Held in New Zealand from 20 to 23 October 2013.

Elucidating the underlying biological causes of differences in breech strike between resistant and susceptible sheep

Differences in moisture, wax, suint and dust content between resistant and susceptible sheep

Background

Anecdotal comments from breeders indicated that moisture content in greasy wool could be associated with yellowing, fleece rot and flystrike (Raadsma, 1989). Moisture content is related to suint, wax and dust content. Dowling et al (2006) showed that moisture content is a heritable trait and is correlated with yield and suint content of the fleece. As yield is mostly dust and wax content (Ladyman *et al,* 2003), this study investigated whether there is a relationship between these four traits and breech strike.

Methodology

Mid-side wool samples were collected from 671 hogget ewes and rams from the Breech strike experiment at the Mt Barker research station.

Greasy wool samples were conditioned at a temperature of 20◦C and a relative humidity of 65% for 24 h (IWTO 1996), measured for yield (AS/NZS 2000). Dust penetration was measured on 10 staples and the results averaged. Wax, suint and dust content were determined using a modification of the column extraction method outlined by Hemsley and Marshall (1984). The method was modified to calculate weight loss of wax, suint and dust following extraction rather than centrifugation. Wax, suint and dust content were expressed as percentages of clean, dry wool and termed wax, suint and dust indexes. Moisture index was determined by exposing clean wool dried at 105°C for 16 h to a conditioned environment at a temperature of 20°C and a relative humidity of 65% for 24 h (IWTO 1996(Dowling *et al*. 2006)

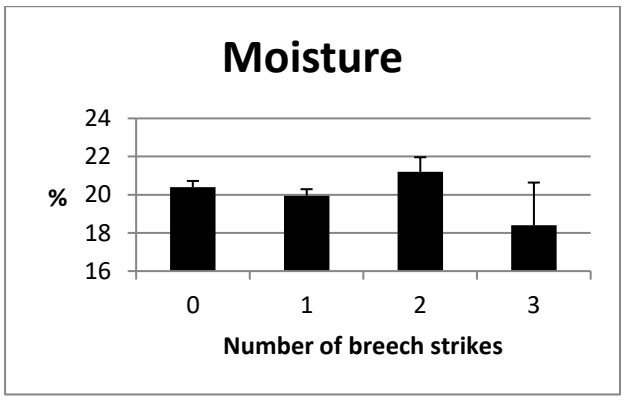
Statistical analysis

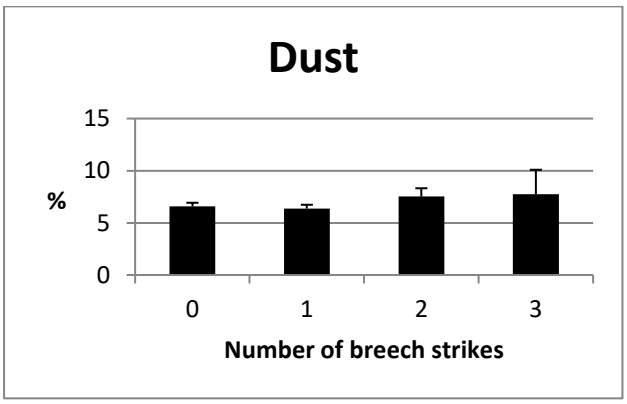
The data were analysed with analysis of variance procedures to determine whether there are significant differences between resistant and susceptible groups for these traits, where groups consists of animals that were not struck, struck once, twice or three times from birth to hogget shearing at approximately 17 months of age.

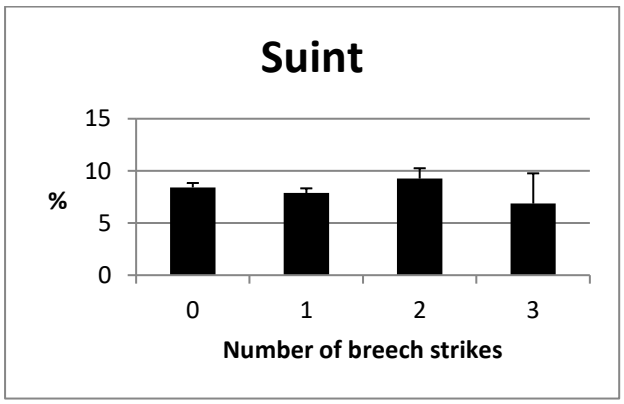
Results

Figure 11 shows the relationship between these four traits and number of breech strikes from birth to hogget shearing.

Figure 11 clearly shows that there was no relationship between moisture, wax, suint and wax content of wool sampled from the mid-side site and number of breech strikes experienced. This supports James (2006) that it is unlikely that these traits contribute directly to breech strike. However, Steer (2015) showed that breech wool from the resistant line had a higher proportion of wax than the control line (16.4% vs 12.7%).







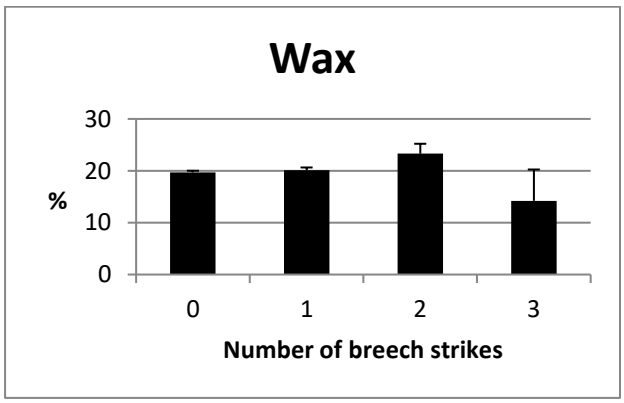


Figure 11. Relationship between moisture, dust, suint and wax content in midside wool samples and numbers of strikes.

Conclusion

These results suggest that there is no relationship between moisture, wax, suint and wax content of wool sampled from the mid-side site and number of breech strikes experienced.

References

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Differences in microclimate in the breech of resistant and susceptible sheep for breech strike

Background

Wool is more hygroscopic than any other textile raw material. Young (1955) showed that the amount of moisture varies across the fleece with the wool on the upper parts of the body having lower amounts of moisture. However, no one studied whether differences in moisture content exist between different sheep that are genetically different in breech strike. This study was carried out to determine whether there are differences in moisture content and temperature between resistant and susceptible sheep for breech strike.

Material and methods

Differences in the micro-climate at regular intervals in the breech, was assessed by using micro data loggers that record temperature and humidity over different times on the sheep. This was done to elucidate the biological causes of breech strike. Data were collected on ewes and rams. The data were collected repeatedly over a 2 month period during the breech strike season on 19 ewes from the resistant line and on 19 ewes from the susceptible line and that were born in 2013, and on 14 mature rams with a range of breech values for breech strike from October to December in 2014**.** The data loggers were fitted in an open plastic clamp and tied to the wool staple with a cotton string as close as possible to the skin. It was left on the sheep for 2 weeks to record the changes in humidity and temperature in the breech over this period. Four sheep were tested per line during each 2 week period. After completion, the data were downloaded, and the loggers fitted again on another four sheep per sheep per line.

The following photo shows the data loggers and how they were fitted to the wool in the breech area.

Picture of the data loggers and how they were fitted to the wool in the breech area

Statistical analysis

The temperature and humidity data were analysed with ASREML (Gilmour et al. 2007). Line, day and the time of day, and their interactions were fitted in a linear model. The data from the mature rams were regressed against their Breech strike ASBV.

Results

Figures 12 and 13 show the changes in humidity and temperature in the breech of known resistant and susceptible ewes over a 24 hour period.

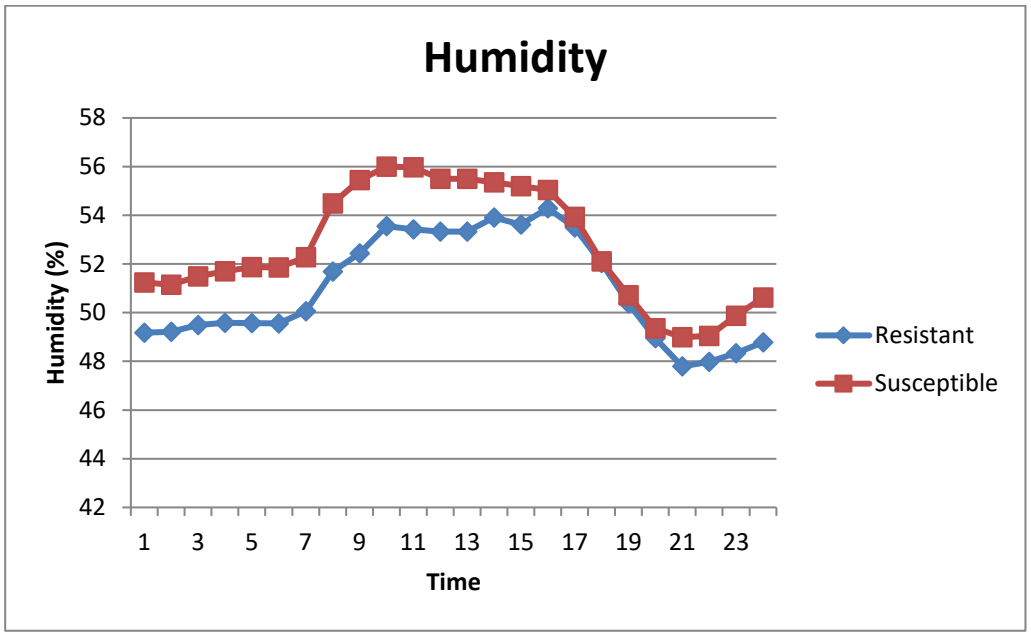


Figure 12. Changes in humidity in the breech at skin level of resistant and susceptible mature ewes over a 24 hour period.

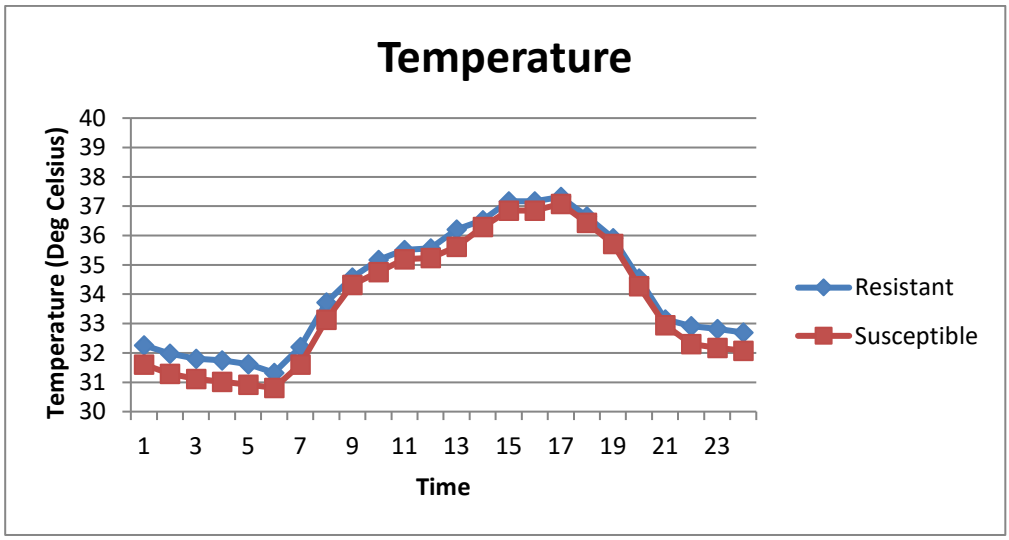


Figure 13. Changes in temperature in the breech at skin level breech of resistant and susceptible mature ewes over a 24 hour period.

The largest difference between the lines was found during the night and the morning up to early afternoon, when the difference disappeared. However, the difference started to appear again from 8-9pm. A similar pattern was found for temperature but less pronounced. A possible solution for this pattern is that the fleece dries out during the afternoon but as soon as the temperature drops then the difference in humidity starts to appear again. These results show that differences in micro-climate exist between resistant and

susceptible sheep and supports the work of Mulcock and Fraser (1958). However, they found that the difference was only present during dry times. They concluded that susceptible fleeces have more bacteria but with a lower diversity than immune fleeces.

Figures 14 and 15 shows the relationship between the average humidity and Breech strike ASBV and between the average temperature and Breech strike ASBV in mature rams that were sampled over a 2 week period.

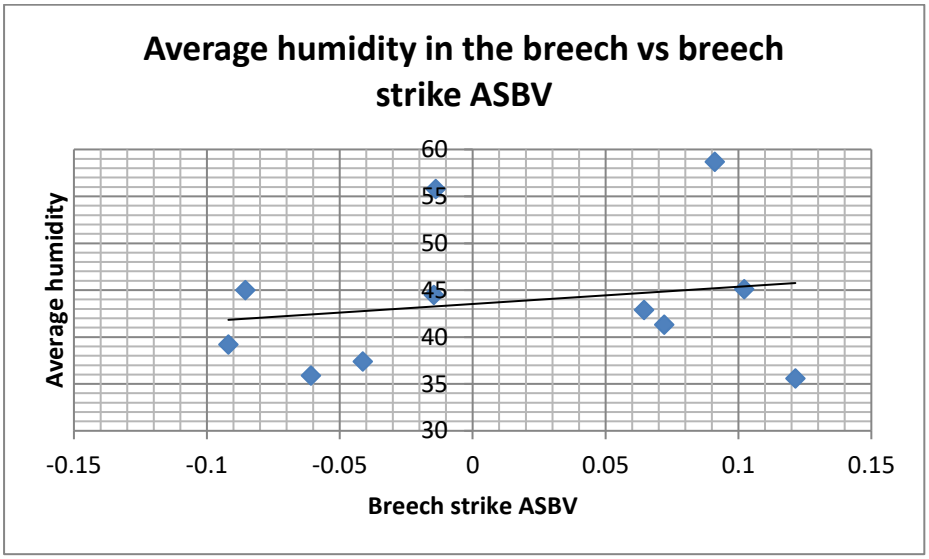


Figure 14. The relationship between the average humidity in the wool in the breech area and Breech strike ASBV in mature rams.

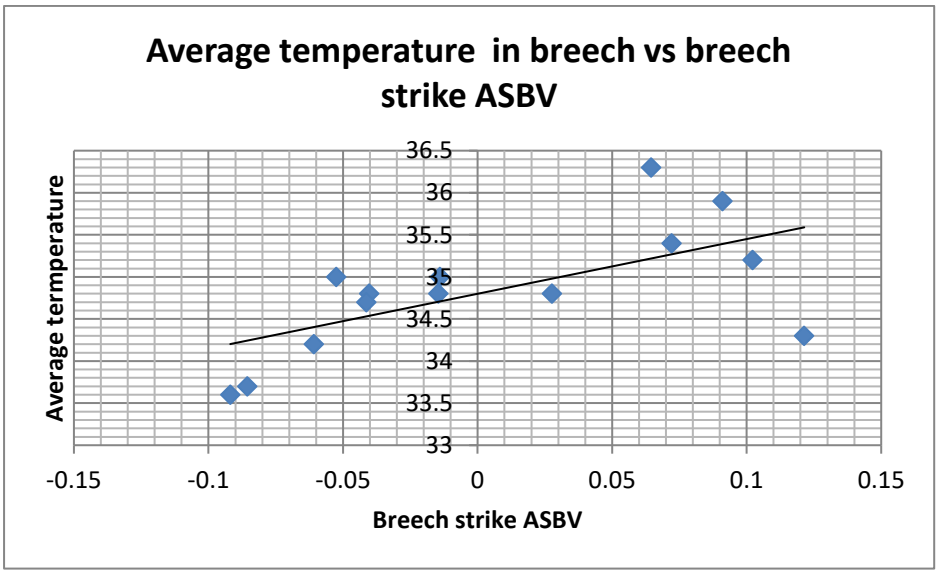


Figure 15. The relationship between the average temperature in the wool in the breech area and Breech strike ASBV in mature rams

The relationship between humidity and Breech strike ASBV in mature rams was positive (b = 18.3 ± 31.2; R^2 = 0.03) but this trend was not significant (P = 0.34). It was however, in the same direction as found in the ewes.

The relationship between temperature and Breech strike ASBV (b = 6.5 3.2; R^2 = 0.40) was highly significant (P=0.015). But this trend was in the opposite direction to that found between the ewe lines. It is unclear as to why this could be the case.

These results indicate that there are differences in temperature and humidity of wool in the breech area of resistant and susceptible sheep, but more investigations are required to elucidate the relationships of breech strike ASBV with humidity and temperature. These results support the role of moisture in breech strike where dag moisture has been found to play a significant role in breech strike.

References

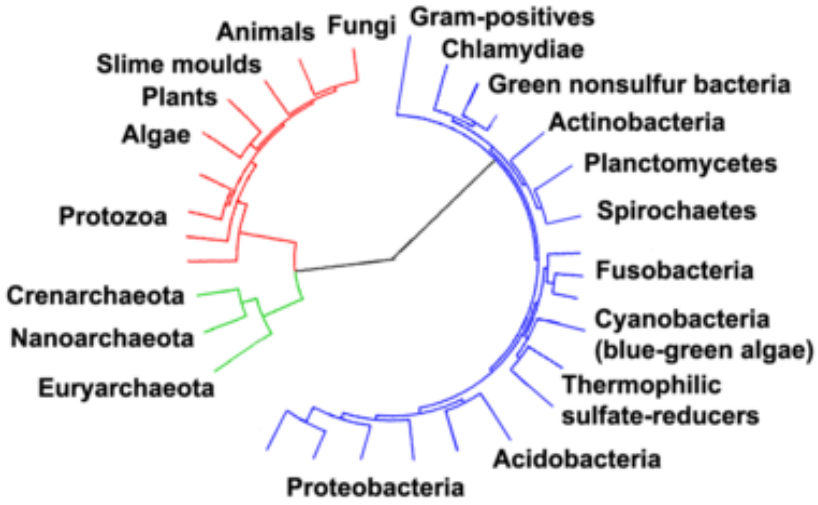
Young SSY (1955) A survey of moisture content in some greasy wool from New South Wales. **Australian Journal of Agricultural Research 6**, 624–639.

Mulcock AP, Fraser IEB (1958) Total counts of microorganisms in the fleece of two Corriedale fleece types. **Australian Journal of Agricultural Research 9**, 704–707.

Differences in microbial populations between resistant and susceptible lines for breech strike

Background

Previous research with sniffer dogs (Greeff *et al*. 2013) have shown that there is a difference in odour between resistant and susceptible sheep. Mulcock and Fraser (1958) showed that significant differences exist between the numbers of bacteria in the fleece of sheep which have been selected as immune and susceptible respectively to bacterial discoloration. This study aims to determine whether differences exist in the microbial populations in the breech of resistant and susceptible sheep that could give rise to differences in odour. The following phylogenetic tree diagram shows the diversity of bacteria compared to other organisms. Eukaryotes are coloured red, archaea green and bacteria blue.



Material and Methods

Two different methods were used to collect tissue samples for evaluation.

1. Swabbing the skin in the breech

Two different studies were carried out.

Study 1

Eight extreme ewes from both resistant and susceptible lines that were born in 2008 were swabbed with sterile Amies swabs on their rump when it was dry in late autumn and again in early spring prior to the fly season after the animals were regularly wetted by rain. The samples were cultivated in the DAFWA laboratory in South Perth and tested for the presence of a range of bacterial species. The pH on the skin was also measured to determine whether differences in environment exist between resistant and susceptible sheep. This was part of an honour's project by Josh Hendry in his final year in Agricultural Science at the University of Western Australia.

Study 2

Eighty six extreme resistant and susceptible animals from the 2013 born ewe and ram hoggets were identified using their ASBV for breech strike. The skin of sheep were swabbed with sterile Amies swabs and cultivated in the DAFWA microbial laboratory.

Culture for aerobic and anaerobic bacteria of the above samples was conducted at the bacteriology laboratory, Animal Health Laboratories, Department of Agriculture and Food WA, (a NATA-accredited laboratory) using standardised methods.

Material on Amies swabs was cultured to blood agar and MacConkey agar plates and incubated at 36^{o}C in an atmosphere containing 5% carbon dioxide for growth of aerophilic organisms. Plates were examined daily for five days. Culture for Gram-negative and Gram-positive anaerobic bacteria was done using Wilkins chalgren agar (Oxoid) containing equine blood, and for Gram-negative anaerobes only, G-N selective supplement (Oxoid) was added to the medium. Plates were incubated in anaerobic boxes (Oxoid) containing a GasPak anaerobic pouch (Becton-Dickinson). Plates were examined for colonies at 24 and 48 hours. Identification of colonies was done using matrix-assisted laser desorption time of flight mass spectrometry (Bruker Daltonics) or conventional biochemical methods.

2. Species profiling using DNA technology

An initial study was carried out on 2 mature resistant rams and two mature susceptible rams in a preliminary study. The skin samples of these rams were tested for microbial activity using DNA profiling. Promising results were found which resulted in a larger sampling of hogget rams and ewes that were born in 2012, 2013 and 2014.

Skin samples were collected in the breech of each sheep born in 2012, 2013 and 2014 in the Mt Barker breech strike flock prior to the onset of the breech strike season. These samples were frozen and stored until after the flystrike season was completed. The breeding values for breech strike were used to identify the most resistant and susceptible rams and ewes in each drop. Fifteen of the most resistant rams and ewes, and 15 of the most susceptible hogget rams and ewes were identified per year of birth. Following the results from this analysis on the hoggets, another small set of mature rams similar as in the preliminary study (2 resistant and 2 susceptible), were again skin sampled for testing. All the skin samples were forwarded to Australian Genome Research Facility (AGRF) for the profiling of the microbial populations in and on the skin using 16S DNA technology.

Bioinformatics methods

Image analysis was performed in real time by the MiSeq Control Software (MCS) v2.6.1.1 and Real Time Analysis (RTA) v1.18.54, running on the instrument computer. RTA performs real-time base calling on the MiSeq instrument computer. Then the Illumina bcl2fastq 2.17.1.14 pipeline was used to generate the sequence data. The target was 27F-519R and the Forward (27F) and Reverse Primers used were AGAGTTTGATCMTGGCTCAG and GWATTACCGCGGCKGCTG, respectively with a read length of 300 base pairs(bp).

Paired-ends reads were assembled by aligning the forward and reverse reads using PEAR (version 0.9.5) (Zhang et al. 2014). Primers were trimmed using Seqtk (version 1.0). Trimmed sequences were processed using Quantitative Insights into Microbial Ecology (QIIME 1.8) (Caporaso et al. 2010) USEARCH (version 8.0.1623) and UPARSE software (Edgar et al. 2010; Edgar et al. 2011).

Using USEARCH tools sequences were quality filtered, full length duplicate sequences were removed and sorted by abundance. Singletons or unique reads in the data set were discarded. Sequences were clustered followed by chimera filtered using “rdp_gold” database as reference. To obtain number of reads in each OTU, reads were mapped back to OTUs with a minimum identity of 97%. Using Qiime taxonomy was assigned using Greengenes database (Version 13_8, Aug 2013) (DeSantis et al. 2006). The data generated here meet the Australian Genome Research Facility (AGRF) quality standards. The data yield is shown in Appendix 3 for the different samples.

Statistical analysis

Different diversity indices were calculated from the data obtained from the swabbed skin samples using the methods described by ecologists (Ludwig and Reynolds, 1988). The data from the swabbed skin samples

were analysed with analysis of variance fitting line, sex and their interaction using the Genstat computer package.

Species profiling using DNA technology

The data on the 2012 and 2013 born animals were analysed separately from that of the 2014 born animals as the 2012 and 2013 drop animals were crutched while the 2014 drop were not crutched. This resulted in a large difference in the incidence of breech strike between these two groups. The 2012 and 2013 born groups were categorized in resistant and susceptible on the basis of the breeding value for breech strike resistance because of the low incidence of flystrike experienced. The 2014 born animals were grouped in resistant and control based on whether they were struck by flies or not. The effects of line (resistance vs susceptible), year (2012 and 2013) and sex (male and female) and their interactions were fitted in a linear model on the 2012 and 2013 dataset while only line, sex and the interaction was fitted on the 2014 microbial data to determine whether differences exist between resistant or susceptible sheep for breech strike.

Results

Swabbed skin samples

Study 1 (Josh Hendry Honour's project)

No significant differences were found in pH levels between resistant and susceptible sheep. The average pH (±SE) of the skin was 8.6 ± 0.35 and 8.5 ± 0.46 for the resistant and susceptible groups respectively.

The presence of bacteria between the resistant and susceptible ewes born in 2008 are shown in Table 11.

Table 11. Number of isolates (%) between resistant and susceptible ewes for breech strike in dry and wet conditions in WA.

	Dry conditions		Wet conditions	
Resistance Level in sheep	***Resistant***	***Susceptible***	***Resistant***	***Susceptible***
Total number of isolates obtained	**99**	**93**	**89**	**87**
Bacterial species				
Staphylococcus sp.	*32 (32.2)*	*29 (31.2)*	*30 (33.7)*	*27 (31)*
Micrococcus sp.	*9 (9.1)*	*14 (15.1)*	*16 (18)*	*14 (16.1)*
Bacillus cereus sp.	*14 (14.1)*	*9 (9.7)*	*5 (5.6)*	*12 (13.8)*
Bacillus licheniformis	*18 (18.2)*	*13 (14)*	*10 (8.9)*	*11(12.6)*
Bacillus sp.	*1 (1)*	*10 (9.3)*	*6 (6.7)*	*9 (10.3)*
Neisseria sp.	*0 (0)*	*3 (3.2)*	*0 (0)*	*0 (0)*
Paeni-bacillus sp.	*0 (0)*	*0 (0)*	*0 (0)*	*1 (1.1)*
Moraxella sp.	*0 (0)*	*1 (1.1)*	*1 (1.1)*	*2 (2.3)*
Acinetobacter sp.	*7 (7.1)*	*1 (1.1)*	*7 (7.9)*	*0 (0)*
Lactobacillus sp.	*0*	*1 (1.1)*	*0 (0)*	*0 (0)*
Corynebacterium sp.	*7 (7.1)*	*1 (1.1)*	*0 (0)*	*0 (0)*
Bifidobacter sp.	*2 (2)*	*0 (0)*	*2 (2.2)*	*3(3.4)*
Elizabethkingia sp.	*1 (1)*	*0 (0)*	*0 (0)*	*0 (0)*
Sphingobacterium sp.	*1 (1)*	*0 (0)*	*0 (0)*	*0 (0)*
Enterobacter sp.	*1 (1)*	*0 (0)*	*0 (0)*	*0 (0)*
Chryseobacterium sp.	*5 (5.1)*	*0 (0)*	*0 (0)*	*1 (1.1)*
Flavobacterium sp.	*0 (0)*	*0 (0)*	*3(3.4)*	*1 (1.1)*
Actinomyces sp.	*0 (0)*	*3 (3.2)*	*3 (3.4)*	*0 (0)*
Streptococcus sp.	*0 (0)*	*0 (0)*	*1 (1.1)*	*1 (1.1)*

Table 11 shows that the number of isolates increased from dry to wet conditions. However, no significant (P>0.05) differences were found in the number of isolates between resistant and susceptible ewes in the wet or in the dry times of the year.

Study 2

No significant differences were found in the number and type of cultured aerobic or cultured anaerobic bacteria between resistant and susceptible rams from both lines.

Table 12 shows the results of different diversity indices between the resistant and susceptible lines and sexes from the swabbed skin. The different indices are described in Ludwig JA and Reynolds J (1988; Statistical ecology. John Wiley and Sons Brisbane). These different diversity scores measure effectively the same phenomenon but does it in different ways. No significant differences were found between lines or between male and female sheep for any of the diversity indices indicating that there are no differences in diversity of species in this experiment.

Table 12. Species diversity indices of resistant and susceptible breech strike sheep from swabbing the skin.

Diversity statistic	Female Rest	Female Suscept	Male Rest	Males Suscept	SED Line	SED Sex
Richness index (R1)	1.81	1.92	1.840	1.927	0.089	0.089
Richness index (R2)	1.60	1.62	1.611	1.666	0.051	0.051
Simpson's Diversity	0.12	0.11	0.115	0.102	0.008	0.008
Hill diversity index (N0)	5.43	6.06	5.571	5.824	0.327	0.326
Hill diversity index (N1)	5.32	5.87	5.451	5.631	0.33	0.335
Hill diversity index (N2)	9.61	10.02	9.594	10.333	0.658	0.655
Shannon's index (H')	1.63	1.72	1.670	1.707	0.064	0.064

SED = standard error of difference

Species profiling using DNA technology

Bacteria

Mature rams in Groups 1 and 2.

Table 13 shows the different phyla present in group 1 for the preliminary study and for group 2 in the subsequent follow up study. The resistant sample in Group 1 showed a larger diversity of phyla relative to the susceptible group. Ten different phyla were found in the resistant group that were absent in the susceptible group. This resulted in a larger study of which the results are shown in Tables 14 and 15.

Following the results from the subsequent studies, a follow up study was carried out on another set of mature rams. Their microbial profiling results are also shown next to the initial study in Table 12.

The resistant rams in Group 2 had a larger number of phyla relative to that of the susceptible group. The resistant group had 3 phyla extra that were absent in the susceptible group. However, there is a clear pattern in that the susceptible rams had a higher proportion of Fermicutes, Lentispaerae, Spirochaetes, Tenericutes and Verrucomicrobia that the resistant rams in both Groups 1 and 2.

Table 13. Relative proportions of Phyla bacterial profiles in two groups of resistant (Rest) and susceptible (Suscept) rams sampled at different times.

Phyla	Group 1 (Preliminary study)		Group 2 (Follow-up study)	
	Suscept/Rest	Rest/Suscept	Suscept/Rest	Rest/Suscept
Acidobacteria	0.18	5.64	0.40	2.53
Actinobacteria	0.44	2.29	1.16	0.86
Armatimonadetes	0.00	**only Rest**	0.45	2.20
BRC1	0.60	1.68		Only Rest
Bacteroidetes	1.02	0.98	1.57	0.64
Chlorobi	0.00	**only Rest**	0.81	1.23
Chloroflexi	0.30	3.37	0.39	2.58
Cyanobacteria	0.16	6.32	0.18	5.63
Deferribacteres	3.19	0.31		0.00
Elusimicrobia	2.50	0.40	Only Susc	0.00
FBP				Only Rest
Fibrobacteres	0.76	1.32	1.45	0.69
Firmicutes	4.22	0.24	5.69	0.18
Fusobacteria	0.81	1.24		Only Rest
Gemmatimonadetes	0.19	5.26	0.56	1.78
Lentisphaerae	10.62	0.09	17.11	0.06
NKB19	0.00	**only Rest**		
Nitrospirae	0.00	**only Rest**	0.00	
OD1	0.00	**only Rest**	0.00	
OP11			0.42	2.40
Planctomycetes	0.14	7.10	0.39	2.58
Proteobacteria	0.49	2.02	0.81	1.24
SR1	0.00	**only Rest**		
Spirochaetes	4.61	0.22	14.91	0.07
TM6	0.00	**only Rest**		
TM7	1.26	0.79	1.47	0.68
Tenericutes	8.26	0.12	23.74	0.04
Thermi	0.08	12.42	0.71	1.40
Verrucomicrobia	4.97	0.20	3.82	0.26
WPS-2	0.00	**only Rest**		
WS2	0.00	**only Rest**	0.00	
WWE1			0.00	
WYO	0.00	**only Rest**		

Mt Barker hoggets

Table 14 shows the proportion of microbial phyla in the skin, taken from the breech on resistant and susceptible sheep born in 2012 and 2013 and that were categorized using the breeding values for breech strike. It also shows the proportion of microbial phyla for the 2014 born rams and ewes that were not struck and for the susceptible sheep that were struck during the 2015 blowfly season. In both data sets, a large proportion (~0.55) of DNA reads could not be assigned to a phylum because this DNA is as yet unassigned to different phyla and remains undescribed. The coded phyla (ie FBP) are currently identified as candidate phyla until more information on them become available which will to allow them to be validated.

In the 2012 and 2013 born sheep (Table 13), significant differences ($P<0.05$) were found between resistant and susceptible sheep for the Acidobacteria and Actinobacteria phyla. The amount present was very small for Acidobacteria, whereas the Actinobacteria contributed a relatively large proportion to the total amount, i.e. 24% and 18% for resistant and susceptible groups, respectively.

Significant differences $P<0.05$) were found between the resistant and susceptible lines that were born in 2014 (Table 14) for two unknown phyla (Unknown [1 and 2]), Armatimonadetes, Chloroflexi, Elusimicrobia and the candidate phylum, FBP. Armatimonadetes and Chloroflexi phyla are aerobic and plant-based phyla while the Elusimicrobia is an anaerobic phyla found in ecosystems like marine environment, sewage sludge, soils and termites. Although significant differences ($P=0.03$) exist between resistant and susceptible sheep with an average of 0.008% vs 0.018% per line respectively, the amount of Armatimonadetes present was very small. The resistant line had 0.145% Chloroflexi while the average amount in the susceptible line was significantly higher, i.e. 0.235%. A very small amount of Elusimicrobia was found in the resistant (0.004%) line and also in the susceptible (0.001%) line. Similarly, for the FBP phylum.

The phyla that differ significantly within the two datasets are not the same as across the 2012 and 2013 and the 2014 born animal datasets. Thus, it appears that these phyla, although different, do not consistently contribute to breech strike. However, whether these phyla contribute to differences in susceptibility to breech strike will require further studies to validate their effects, but it appears unlikely.

The microbial pattern that was found in the mature rams tested in the preliminary and follow up studies, which showed that the susceptible rams had a larger proportion of certain phyla than the resistant rams were not found in the 2014 and in the 2012 and 2013 born hogget ewe and ram groups. Thus, this effect may be due to a paddock effect and/or to an age effect.

Table 14. Proportion of phyla present on and in the skin sampled from the breech area in rams and ewes from the resistant and susceptible lines for breech strike that were born in 2012 and 2013 at Mt Barker.

Phylum	Resistant	Susceptible	SED	Female	Males	SED	2012	2013	SED
Unassigned	0.5045	0.5430	0.0520	0.5322	0.5050	0.0497	0.6451[a]	0.4226[b]	0.0514
Unknown [1]	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Acidobacteria	0.00002[a]	0.0001[b]	0.0001	0.0001	0.0000	0.0001	0.0001	0.0001	0.0001
Actinobacteria	0.2393[a]	0.1838[b]	0.0298	0.2039	0.2322	0.0284	0.1594	0.2618	0.0293
Armatimonadetes	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Bacteroidetes	0.0162	0.0174	0.0032	0.0181	0.0151	0.0031	0.0133[a]	0.0193[b]	0.0032
Chlorobi	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Chloroflexi	0.0000	0.0002	0.0001	0.0001	0.0000	0.0001	0.0001	0.0001	0.0001
Cyanobacteria	0.0002	0.0002	0.0002	0.0001	0.0003	0.0002	0.0002	0.0002	0.0002
FBP*	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Fibrobacteres	0.0004	0.0002	0.0002	0.0004	0.0002	0.0002	0.0003	0.0003	0.0002
Firmicutes	0.0823	0.0911	0.0180	0.0926	0.0781	0.0172	0.0469[a]	0.1161[b]	0.0178
Fusobacteria	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Gemmatimonadetes	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
GN02*	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Lentisphaerae	0.0002	0.0004	0.0002	0.0003	0.0002	0.0001	0.0002	0.0003	0.0002
OD1*	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
OP11*	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
OP8*	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Other (known)	0.0002	0.0001	0.0001	0.0002	0.0001	0.0001	0.00003[a]	0.0002[b]	0.0001
Planctomycetes	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Proteobacteria	0.1501	0.1500	0.0281	0.1395	0.1621	0.0268	0.1287	0.1666	0.0277
Spirochaetes	0.0003	0.0003	0.0002	0.0002	0.0003	0.0002	0.0005	0.0001	0.0002
Synergistetes	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Tenericutes	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0000	0.0001
TM6*	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
TM7*	0.0038	0.0049	0.0014	0.0058[a]	0.0023[b]	0.0013	0.0026[a]	0.0054[b]	0.0013
Verrucomicrobia	0.0019	0.0074	0.0032	0.0057	0.0024	0.0031	0.0015	0.0062	0.0032
WPS*	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000

[ab] Columns with different superscript differ significantly (P<0.05) for the same factors

*The coded phyla are candidate phyla and to date have not been named.

Figure 16 shows the relationship between an unknown genus of the family Geodermatophilaceae. A clear relationship is found in that the susceptible animals with the positive ASBVs for breech strike having significantly (P<0.01) higher amounts (b=0.000242 ± 0.00006) of this unknown genus than the resistant line.

This family is different to the Dermatophiloceace family which hosts *Dermatophilosis congolensis* which is a major predisposing factor for body strike in the winter rainfall regions (Gherardi *et al*. 1983) but falls in the same Order.

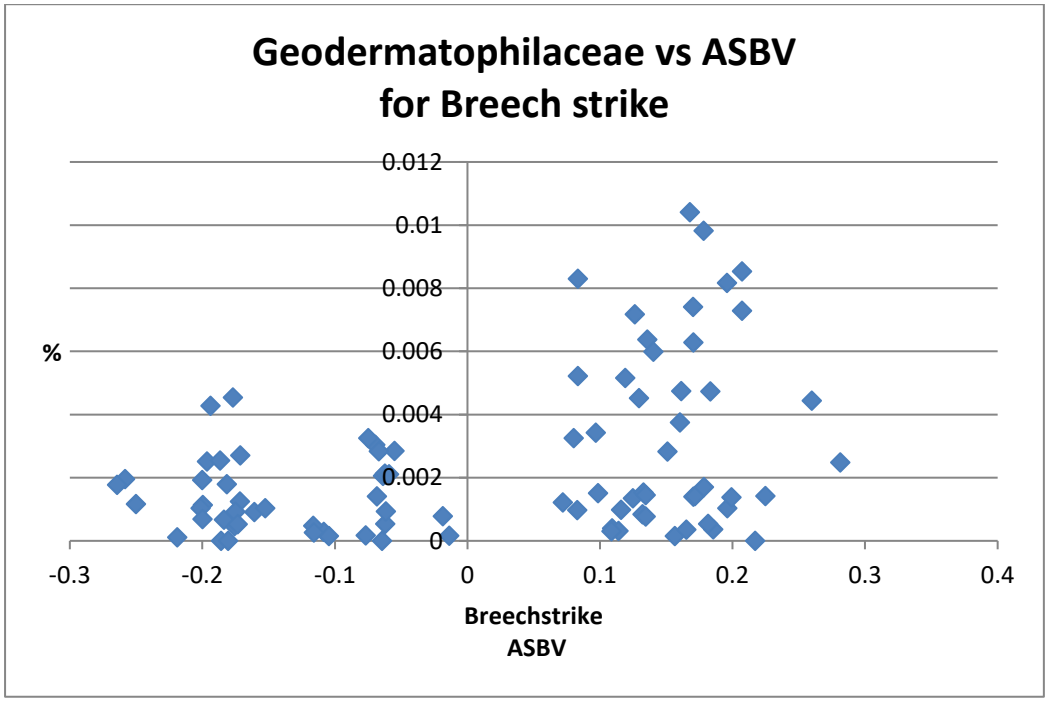


Figure 16. Relationship between Geodermatophilaceae and resistance to breech strike in the 2014 born sheep at Mt Barker research station.

CSIRO hoggets

The CSIRO results are shown in Figure 17 for the groups that had a significant relationship with ASBV of breech strike.

Although a number of significant relationships were found, Figure 17 shows that it is due to a relatively few outliers that lie in the right direction. It is unlikely that these organisms contribute to breech strike in the Armidale flock.

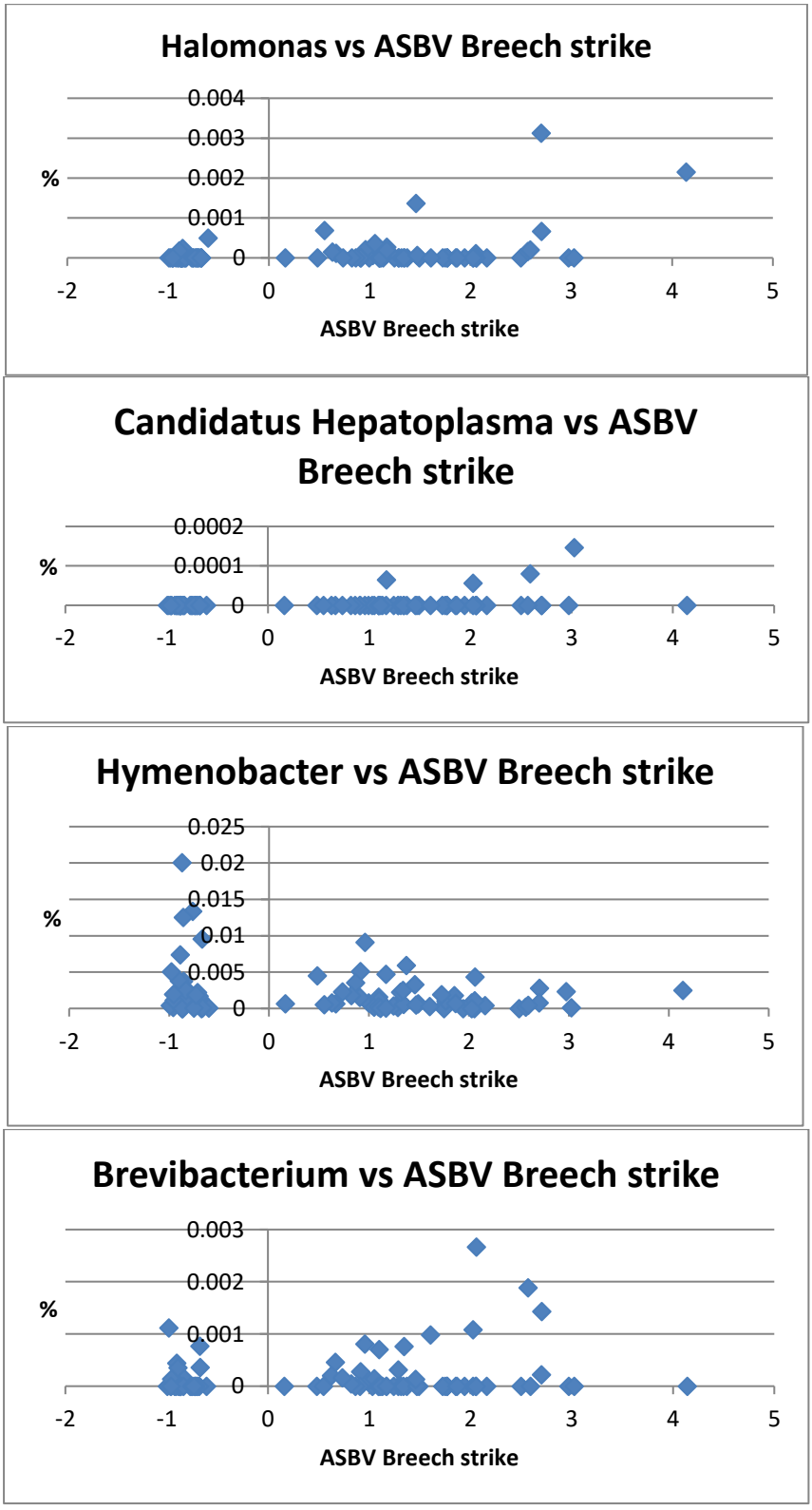


Figure 17. Relationship between different bacteria genuses and ASBV for Breech strike in the CSIRO flock from Armidale

Fungi

One hundred and eighty-one species were identified to be present on the sheep from the CSIRO flock. Of these only *Cladosporium grevilleae*, a member of the Ascomycota phylium had a significant relationship with ASBV of breech strike in the Armidale flock which is shown in Figure 18. However, it is clear, that the relationship is not particularly strong, and it is unlikely that this organism contributes to breech strike susceptibility

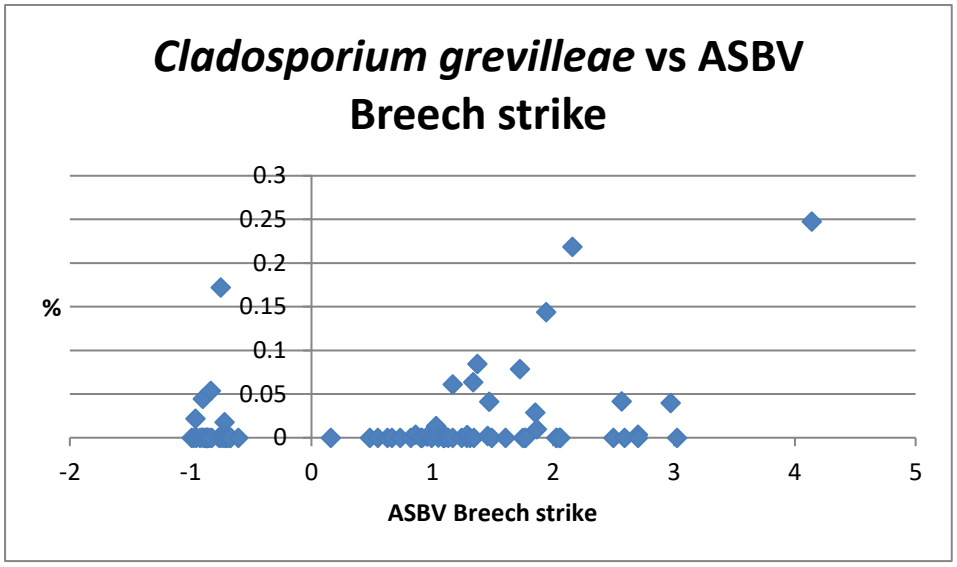


Figure 18. Relationship between the presence of *Cladosporium grevilleae* and ASBV of breech strike in the CSIRO flock

In the Mt Barker breech strike flock 282 Fungi genus and species were identified. However, none of these showed any significant relationship with ASBV for breech strike.

Conclusion

These results indicate that it is unlikely that different phyla of the bacteria and fungi contribute to differences in odour. However, an unknown member of the Bacteria family Geodermatophilaceae appears to have a positive relationship with breech strike in the Mt Barker research flock as the more susceptible sheep have higher amounts of the unknown member of this family. No clear relationships were found between any bacteria species and breech strike in the CSIRO flock in Armidale.

Table 15. Proportion of microbial phylums present in and on the skin, taken prior to the breech strike season, from the breech of unstruck resistant and struck susceptible male and female sheep that were born in 2014. The coded phyla are candidate phyla and to date have not been named.

Phylum	Resistant	Susceptible	Female	Males	SE	P-value Line	P-value Sex	P-value LxS
Unassigned	0.55650	0.52835	0.57765	0.50720	0.06914	0.562	0.155	0.919
Unknown[1]	0.00021	0.00015	0.00014	0.00021	7.49E-05	0.281	0.243	0.405
Unknown[2]	0.00015	0.00058	0.00038	0.00035	0.000216	0.006	0.835	0.997
Acidobacteria	0.00030	0.00044	0.00039	0.00034	0.000161	0.21	0.633	0.372
Actinobacteria	0.18505	0.21930	0.18915	0.21520	0.03732	0.208	0.311	0.552
Armatimonadetes	0.00008	0.00018	0.00015	0.00011	6.71E-05	0.031	0.364	0.183
Bacteroidetes	0.03048	0.03312	0.02706	0.03654	0.007656	0.604	0.089	0.509
BRC1	0.00005	0.00012	0.00011	0.00006	6.87E-05	0.178	0.328	0.925
Chlorobi	0.00004	0.00002	0.00004	0.00003	4.19E-05	0.594	0.824	0.167
Chloroflexi	0.00145	0.00235	0.00170	0.00210	0.000643	0.049	0.378	0.955
Cyanobacteria	0.00095	0.00109	0.00083	0.00121	0.000371	0.531	0.178	0.037
Elusimicrobia	0.00004	0.00001	0.00001	0.00004	1.94E-05	0.039	0.122	0.077
FBP	0.00018	0.00042	0.00042	0.00018	0.000113	0.003	0.003	0.054
Fibrobacteres	0.00060	0.00069	0.00067	0.00062	0.000602	0.786	0.871	0.375
Firmicutes	0.12055	0.08995	0.08140	0.12910	0.02427	0.077	0.007	0.927
Fusobacteria	0.00001	0.00004	0.00001	0.00004	4.58E-05	0.375	0.293	0.195
Gemmatimonadetes	0.00026	0.00031	0.00029	0.00029	0.000147	0.644	0.979	0.981
GN02	0.00000	0.00002	0.00001	0.00001	1.23E-05	0.157	0.902	0.563
Lentisphaerae	0.00055	0.00031	0.00018	0.00067	0.000229	0.15	0.004	0.288
Nitrospirae	0.00002	0.00000	0.00000	0.00002	2.36E-05	0.348	0.442	0.222
NKB19	0.00001	0.00000	0.00000	0.00001	1.11E-05	0.392	0.663	0.255
OD1	0.00001	0.00004	0.00002	0.00004	3.27E-05	0.217	0.499	0.292
OP3	0.00000	0.00000	0.00000	0.00000	4.37E-06	0.356	0.309	0.333
OP8	0.00001	0.00000	0.00000	0.00001	8.44E-06	0.284	0.284	0.261
Planctomycetes	0.00073	0.00092	0.00074	0.00090	0.000317	0.38	0.48	0.496
Proteobacteria	0.08990	0.11465	0.11300	0.09155	0.02138	0.097	0.147	0.412
Spirochaetes	0.00074	0.00042	0.00021	0.00095	0.000284	0.131	0.001	0.053
SR1	0.00001	0.00000	0.00001	0.00000	9.02E-06	0.279	0.238	0.965
Tenericutes	0.00344	0.00077	0.00057	0.00364	0.0033	0.281	0.212	0.215
Thermotogae	0.00001	0.00001	0.00000	0.00001	9.45E-06	0.854	0.485	0.123
TM7	0.00464	0.00314	0.00317	0.00461	0.001954	0.286	0.311	0.7
Verrucomicrobia	0.00277	0.00223	0.00136	0.00364	0.000966	0.463	0.001	0.283
WPS	0.00014	0.00034	0.00029	0.00019	0.000164	0.073	0.389	0.34

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Investigating the phenotypic relationships between diarrhoea and immune parameters

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Background

Dags is the most important predisposing trait to breech strike susceptibility (Greeff *et al.* 2013). It is generally caused by worms and the Australian sheep industry loses A$600m pa (A$370 m for nematode infection; A$147 m for breech flystrike and A$80-160 m for breech soiling) due to losses in the quantity and quality of wool, cost penalties for soiled wool, animal mortality from worms and flystrike, and the chemicals and the labour associated with monitoring, prevention and treatment.

Diarrhoea is the outcome of a series of processes along the intestine. Apart from damage caused by nematodes in the intestinal tract, nematode infection also elicits an immune response which is associated with hypersensitivity and allergic reaction. The most powerful trigger of hypersensitivity is immunoglobulin E (IgE) (Meeusen 1999). During helminth infection, total IgE concentration increases, and more parasite molecules bind to IgE on the surface of mast cells, triggering degranulation, probably by crosslinking IgE, and thus releasing mediators of inflammation. Importantly, this process is under genetic control, as evidenced by the strong values for heritability (h^2):

i) h^2 = 0.39 for the IgE specific for third-stage larvae of *T. circumcincta* in 6-month old Texel lambs (Stear et al. 2011)
ii) h^2 = 0.36 for the IgE specific for *T. colubriformis* in Romney sheep (Shaw, 1999)
iii) h^2 is 0.35 to 0.63 for the peripheral concentration of total IgE against GIT parasites in humans (Grant et al. 2008; Newman et al. 2001).

According to Stear (personal communication) parasite-specific IgA reduces the survival and fecundity of the target parasite in the United Kingdom (Stear et al. 2011; de Cisneros et al. 2014). IgA concentrations are directly correlated in plasma and mucosa, and inversely related to both helminth burden and FEC (Stear et al. 2011; Shaw et al. 2012). Importantly, plasma IgA concentration is highly heritable in sheep (h^2 = 0.56). Modelling studies have suggested that selection for high IgA over seven generations can reduce FEC by 85% whereas selection for low FEC alone can reduce it by only 50% de Cisneros et al. (2014). A novel and effective trait for breeding parasite-resistant sheep, based on a combination of IgA concentration and FEC, could therefore accelerate genetic progress towards resistance.

This study was carried out to determine whether the two immunoglobulins IgE and IgA are phenotypically and genetically correlated with diarrhoea as measured by dag score or faecal consistency score, and faecal worm egg count.

Material and methods

Two Chinese post-doctoral researchers from China, Associate Professor Dr Mengzhi Wang and Dr Zhongquan Zhao were available to investigate the relationships between IgE and IgA with dags. The experimental work was carried out in the laboratories of the Department of Agriculture and Food and at the University of Western Australia. Serum from the breech strike experiment at the Mt Barker research station was available on approximately 800 sheep sampled in 2015. The serum was collected after centrifuging the blood samples and removing the buffy coats for DNA studies.

The immune-assay for IgE is shown in Appendix 4.

Results and discussion

To test the hypothesis, ELISA assays of plasma IgE and IgA specifically against *Teladorsagia circumcincta* have been established in the Parasitology Laboratory of Department of Agriculture and Food Western Australia (DAFWA). We are also trying to establish assay for plasma total IgE with great assistance from Dr Richard Shaw of AgResearch, who has supplied the method and the required reagents, ie the IgE reference serum and two monoclonal antibodies.

Samples and analyses

The sheep were born in June/July 2013 and were the progeny of 22 sires. The number of progeny ranged from 11 up to 44 for individual sires. Blood samples were taken from 748 yearling rams and ewes in Sept (the wet season) 2014, and plasma was harvested by centrifugation and the samples were stored at -70°C until the analysis.

The following assays have been carried out:

The titre of plasma IgA specifically against *Teladorsagia circumcincta*;
The titre of plasma IgE specifically against *Teladorsagia circumcincta*.

Analysis of plasma total IgE is in progress.

In these assays (except for the total IgE), L3 larvae were homogenized, protein was purified and then used as the antigen to test the antibody titre. The titres of IgA and IgE were expressed as % to a reference sample (assigned 100%) which had almost the highest reading amongst the first 50 blood samples analysed in ELISA. The detailed assays for nematode specific IgA and IgE, and the total IgE are shown in Appendix 4.

The titres of nematode specific IgE and IgA in plasma

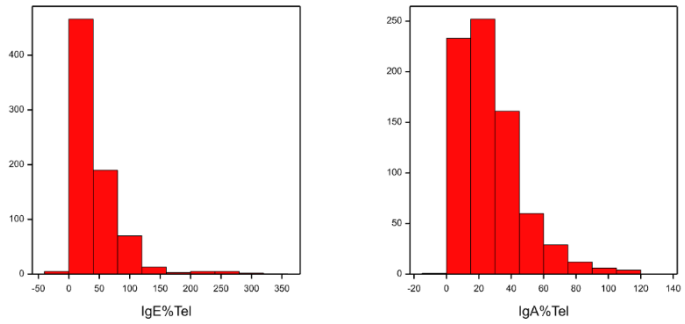

Figure 19. Distributions of the titres of nematode specific IgE and IgA in plasma of 748 Merino sheep against *Teladorsagia circumcincta*.

Distributions of the titres of nematode specific IgE and IgA in plasma are shown in Figure 19. The IgE titration had a biased distribution towards the low end, and among 748 samples, 160 sheep had their values lower than 10%, while a small fraction of sheep fell in the high end, indicating that the number of sheep with very high IgE responses to L3 larvae of Teladorsagia was small. As for the IgA titres, the trends of the nematode specific IgA distribution were also biased slightly towards the low end.

As the IgE and IgA titre distributions were skewed, a transformation to the power of 0.2 was carried out. The transformed data (ie, $IgE^{0.2}$ and $IgA^{0.2}$) were then used for further analyses of the variance and regression.

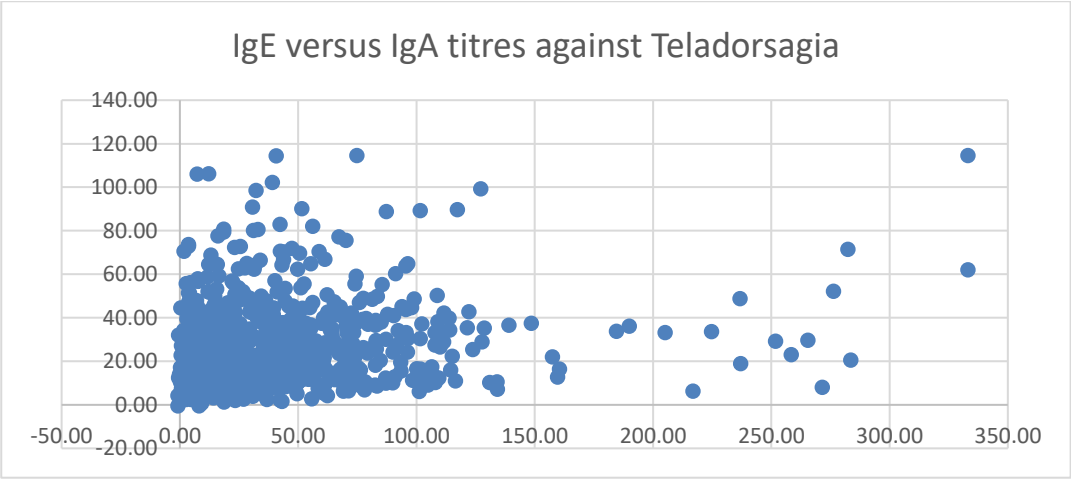

Figure 20. Relationships between the specific IgE and specific IgA titres against Teladorsagia.

There was no relationship between IgE and IgA titres against Teladorsagia (Figure 20). This could imply that the responsive patterns of the specific IgA and IgE differed in response to nematode infection, and that IgA and IgE play different roles against nematode infection, which warrants further investigation.

Differences between males and females in the specific IgE and IgA against Teladorsagio

Table 16 shows the average specific IgE and specific IgA against Teladorsagia and Trichostrongylus in plasma of males and female hoggets. Females had higher IgE and higher IgA against Teladorsagia than males ($P < 0.01$), but no difference exist between males and females in IgA against Trichostrongylus ($P = 0.155$).

Table 16. Average specific IgE and specific IgA against Teladorsagia between males and females.

Sex	No. progeny	IgE-Tel	IgA-Tel
Male	347	18.12	23.09
Female	401	62.36	30.96
P value		0.001	0.001

Relationship of specific IgE and specific IgA with ASBV of late dags

Figures 21 and 22 show that IgE and also IgA do not have a clear relationship with late dags in this flock.

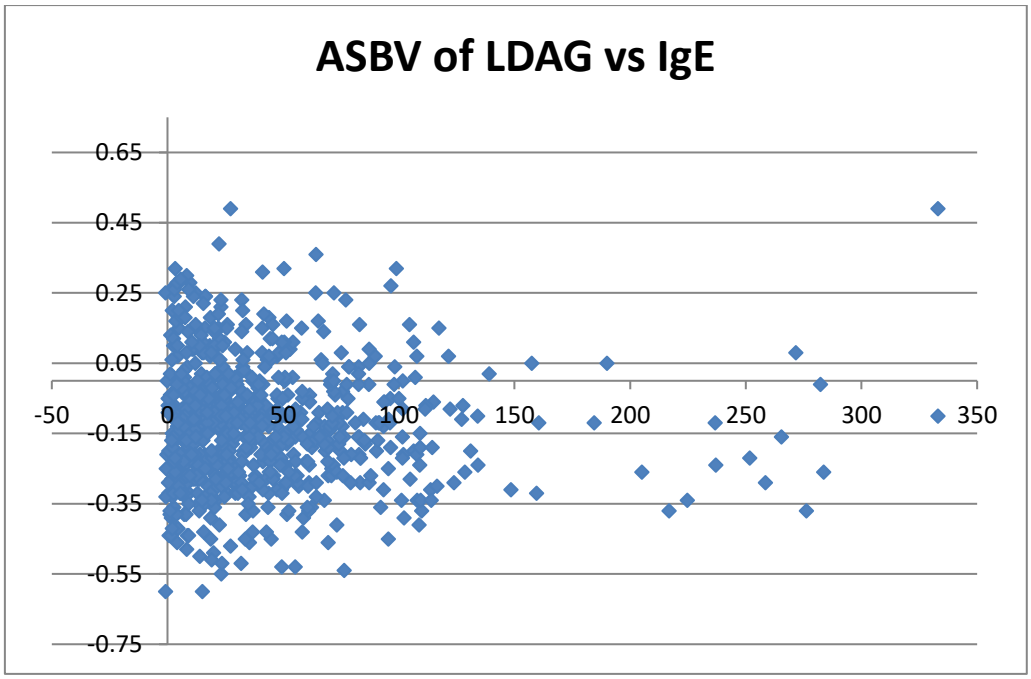

Figure 21. Relationship between ASBV of late dags and specific IgE titres against *Teladorsargia*

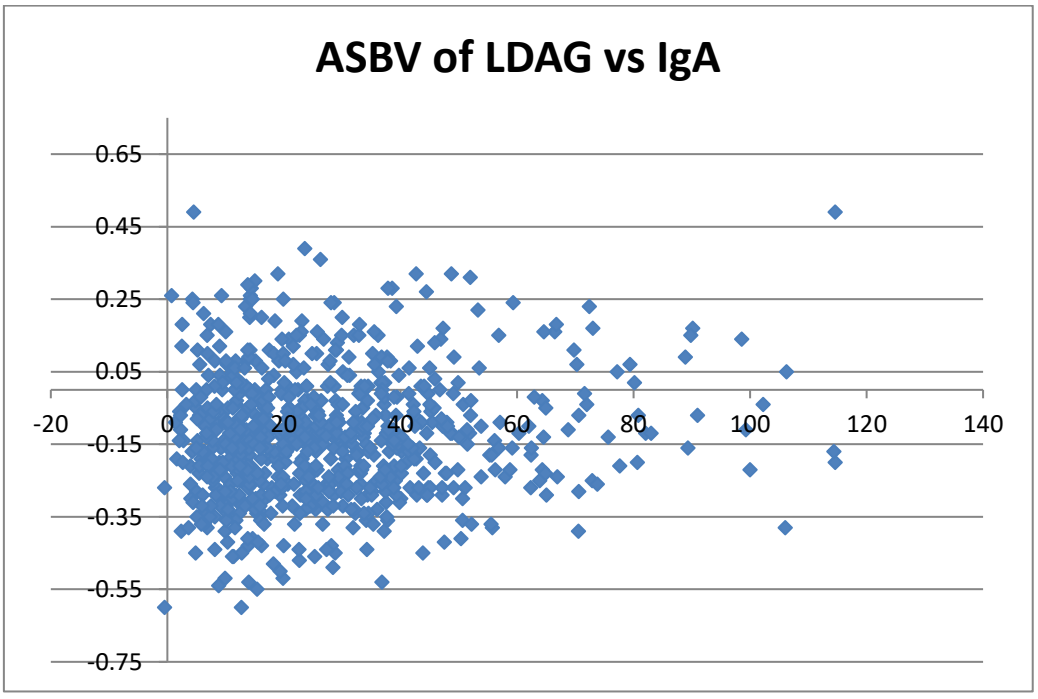

Figure 22. Relationship between ASBV of late dags and specific IgA titres against Teladorsargia.

It is clear, that no clear relationship exists between ASBV of dags and specific IgE and, also with specific IgA.

Relationships of specific IgE and specific IgA with ASBV of FEC at hogget age

Figures 23 and 24 show the relationship between specific IgA and ASBV of FEC. There appears to be a curvilinear negative relationship between ASBV of FEC and IgE. As FEC decreases, the amount of variation in IgE increases due to the tail to the right. This indicates that the high IgE values are found mostly in the resistant animals. However the relationship with IgA is not that clear.

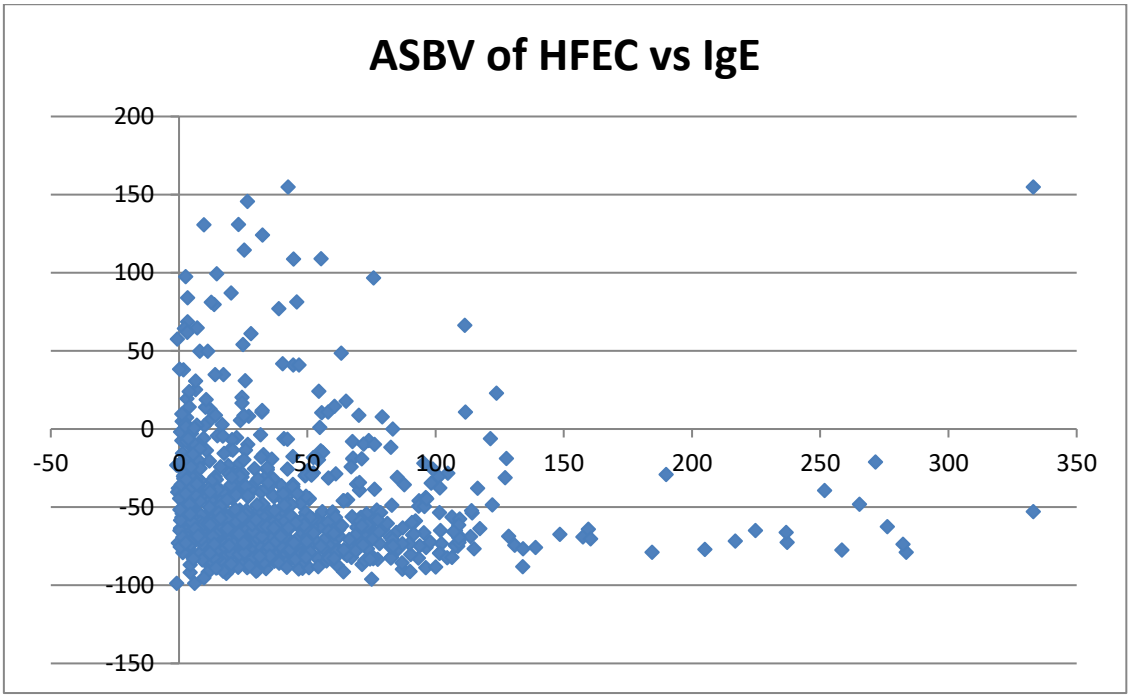

Figure 23. Relationship between ASBV of hogget FEC and specific IgE titres against *Teladorsargia*

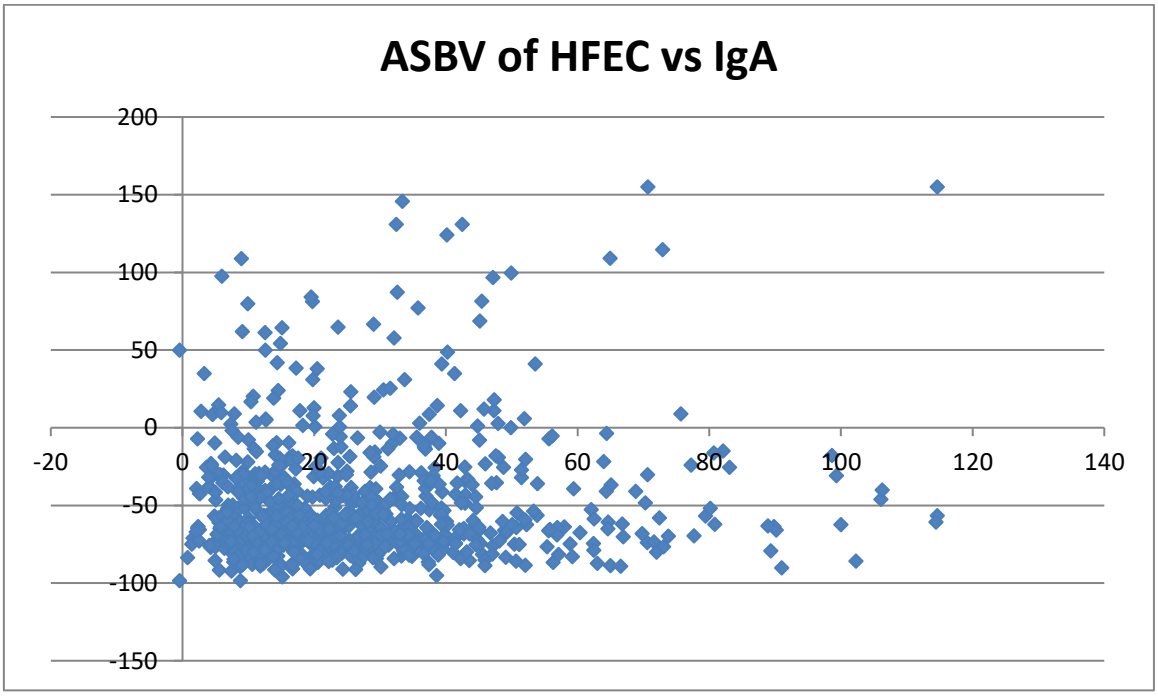

Figure 24. Relationship between ASBV of hogget FEC and specific IgA titres against *Teladorsargia*.

Conclusion

This preliminary study shows that larval antigen specific IgE antibody appears to have a negative relationship with the ASBV for FEC at hogget age. The general accepted theory is that animals with high levels of IgE are more hypersensitive to scour as indicated by Meeusen (1999). However, this appears not to be the case in this study as there was no relationship between larval antigen specific IgE antibodies and late dags in this flock. This indicates that other allergens are not present in the larval extract could be involved in hypersensitivity scouring. For this reason, we plan to determine if there is a relationship between total IgE levels and hypersensitivity scouring.

No relationship was also found between specific IgA and worm resistance in this flock, in spite of the fact that this population probably has the largest spread of animals, from highly susceptible to highly resistant to worms, available. This does not agree with Stear *et al.* (2011) and de Cisneros' *et al.* (2014) findings. This is quite a significant finding and needs to be investigated further.

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DISCUSSION AND RECOMMENDATIONS

Background and establishing of the flocks.

The breech strike flocks were established in 2006 by setting up three lines i.e. an intense selection line, an industry line and an unselected control line to determine how long it will take to breed a resistant flock using the known indicator traits and breech strike in a scenario where no mulesing is carried out. The industry line was included to determine any potential genetic trade-offs in order to advise the industry what genetic gains commercial producers can expect by purchasing rams from studs that breed intensely for breech strike resistance. Halve of each group were mulesed to compare the incidence of breech strike of "resistant" unmulesed sheep against a representative sample of mulesed sheep.

The experimental flocks were established by purchasing of 600 ewe weaners for the project from 10 different producers in Western Australian in the winter rainfall region, and a similar number of ewes from fine wool producers by CSIRO in NSW for the project in the summer rainfall regions. DAFWA and CSIRO also contributed 600 mature ewes from DAFWA and CSIRO research stations to the project. During the first two years, more than 20 industry rams were sourced based on likely indicator traits such as wrinkles, breech cover, dags and wool types to generate as much variation as possible between sire progeny groups. These sires were progeny tested during 2006, 2007 up to 2008 and the first ram progeny were selected for breeding in 2008. Four rams were used across both flocks to generate links between flocks.

During the first three years, it became clear that breeding for breech strike using the known indicator traits was more complex and that larger differences were found between sire progeny groups within lines, than between lines. In addition, it was decided to terminate the mulesed part of the experiment in order to generate more data on unmulesed sheep to estimate genetic parameters for breech strike. The project was therefore modified to identify the underlying causes of differences between sire progeny groups in unmulesed sheep. A resistant line consisting of 250 ewes and a control line of 250 ewes were established using the breeding value for breech strike to identify animals for the two selection lines. The most resistant animals were allocated to the resistant line while the remainder were allocated to the control line.

In 2008 the Rylington Merino flock was included in this project because of its huge database on dags and faecal worm egg count (WEC) information. Since then this flock has been used to progeny test different rams (including industry rams) for breech strike resistance to generate more data to obtain more accurate genetic parameters for breech strike resistance. Rams that were proved to be resistant were considered for breeding in the breech strike resistant line. In addition, all mulesing stopped in this research flock.

Outcomes to date

a. Inheritance of breech strike

Previous results obtained in phase I of the project showed that breech strike is a very heritable trait (>0.50) in an un-mulesed and un-crutched flock. Large differences were found in susceptibility/resistance between sire progeny groups.

The heritability of breech strike up to weaner shearing was 0.21 ± 0.03.

However, where the sheep were crutched, at yearling age prior to the onset of the fly season, the heritability was only 0.11 ± 0.03 from weaner shearing up to hogget shearing. This low heritability when the sheep were crutched has important implications as it implies that in order to identify genetically resistant replacement animals accurately, that the population must be challenged by flies in a production system where the sheep are not crutched. It will be highly inaccurate to identify genetically resistant animals on their own performance where they have been crutched prior to the fly season.

However, as industry will continue to crutch their sheep, the need to identify effective indicator traits for breech strike in a crutched environment, is therefore much more important.

This experiment showed that breech strike is also a repeatable trait as susceptible animals are more prone to be struck again compared to more resistant sheep. The breech strike incidence rate of sire progeny groups rank very similar across years. This implies that any struck animal should be identified and culled. Any ram that have become struck in the breech should also be culled and not used for breeding. As most ram breeding flocks will have sire pedigree information, flystrike records should be used to identify those sires whose progeny groups have the highest strike rate in order to cull any undesirable replacements.

b. Important indicator traits

The most important indicator traits in a winter rainfall region were shown to be dags followed by urine stain, skin wrinkles and breech cover. These indicator traits were also the most important traits in a production system where sheep are crutched regardless of the rainfall distribution.

Wrinkles

In hogget ewes where wrinkles were found to be the most important as breech wrinkles explained 83% of the variation in breech strike in these ewes. This supports the Armidale results which shows that wrinkles is the most important indicator trait for breech strike where dags are not present or as in this study, where it had been removed through crutching. Timing of crutching is thus very important to ensure that animals stay clean for as long as possible during the fly season.

This experiment showed that in a crutched environment body wrinkle at birth was the best indicator trait for breech strike resistance followed by neck wrinkle at marking and then urine stain at weaning. Other wrinkle traits measured at different times and ages, were still important, but they would be less effective compared to body wrinkle at birth.

Dags

This study have shown that high dag content is the most important indicator trait for breech strike in the winter rainfall regions, even in mulesed and crutched flocks, and therefore appears to be the main **putrification factor** involved in attracting flies to susceptible sheep. It contributes moisture which is critical to egg laying and larvae development. However, dags can be due to high worm burdens or due to an immune response which is generally known as hypersensitivity dags.

Dags from high worm burdens can be solved by drenching sheep and/or by breeding sheep for increased worm resistance. However, hypersensitive diarrhoea will continue to be a serious problem unless a clear understanding of the underlying causes of the hypersensitivity scouring is established. This may be related to Irritable Bowl Syndrome (IBS) in humans. The preliminary results in this study which measured specific IgE and specific IgA against Teladorsagia L3 larvae, show that specific IgE was negatively related to the ASBV of FEC but not to the ASBV of late dags. This implies that sheep that are resistant to *Teladorsagia circumcincta* and have a higher specific IgE, does not have a higher propensity to scour than less resistant sheep. No relationship could be found between specific IgA against *Teladorsagia* and ASBV for FEC so far. This may be due to a number of factors that needs to be pursued in future to determine as to whether time of blood sampling may have affected the results. These results contradict other studies and may indicate that the local WA environment may have different causes of scouring than those environments were positive relationships were found with IgE and IgA. It is important to be able to differentiate between these two dag types in order to control it through management and/or to breed more effectively to reduce it.

Urine

Urine stain has also been shown to be an important indicator trait that results in increased breech strike. It is difficult to record urine stain in the presence of dags as one cannot see the stain, however

one can sometimes smell it. But it is unclear whether the increased attractiveness of urine stain is due to the moisture only or whether there is something in the urine that attracts flies.

c. Application in industry

The results contributed to the scoring, measuring, development of breeding values (ASBV) and publication of ASBVS for skin wrinkles, dags and breech cover by industry. Neck wrinkle can be used where animals have been mulesed as it is genetically the same trait as breech wrinkle. These breeding values are now freely available on Sheep Genetics website to assist breeders to identify more resistant and productive rams for their breeding programs. This information has been adopted very well by industry and it has resulted in a dramatic increase in the number of breeders that scored these known indicator traits in industry flocks. The value of this information on dags, wrinkles, and breech cover needs to be disseminated more widely to encourage breeders to adopt using this information in selecting future animals

Progress to find additional indicator traits for practical breeding programs

Although four main indicator traits (wrinkle, urine stain, dags and breech cover) have been identified, the results also indicated that these traits only explained a relatively small proportion (20-25%) of the variation in breech strike in uncrutched sheep and in crutched rams. In crutched ewes, however, breech wrinkle explained 83% of the variation in breech strike from post weaning up to hogget shearing.

Odour

The role of odour in attracting or repelling flies, had been investigated using dogs trained by Hanrob Dog Academy, Sydney. The dogs were trained to identify resistant animals on odour from crutched wool samples from the Mt Barker flock. The dogs were then tested to determine whether they can differentiate between resistant and susceptible sheep. They were very accurate in identifying the target group on which they have been trained. Similar results were also recorded by using crutched wool samples from the Armidale flock from CSIRO, to which the dogs have never been exposed to. The dogs were 82% accurate in identifying the target samples and 92% accurate in ignoring the non-resistant samples in the Armadale flock. These results strongly indicate that odour may be an important factor in attracting flies.

A joint project was initiated with the University of Western Australia to identify the components of odour using gas chromatography (GC) and mass-spectrometry. A PhD student, Joe Steer, was recruited to carry out this work. Positive trends have been found and a number of potential components have been identified on crutched wool samples from ewes from the most resistant and most susceptible sire progeny groups born in 2008. These samples originate from the same group of sheep that was used to train and evaluate the dogs. These ewes were subsequently re-tested at different times over 4 years to investigate the repeatability of the odour components measured with the gas chromatograph. A number of compounds were repeatable albeit low (r= 0.10 to 0.23). Whether these compounds will attract flies needs to be confirmed.

In 2015 an additional Ph.D student, Guanjie Yan was recruited to participate in this study.

Origin of the odour - Skin Bacteria

The origin of the odours was initially investigated in a small preliminary study using surplus rams from the resistant (3 rams) and susceptible (3 rams) groups. Subsequently larger groups of resistant and susceptible sheep were tested. The initial results showed that a resistant group of rams had a higher level of bacteria diversity than a susceptible group. However, this could not be verified on hogget ewes or rams, but susceptible sheep had higher levels of the bacterial family Geodermatophilacease. As only one bacterial library was used to identify potential bacterial species, other libraries are also available

which should be investigated to determine whether other bacterial species could contribute to differences in odour between resistant and susceptible groups.

Fly behaviour studies: Dr AC Schlink.

Fly behaviour studies have been carried out using different methods. The Y-tube choice test was first used and wool from resistant and susceptible sheep was extensively tested. Excellent results were obtained with the very first test which showed a strong relationship between flies visiting the wool of susceptible sheep compared to resistant sheep. However, this could not be repeated with the same wool samples. Extensive tests were subsequently carried out to find the causes that could explain this inconsistent behaviour. However, after many weeks and testing 1000s of flies, this test was dropped as consistent results could not be obtained. This test was also too time consuming and labour intensive.

An alternative test, the arena test was developed and extensively tested to validate this test. Although the arena test provides more consistent results, the results were not good enough to develop a reliable test for industry. It was also labour intensive, but the test allowed itself to be automated with video imaging that could tract individual flies. Eventually this test was abandoned as the results obtained could not be repeated when the same wool samples were tested again.

Invited Scientist- Dr Bekka Brodie

Dr Bekka Brodie from America developed a batch test which uses large numbers of gravid flies in testing regimes. In her PhD study, she identified the specific odours that attract *L. sericata* flies to a food source and also elucidating their egg laying behaviour and the semio-chemicals involved. She developed the cage batch test and showed that although certain substances attract *L. sericata* to a food source, they will not deposit their egg where another substance, in particularly where indole is present. Thus, there is a difference in attractiveness and egg laying behaviour between different substances. She was therefore invited in April 2016 to participate in a joint activity with UWA to replicate her work with *L. sericata* on *L. cuprina* to determine whether there were similarities between these two fly species. She quickly showed that *L. cuprina* has a different identification system in place as they were not attracted to the same volatile substances that attract *Lucilia sericata.* This was unexpected and explains *L. cuprina's* attractiveness to live wool sheep rather than carrion. It is imperative that this difference be investigated to elucidate the biology of the Australian blowfly.

This is critical as this will provide the data and chemicals necessary before the electro-antennagram can be used to identify the specific semio-chemicals that attract flies to sheep.

Under Dr Brodie's guidance the cage batch test was modified and adapted to *L. cuprina* to ensure that their natural responses to semio-chemicals are elicited. This test was implemented, and good results were obtained with this test and the results were more consistent. Using fresh wool from resistant and susceptible sheep from the Mt Barker, the flies' attractiveness to wool explained 50% of the variation in ASBV of breech strike.

Procedures are now in place to test flies for their attractiveness to different wool types. In addition, previous work on flies' ability to lay eggs on wool have been revisited with the final aim to get the flies to lay eggs on wool of sheep. Preliminary results have found that *L. cuprina* will lay eggs on wool if sufficient moisture is present in part of the wool profile. This research needs to continue with the final aim to determine whether it would be possible to use egg laying ability on individual wool samples, to rank sheep's level of resistant to fly strike.

Maintaining the genetic resources

The research flocks at DAFWA and CSIRO have been generated at very high cost. A huge body of phenotypic data had been collected on all the animals in these two fully pedigreed flocks which makes it possible to retrospectively source genetically resistant or susceptible animals for a wide range of traits.

A large library of tissue samples (wool and blood) have been collected prior to sheep being struck which is also available for future research.

However, future studies will need fresh wool samples from sheep with known levels of breech strike resistance/susceptibility on a continuous basis in order to identify the elusive semiochemicals that attract flies to susceptible sheep. These resources are critical for the success of this project and therefore the flocks at Katanning and Armidale need to be maintained until a reliable external test for breech strike susceptibility/resistance had been developed and validated in industry flocks.

As the heritability of breech strike where sheep have been crutched was very low (h^2= 0.11), it is important that replacement sheep are only selected under a scenario where the sheep have not been crutched. If this is not done, then it would not be possible to identify genetically resistant sheep for breeding purposes. Modern reproductive technologies should be considered to established genetically diverse lines by super-ovulating extreme resistant and susceptible ewes and inseminating them with semen from similarly extreme rams for breech strike resistant/susceptibility. Having diverse populations will contribute greatly to elucidating the underlying causes of breech strike resistance and susceptibility.

Environmental factors affecting the "new" indicator traits
It is important to elucidate the environmental factors that could impact on the expression of the novel indicator traits for breech strike. Identifying the best time to measure or sample sheep for the new novel traits should be carried out to establish the earliest age when one would be able to differentiate between resistant and susceptible animals, whether differences exist between sexes, and whether the trait is expressed differently in summer, autumn, winter and spring. This information is important to develop the best measurement protocols to identify genetically resistant animals more accurately. This can only be done on a dedicated research flock for breech strike.

Future activities and recommendations

The results from this study and that of Mackerras and Mackerras (1944) show that the following three factors need to be present for an effective flystrike, (1) a sheep that contributes some elusive factor, (2) a putrification environmental factor and (3) a wool moisture factor.

Elucidating the **sheep** factor in blowfly strike should focus on the following.

1. Maintaining the current research populations in DAFWA and Armidale.
2. Generate larger differences between resistant and susceptible sheep by challenging the animals with blowflies when the animals are not crutched.
3. Multiplying the most diverse resistant and susceptible genotypes with modern reproductive technologies to provide unique tissues for in-depth research activities,
4. Use diverse resistant and susceptible genotypes to determine differences in immune parameters of the skin in the breech. This has been carried out previously by Smith *et al*. (2008) who showed that genetic variation exists amongst sheep in their ability to restrict the growth and development of blowfly larvae on the skin and that anti-larval factors may be present in serum and eosinophils. This need to be investigated in extreme diverse animals to determine whether immune factors in the skin may inhibits the development of blowfly larvae in the breech. This could explain the differences which the dogs recognized. Thus, it may not have been odour *per se* which the dogs have detected but rather differences in immune substances.
5. Validating the Brodie test with fresh wool samples to determine whether flies will lay less eggs on wool from resistant than on wool from more susceptible sheep in a laboratory situation.

6. Continue with the program to identify the unique odour signature that attracts flies to susceptible sheep and/or that causes flies to avoid attacking resistant sheep.
7. Determine whether the different odours act as attractants or repellents to develop specific management systems to protect sheep from being struck in the absence of mulesing.
8. Test the fly's olfactory neural responses to potential chemical compounds with the electro-antennagram to determine for which compounds flies have special odour receptors, and whether these may be semiochemicals.
9. Elucidating the search patterns *L. cuprina* follows in identifying susceptible sheep.

Elucidating the role of **wool moisture** in egg laying by the fly, hatching of the eggs and development of the larvae is critical in this study. Following the finding that differences exist between resistant and susceptible sheep in humidity and moisture content in breech wool, the following activities should be carried out.

1. Determine the role of urine in wetting the breech area in attracting flies.
2. Determine whether there are differences in sweating rates between resistant and susceptible sheep and if so, is it correlated with breech strike.
3. Does the wool from the breech of resistant sheep have a different drying pattern than that of susceptible sheep and if so, is it correlated with breech strike.

Elucidating the underlying causes of diarrhea, which causes dags and which is a major component of the **putrification factor.**

1. Determine the role of the immunoglobulins IgE and IgA as indicator traits for faecal dags and for worm egg count where *Teladorsagia* and *Trichostrongulus* are the dominant as in the winter rainfall regions of Australia, to clarify the relationship between high worm burden and hypersensitivity diarrhoea.
2. As dags is the most important indicator trait in breech strike, dags may involve other characteristics such as its colour and perhaps also its odour, both of which could also play a role in the attraction of blowflies to daggy sheep. This could be due to difference in the gut microbiome which have been shown to play a role in human irritable bowel syndrome.
3. Determine whether differences exist in the composition of the urine from susceptible and resistant sheep

Fly biology and behaviour

Having well characterized resistant and susceptible sheep is critically important to understand the biology of what attracts blowflies to specific sheep. This information is also imperative for the molecular genetic studies at Melbourne university. Knowledge of the biology of the fly will make it possible to determine the behaviour consequences of knocking out specific genes in the fly by using the new CRISP Cas9 technologies. The following should be carried out.

1. Hold a workshop on blowfly behaviour
2. Invite leading authorities on blowfly behaviour and methodologies on how to measure and record the searching patterns in insect species.

Genomic studies.

The genomic studies need to continue to determine whether SNP markers could be found to increase the accuracy of genomic breeding values for breech strike. For this approach to work indefinitely, a genetic reference flock, in which sheep are challenged to differentiate between susceptible and resistant sheep, is crucial to phenotype sire progeny groups for breech strike.

A large number of blood samples have been collected on all the sheep born in this experiment at both CSIRO and at Mt Barker research stations. Complete phenotypes are available on all the sheep born in these research flocks. These resources should be maintained to identify genetic markers that could be used to estimate genomic breeding values to identify genetically resistant sheep for breech strike.

Benchmarking industry rams

The two breech strike flocks at Mt Barker and Chiswick in NSW are the only animal resource flocks involved in breech strike studies. It is important to expand these resources. This can be done in any flock with complete pedigrees and that can monitor sheep for breech strike. The Sire Referencing flocks of AMSEA should be considered where industry rams are progeny tested. The progeny can be evaluated for breech strike resistance after their test for the production traits had been completed. Proven sires from the Breech strike flocks should be progeny tested in these industry flocks as reference sires to identify genetically resistance rams. This will demonstrate that large differences exist between sire progeny groups for breech strike resistance and will emphasized that breeding for breech strike resistance is possible. It should therefore also encourage the collection of objective breech strike measurements on fully pedigreed flocks across Australia to further identify genetically resistant sheep.

References

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APPENDIX 1 FIXED FACTORS – BIRTH TO WEANER SHEARING

Number of records, minimum, maximum, mean and standard deviation (SD) and the F-values of the significant fixed effect for all the traits measured on all the sheep in the Breech strike flock including the Rylington Merino flock at the Mt Barker research station from 2006 to 2014 up to weaner shearing. (P<0.01; *P<0.05); ns = not significant)**

Trait	n	Min	Mean	Max	SD	Yr of birth	Birth type	Sex	Age of dam	Yr x date of birth
EBRSTRWEAN	7703	0	7.0	4	2.7	**	ns	ns	ns	*
BBDWR	6595	1	2.84	5	1.05	**	**	ns	ns	ns
BIRTHCOAT	7609	1	3.02	6	1.23	**	**	**	**	**
BIRTHWT	7611	1	4.42	7.9	0.85	**	**	**	**	**
MANBALE	4486	2	4.81	10.5	1.40	**	**	**	ns	**
MANBAWD	4486	2	4.40	12	1.56	**	**	**	**	**
MHAIR	4281	1	1.25	4	0.46	**	**	**	ns	**
MBCOV	6268	1	3.38	5	0.83	**	**	**	ns	ns
MBDWR	5882	1	1.73	5	0.93	**	**	**	**	**
MBFLUF	4099	1	3.46	5	0.73	**	**	**	ns	ns
MBRWR	5882	1	1.47	5	0.83	**	**	**	ns	ns
MCCOV	4099	1	3.21	5	0.68	**	ns	**	ns	**
MCOL	4911	1	1.92	5	0.51	**	*	**	ns	**
MDAG	7137	1	1.20	5	0.51	**	ns	**	ns	**
MDAGDM	3419	1	1.72	5	1.06	**	ns	ns	ns	ns
MFACE	4099	1	2.52	5	0.36	**	**	**	ns	**
MNKWR	6269	1	2.19	5	0.94	**	**	**	**	**
MTABALE	4471	5	10.81	25	1.76	**	**	**	ns	**
MTABAWD	4483	2	4.05	7.5	0.86	**	**	**	**	**
MTALE	7022	2.5	25.23	44	3.86	**	**	**	**	**
MTALESC	6663	1	3.72	5	0.60	**	**	ns	**	**
MTAWDTH	4483	2.5	4.63	8	0.92	**	**	**	**	**
MTAWR	5881	1	1.84	5	0.94	**	**	**	**	**
MUM	7542	1	4.25	6	1.36	**	**	ns	**	**
MURINE	6992	1	1.13	5	0.45	**	**	**	ns	ns
W1FEC	6638	25	854	43160	2068	**	ns	ns	**	ns
W2BCOV	5754	1	3.09	5	0.58	**	**	**	ns	ns
W2BDWR	6674	1	1.23	5	0.49	**	**	ns	ns	**
W2BECOV	4877	1	3.05	4.5	0.44	**	**	**	ns	*
W2BRWR	6674	1	1.27	4	0.57	**	**	ns	ns	*
W2CCOV	4877	2	3.06	4.5	0.42	**	**	**	ns	**
W2CHAR	4070	1.5	2.97	5	0.72	**	ns	ns	ns	*
W2COL	4877	1	2.53	5	0.47	**	ns	*	ns	**
W2CS	1955	1.5	3.15	4	0.44	**	**	**	ns	**
W2DAG	3862	1	1.18	5	0.42	**	ns	**	ns	**
W3DAGDM	3020	1	2.00	5	1.09	**	ns	ns	ns	ns
W3DAGS	7002	1	1.24	5	0.51	**	**	**	ns	**
W2DUST	4070	1	1.66	4	0.54	**	**	ns	ns	**
W2FACE	5754	1	2.48	5	0.51	**	**	**	ns	**
W2FLROT	4877	1	1.04	4	0.20	**	*	ns	ns	ns

W2NKWR	6674	1	1.53	5	0.65	**	**	**	ns	**
W2SHLDR	4876	1	1.26	5	0.52	**	*	ns	ns	**
W2SSTRC	4069	1	2.18	4	0.43	**	ns	**	ns	**
W2TAWR	6674	1	1.39	5	0.62	**	**	ns	ns	**
W2TOES	1757	1	1.47	5	0.54	**	ns	ns	**	ns
W2URINE	5032	1	1.17	4	0.42	**	ns	**	ns	ns
W2WAX	4070	1	2.06	3.5	0.42	**	**	**	**	**
W2WT	2910	9.5	26.78	43	5.14	**	**	**	**	**
W3CS	6582	1	2.76	4	0.44	**	ns	**	ns	ns
W3WT	7474	10.5	27.28	51.4	5.47	**	**	**	**	**
WFMOIST	6985	1	2.28	5	0.89	**	**	**	ns	**
DERMO	1772	1	0.32	5	0.66	**	**	ns	*	**
E1BCOV	4295	1	3.12	5	0.65	**	**	**	ns	**
E1BDWR	4295	1	1.27	5	0.42	**	**	ns	ns	ns
E1BECOV	4710	1	2.96	6	0.61	**	ns	**	ns	ns
E1BFLUF	3912	1	2.90	4	0.52	**	**	**	ns	**
E1BRWR	4295	1	1.13	5	0.28	**	**	**	ns	ns
E1CCOV	4302	1	2.93	6	0.43	**	**	**	*	ns
E1CS	2980	1.5	2.80	3.75	0.41	**	**	ns	ns	**
E1DAG	2936	1	1.23	4	0.53	**	ns	**	ns	*
E1DAGDM	1474	1	1.64	5	0.91	**	ns	ns	ns	ns
E1FACE	5172	1	2.29	5	0.61	**	*	**	ns	ns
E1NKWR	4295	1	2.07	4.5	0.56	**	**	**	ns	ns
E1SC	1481	11	18.66	30	3.83	**	**	ns	ns	**
E1TALE	941	5	8.98	12	1.16	ns	**	**	ns	ns
E1TAWDTH	941	4	7.78	11	0.98	ns	**	**	ns	ns
E1TAWR	3992	1	1.49	4	0.41	**	**	**	ns	**
E1TOES	2088	1	2.07	4	0.26	ns	ns	**	ns	**
E1URINE	1983	1	1.03	3	0.18	**	ns	**	ns	*
E1WAX	852	1	2.35	3	0.29	ns	ns	**	ns	ns
E1WCOL	3911	1	2.40	5	0.58	**	ns	**	ns	ns
E1WT	3984	10.5	26.26	57.2	5.78	**	**	**	**	**
E2BCOV	864	1.5	2.78	4	0.43	ns	ns	**	ns	**
E2BDWR	864	1	1.12	3	0.27	ns	**	ns	ns	ns
E2BRWR	864	1	1.04	3	0.16	ns	**	**	ns	ns
E2CS	2863	1.75	2.71	3.5	0.33	**	**	**	ns	**
E2DAG	2834	1	1.25	4.5	0.53	**	*	ns	ns	*
E2DAGDM	619	1	1.38	5	0.87	**	ns	**	ns	**
E2FACE	864	1	2.28	3.5	0.42	ns	ns	**	ns	ns
E2NKWR	864	1	1.77	4	0.61	ns	**	**	ns	ns
E2TAWR	864	1	1.23	3	0.32	ns	**	ns	ns	ns
E2WT	4389	13.5	28.81	53.8	5.59	**	**	**	**	**
E3CS	3704	1.5	2.68	3.75	0.33	**	**	**	ns	**
E3DAG	3202	1	1.31	5	0.61	**	ns	*	ns	ns
E3DAGDM	1235	1	1.47	5	0.80	**	ns	**	ns	ns
E3WT	3688	10.5	29.60	54	5.71	**	**	**	**	**

APPENDIX 2 FIXED FACTORS – WEANER SHEARING TO HOGGET SHEARING

Number of records, minimum, maximum, mean and standard deviation (SD) and the F-values of the significant fixed effect for all the traits measured from 2010 to 2014 on all the sheep in the Breech strike flock including the Rylington Merino flock at the Mt Barker research station, from weaner shearing until hogget shearing. (P<0.01; *P<0.05), ns = not significant)**

Trait	n	Min	Mean	Max	SD	Yr of birth	Birth type	Age of dam	Yr.Sex	Yr.Day of birth
BRSTRWEAN	4200	1.0	2..6	3.0	1.7	**	Ns	ns	*	ns
BRSTRHOG	4200	0	8.1	3.0	3.1	ns	Ns	**	**	ns
P1CS	1652	1.8	3.1	4.5	0.67	**	**	**	ns	**
P1DAG	1225	1.0	1.7	5.0	1.00	*	ns	**	ns	ns
P1DAGDM	517	1.0	1.5	5.0	0.97	**	ns	**	**	ns
P1URINE	794	1.0	1.0	2.0	0.11	ns	ns	ns	**	ns
P1WT	1652	3.5	34.3	59.4	6.75	**	**	ns	**	**
P2CS	3513	1.8	2.9	4.0	0.49	**	ns	**	**	ns
P2DAG	3525	1.0	1.4	5.0	0.73	ns	ns	ns	**	**
P2DAGDM	1057	1.0	1.6	5.0	0.83	ns	ns	**	**	**
P2URINE	783	1.0	1.1	3.0	0.19	ns	**	**	**	**
P2WT	3510	20.5	34.7	64.0	6.00	**	**	ns	**	**
P3CS	2648	1.5	3.0	3.8	0.32	**	**	**	ns	**
P3DAG	1874	1.0	1.4	4.5	0.72	**	ns	ns	**	**
P3DAGDM	613	1.0	1.8	5.0	0.89	**	**	ns	ns	ns
P3FACE	955	1.5	2.7	4.0	0.44	ns	ns	ns	**	*
P3URINE	917	1.0	1.0	2.0	0.16	ns	ns	ns	ns	**
P3WT	2633	21.0	35.8	58.5	5.74	**	**	**	**	**
P4BCOV	3501	1.0	2.9	4.0	0.41	**	**	**	ns	ns
P4BDWR	3501	1.0	1.0	3.0	0.12	ns	**	ns	*	ns
P4BECOV	955	2.0	3.0	3.5	0.26	ns	ns	ns	**	**
P4BEPLUC	955	1.0	1.0	2.0	0.18	ns	**	**	ns	ns
P4BFLUF	955	1.5	2.8	3.5	0.32	ns	ns	**	ns	**
P4BRWR	3501	1.0	1.0	2.0	0.03	ns	ns	ns	ns	**
P4CCOV	3501	1.5	3.1	4.0	0.34	**	ns	**	ns	ns
P4CHAR	955	1.5	2.5	3.5	0.37	*	ns	ns	ns	ns
P4COL	3499	1.5	2.5	5.0	0.40	**	ns	**	ns	ns
P4DAG	3503	1.0	1.7	5.0	0.83	**	**	ns	ns	**
P4DAGDM	1833	1.0	3.0	4.5	0.86	**	ns	**	ns	ns
P4DERMO	3501	1.0	1.0	2.0	0.02	ns	ns	**	*	**
P4DUST	955	1.5	2.1	3.0	0.23	ns	ns	ns	**	**
P4FLROT	3501	1.0	1.0	3.0	0.16	**	*	**	ns	**
P4NKWR	3501	1.0	1.3	4.0	0.40	**	**	ns	ns	ns
P4SHLDR	3501	1.0	1.2	3.0	0.30	**	ns	ns	**	ns
P4SSTRC	955	1.0	2.1	3.5	0.34	ns	**	**	ns	ns
P4TAWR	3501	1.0	1.0	2.0	0.09	*	ns	ns	ns	ns
P4TOES	2727	1.0	1.5	4.0	0.76	**	**	ns	**	ns
P4URINE	2621	1.0	1.4	4.0	0.55	**	ns	ns	**	**
P4URINEDM	695	2.0	2.9	4.0	0.69	ns	**	**	ns	**

P4WAX	955	1.5	2.2	3.0	0.26	ns	ns	**	**	ns
Y1CS	3026	2.0	3.2	4.0	0.34	**	**	ns	**	ns
Y1DAG	3490	1.0	2.1	5.0	0.99	**	ns	**	**	ns
Y1DAGDM	2372	1.0	2.7	5.0	1.21	**	**	ns	**	**
Y1URINE	2279	1.0	1.2	3.5	0.44	**	**	ns	**	ns
Y1WT	3484	21.5	43.7	80.5	8.91	**	**	*	**	**
Y2BCOV	2534	1.5	3.2	5.0	0.69	**	ns	ns	**	ns
Y2BRWR	2535	1.0	1.2	3.0	0.30	**	*	ns	**	ns
Y2CS	770	2.5	3.0	3.5	0.24	ns	ns	**	ns	ns
Y2DAG	770	1.0	1.8	4.0	0.78	ns	ns	ns	ns	ns
Y2DAGDM	509	1.0	3.1	4.0	0.51	ns	ns	ns	**	**
Y2DERMO	4203	0.0	0.0	0.0	0.00	ns	**	ns	**	*
Y2TAWR	2535	1.0	1.5	3.0	0.40	**	ns	**	*	ns
Y2URINE	770	1.0	1.0	3.0	0.17	ns	ns	**	**	ns
Y2WT	761	33.5	54.0	88.0	8.72	**	ns	**	ns	ns
Y3CS	2719	1.0	3.2	4.0	0.31	**	ns	ns	**	**
Y3DAG	2719	1.0	2.0	5.0	1.11	**	ns	ns	**	ns
Y3DAGDM	1711	1.0	2.8	5.0	1.15	**	ns	ns	ns	**
Y3URINE	948	1.0	1.1	4.0	0.29	ns	**	**	ns	**
Y3WT	2719	26.5	46.6	81.5	7.28	**	**	ns	**	ns
H1CS	3124	2.5	3.4	4.0	0.28	**	ns	ns	**	ns
H1DAG	1766	1.0	1.7	4.5	0.72	**	ns	ns	**	ns
H1DAGDM	1145	1.0	1.9	4.0	0.80	ns	ns	**	ns	ns
H1URINE	910	1.0	1.0	2.0	0.15	ns	ns	**	**	**
H1WT	3123	29.0	55.8	88.5	8.56	**	**	*	**	**
H2CS	1381	2.0	3.4	4.0	0.34	**	ns	**	ns	**
H2DAG	1846	1.0	1.4	3.5	0.54	**	ns	**	ns	ns
H2DAGDM	911	1.0	1.9	4.0	0.72	**	**	ns	**	**
H2WT	1852	27.0	57.5	81.5	8.41	**	**	**	ns	**
H3BCOV	1709	1.0	2.5	25.0	0.92	ns	ns	ns	**	ns
H3BDWR	1708	1.0	1.3	5.0	0.61	**	ns	*	ns	**
H3BECOV	949	1.5	2.8	4.0	0.32	ns	ns	**	**	ns
H3BEPLUC	949	1.0	1.3	3.0	0.51	ns	**	**	ns	**
H3BFLUF	949	1.5	2.5	4.0	0.46	ns	**	**	ns	ns
H3BLK	1667	1.0	1.0	5.0	0.42	**	ns	ns	**	ns
H3BRWR	1709	1.0	1.7	15.0	0.95	**	**	ns	**	**
H3CCOV	1709	1.0	2.9	5.0	0.85	**	**	ns	ns	ns
H3CHAR	3477	1.0	2.9	5.0	0.58	**	*	**	ns	ns
H3COL	3477	1.0	2.8	5.0	0.56	**	ns	ns	**	*
H3CS	769	2.5	3.2	3.8	0.23	ns	**	ns	ns	**
H3DAG	3473	1.0	1.6	4.0	0.68	**	**	ns	**	**
H3DAGDM	2052	1.0	2.6	4.5	0.63	**	**	ns	ns	ns
H3DERMO	3477	1.0	1.0	1.5	0.01	ns	**	**	ns	ns
H3DUST	3477	1.0	1.6	3.5	0.45	**	**	ns	ns	**
H3FACE	1709	1.0	2.3	4.0	0.46	**	ns	ns	**	ns
H3FLROT	3477	1.0	1.1	3.0	0.23	**	**	ns	ns	*
H3NKWR	1709	1.0	1.8	5.0	0.92	**	ns	ns	**	ns
H3SHLDR	3477	1.0	1.1	3.0	0.28	*	**	ns	ns	**

H3SPOT	1671	1.0	1.0	5.0	0.40	**	ns	**	ns	**
H3SSTRC	3477	1.0	2.6	4.0	0.68	**	**	ns	ns	**
H3TAWR	1709	1.0	2.1	5.0	0.95	**	ns	**	ns	**
H3TOES	2573	1.0	2.1	5.0	0.86	**	**	ns	ns	**
H3URINE	3476	1.0	1.1	3.0	0.25	ns	**	ns	**	**
H3WAX	3477	1.5	2.8	4.0	0.64	**	**	ns	ns	**
H3WEATH	2528	1.0	1.8	4.0	0.45	**	**	ns	**	ns
H3WT	758	29.0	59.8	88.5	10.66	**	ns	ns	ns	**
H4Belly_Wt	3442	21	298	635	66.95	**	ns	*	ns	ns
H4BULK	930	3.7	6.1	8.7	0.80	**	ns	**	**	**
H4CEM	3448	4.7	7.0	14.5	0.96	**	*	ns	ns	**
H4CFW	3436	1.6	2.9	5.0	0.52	**	**	**	**	ns
H4CURV	3448	55.2	95.8	147.8	11.13	**	**	ns	**	**
H4CURVESD	3448	37.5	56.4	81.9	6.11	**	**	ns	**	ns
H4FD	3448	14.9	19.0	25.4	1.48	**	ns	**	*	**
H4FD15	3448	0.9	14.9	54.2	8.56	**	ns	**	**	ns
H4FD30	3180	0.1	0.8	13.8	1.18	**	**	**	ns	**
H4FDCE	3429	0.1	0.8	8.3	0.74	**	**	ns	ns	**
H4FDCV	3448	14.1	20.6	33.2	2.45	**	ns	**	**	ns
H4FDSD	3448	2.6	3.9	6.8	0.49	**	*	**	ns	**
H4FDSF	3448	14.6	18.5	24.5	1.39	**	**	*	ns	ns
H4FEM	3448	4.4	6.6	10.7	0.78	**	ns	ns	ns	**
H4FFC	3448	86.2	99.3	100.0	1.15	**	**	**	ns	ns
H4GFW	3452	2.2	4.1	6.9	0.70	**	**	**	**	ns
H4GFW_belly	2600	2.0	3.8	6.5	0.68	**	**	**	**	**
H4pRtoC	2515	1.0	5.5	8.6	0.86	**	**	**	ns	**
H4SL	3445	47.0	93.4	158.0	12.62	**	**	ns	**	**
H4SS	3445	3.4	29.0	54.9	7.06	**	**	ns	**	**
H4YLD	3448	55.4	70.5	90.2	3.93	**	*	ns	ns	**
H7BCOV	2707	1.0	2.6	3.5	0.41	ns	**	ns	ns	ns
H7BDWR	2707	1.0	1.2	3.0	0.34	**	**	ns	ns	**
H7BECOV	2707	1.0	2.6	3.5	0.28	**	**	ns	**	**
H7BFLUF	1385	1.0	2.4	3.5	0.43	**	ns	ns	ns	ns
H7BRWR	2707	1.0	1.1	2.5	0.19	ns	ns	**	**	ns
H7CCOV	2707	1.0	2.6	3.5	0.33	**	ns	**	**	ns
H7COL	3464	1.0	2.8	5.0	0.58	**	**	ns	ns	**
H7CS	2693	2.0	3.3	4.0	0.30	**	**	ns	**	ns
H7FACE	2707	1.0	2.3	4.5	0.32	**	ns	**	ns	ns
H7HORN	1271	1.0	3.2	5.0	1.57	ns	ns	**	ns	ns
H7NKWR	2707	1.0	1.9	4.0	0.60	**	**	ns	ns	**
H7SC	1344	16.0	30.0	40.0	2.68	**	ns	**	**	ns
H7SHLDR	2148	1.0	1.3	3.5	0.43	**	ns	ns	**	**
H7TAWR	2707	1.0	1.3	3.5	0.41	**	ns	ns	ns	ns
H7TOES	2707	1.0	2.8	5.0	0.58	**	**	ns	**	ns
H7WT	2692	30.5	54.2	87.2	9.48	**	**	ns	**	**
H8FEC	2672	0.0	227	4950	390	**	**	ns	**	ns
H8FMOIST	2953	1.0	3.2	5.0	0.69	**	ns	**	ns	**
pH9CS	1936	2.0	3.4	5.0	0.45	**	ns	ns	ns	**

pH9EMD	3450	1.0	25.7	37.0	3.26	**	**	ns	**	**
pH9FAT	3442	1.0	3.2	9.4	0.98	**	**	**	ns	ns
pH9WT	2499	28.7	55.9	81.5	8.26	**	**	ns	**	ns
H10Dust_ConWt	393	0.1	4.0	23.1	2.65	ns	**	ns	**	**
H10Suint_ConWt	394	0.4	8.4	36.0	4.15	ns	ns	**	ns	**
H10Water_ConWt	394	13.2	19.0	28.3	2.43	ns	ns	**	ns	ns
H10Wax_ConWt	394	6.6	22.8	58.2	7.28	**	ns	ns	ns	**
H13TALE	944	1.0	8.1	12.0	1.21	**	ns	ns	ns	ns
H13TAWDTH	944	6.0	9.9	14.0	1.06	ns	ns	ns	ns	ns

APPENDIX 3 DATA YIELD FROM SEQUENCING

DNA Data Yield: 300bp Paired End for Fungi (ITS) and Bacteria (16S)

Line	ID	PG - ng/uL	ITS Paired End Reads	ITS Data Yield (base pairs in Gb units) after quality control	16S Paired End reads	16S Data Yield (base pairs in Gb units) after quality control
Resistant	20142200	0.4	169,078	0.1	84,040	0.05
Resistant	20142208	0.0	75,231	0.05	142,939	0.09
Resistant	20142209	0.1	22,719	0.01	98,644	0.06
Resistant	20142213	0.2	34,416	0.02	104,284	0.06
Resistant	20142217	0.0	67,028	0.04	113,339	0.07
Resistant	20142218	0.2	103,185	0.06	102,560	0.06
Resistant	20142252	0.0	26,886	0.02	169,008	0.1
Resistant	20142254	0.1	48,577	0.03	84,098	0.05
Resistant	20142255	0.2	290,161	0.17	166,955	0.1
Resistant	20142266	0.0	704	0	170,229	0.1
Resistant	20142273	0.3	129,348	0.08	65,733	0.04
Resistant	20142305	0.1	62,137	0.04	115,891	0.07
Resistant	20142329	0.9	456,442	0.27	87,039	0.05
Resistant	20142337	0.0	57,849	0.03	170,083	0.1
Resistant	20142354	0.1	102,549	0.06	98,775	0.06
Resistant	20142359	0.4	122,929	0.07	88,654	0.05
Resistant	20142362	1.1	263,228	0.16	55,796	0.03
Resistant	20142365	0.1	17,234	0.01	141,915	0.09
Resistant	20142385	0.2	274,641	0.16	103,601	0.06
Resistant	20142405	0.1	88,225	0.05	89,423	0.05
Resistant	20142419	0.2	256,071	0.15	126,602	0.08
Resistant	20142433	0.1	88,104	0.05	57,474	0.03
Resistant	20142439	0.4	71,634	0.04	70,891	0.04
Resistant	20142452	0.3	483,106	0.29	123,623	0.07
Resistant	20142455	0.1	133,241	0.08	114,962	0.07
Resistant	20142471	0.2	30,469	0.02	81,247	0.05
Resistant	20142615	0.3	119,729	0.07	72,732	0.04
Resistant	20142628	0.3	82,118	0.05	115,211	0.07
Resistant	20144108	0.3	120,570	0.07	80,960	0.05
Resistant	20144213	0.0	11,951	0.01	186,133	0.11
Resistant	20144256	0.3	349,869	0.21	104,270	0.06
Resistant	20144282	0.1	91,973	0.06	113,156	0.07
Resistant	20144337	0.1	11,542	0.01	139,505	0.08
Resistant	20144355	0.2	20,356	0.01	101,442	0.06
Resistant	20144610	0.2	241,869	0.15	113,166	0.07
Resistant	20144611	0.2	60,888	0.04	104,264	0.06
Resistant	20144630	0.1	16,930	0.01	64,075	0.04
Resistant	20144637	0.0	28,151	0.02	101,224	0.06
Resistant	20144680	0.1	88,401	0.05	103,640	0.06
Resistant	20144683	0.1	102,139	0.06	84,889	0.05
Resistant	20144685	0.1	68,936	0.04	83,355	0.05
Susceptible	20140852	0.6	208,647	0.13	98,742	0.06
Susceptible	20140874	0.1	25,112	0.02	114,973	0.07
Susceptible	20142105	0.0	66,813	0.04	109,732	0.07
Susceptible	20142113	0.2	108,348	0.07	85,419	0.05
Susceptible	20142115	0.2	86,767	0.05	106,239	0.06
Susceptible	20142116	0.1	152,291	0.09	162,525	0.1
Susceptible	20142117	0.9	276,165	0.17	84,306	0.05
Susceptible	20142189	0.5	338,377	0.2	90,418	0.05
Susceptible	20142424	0.9	718,939	0.43	128,197	0.08
Susceptible	20142500	0.3	228,945	0.14	73,546	0.04
Susceptible	20142502	0.0	55,037	0.03	176,836	0.11
Susceptible	20142552	0.2	44,306	0.03	115,054	0.07
Susceptible	20142555	0.0	9,740	0.01	120,093	0.07
Susceptible	20142572	0.0	3,839	0	95,034	0.06
Susceptible	20142578	0.0	15,533	0.01	104,667	0.06
Susceptible	20142609	0.4	50,868	0.03	51,176	0.03

Susceptible	20144109	0.3	152,238		0.09	120,840	0.07
Susceptible	20144155	0.1	28,748		0.02	100,723	0.06
Susceptible	20144166	0.1	3,808		0	153,692	0.09
Susceptible	20144167	0.4	175,476		0.11	65,741	0.04
Susceptible	20144185	0.4	632,035		0.38	122,913	0.07
Susceptible	20144186	0.1	22,649		0.01	88,973	0.05
Susceptible	20144187	0.2	261,242		0.16	94,242	0.06
Susceptible	20144300	0.0	73,297		0.04	161,575	0.1
Susceptible	20144307	0.2	10,783		0.01	93,871	0.06
Susceptible	20144326	0.4	112,953		0.07	77,644	0.05
Susceptible	20144368	0.1	27,252		0.02	73,794	0.04
Susceptible	20144403	0.2	16,502		0.01	99,751	0.06
Susceptible	20144501	0.7	493,265		0.3	90,095	0.05
Susceptible	20144513	0.4	440,002		0.26	84,149	0.05
Susceptible	20144514	0.1	16,049		0.01	117,828	0.07
Susceptible	20144516	0.1	14,412		0.01	100,587	0.06
Susceptible	20144519	0.0	2,906		0	119,288	0.07
Susceptible	20144522	0.3	218,409		0.13	87,728	0.05
Susceptible	20144525	0.2	21,470		0.01	121,933	0.07
Susceptible	20144530	0.1	39,866		0.02	144,875	0.09
Susceptible	20144553	0.1	165,168		0.1	130,396	0.08
Susceptible	20144555	0.2	141,504		0.08	103,261	0.06
Susceptible	20144560	0.0	55,039		0.03	139,128	0.08
Susceptible	20144561	0.1	160,446		0.1	129,671	0.08
Susceptible	20144562	0.2	256,770		0.15	101,050	0.06
Susceptible	20144563	0.1	57,773		0.03	96,449	0.06
Susceptible	20144565	0.0	20,971		0.01	105,856	0.06
Susceptible	20144570	0.1	185,071		0.11	113,030	0.07
Susceptible	20144604	0.1	48,103		0.03	84,397	0.05
			12,074,995	7.24		9,894,671	5.94

APPENDIX 4 IgE AND IgA BIOASSAY PROTOCOLS

Methods used in the preparation of 3rd stage homogenate and measuring IgE and IgA

Method for Preparation of Third Stage Larval homogenate

1. Begin with approx. 0.4 million third stage Teladorsagia larvae (from Elanco, Sydney). Larvae can be used straight from refrigerated culture flasks or from frozen at -80˚C.

2. Centrifuge larvae in 50mL falcon tube at 1,000 rpm (replaced with 3214×g) for 15 mins at 4°C. If not all larvae pellet leave on bench to settle for 10 mins. Remove supernatant.

3. Re-suspend larvae in 50 mL PBS, spin at 1000 rpm (replaced with 3214×g) for 15 mins at 4°C. If not all larvae pellet leave on bench to settle for 10 mins. Remove supernatant.

4. Re-suspend larvae in 50 mL PBS/antibiotic solution, spin at 1,000 rpm (replaced with 3,214×g) for 15 mins. If not all larvae pellet leave on bench to settle for 10 mins. Remove supernatant.

5. Re-suspend larvae in 50 mL Tris Poison solution, spin at 1,000 rpm (replaced with 3,214×g) for 15 mins at 4°C. If not all larvae pellet leave on bench to settle for 10 mins. Remove supernatant.

6. At this stage pellet should be frozen -20^{o}C. This aids breakdown of larvae for homogenisation.

7. Before homogenisation re-suspend pellet in an equal volume* of Tris Poison + DOC (sodium deoxycholate) solution.

8. **Homogenisation:** Insert one Ribolyser bead (metal bead lysing matrix 1/8 inch, Ref 6925-500. MP Biomedcals 29525 Foantain Parka Solon Ohil 44139 Germany) into each eppendorf with 1mL of larval suspension. Homogenise using a Ribolyser set at frequency 30Hz for 6 mins (40 sec x 5 in case temperature of homogenate gets high). Check that homogenate is debris free (may be some cuticle particulates remaining). The presence of foam indicates good protein yield.

9. Spin tubes at 13,000rpm for 5 min to recover supernatant – this is the larval antigen prep.

10. Filter homogenate through 0.2μm syringe filter.

11. Spin at 2,000 rpm for 20 mins. Quantify protein concentration by BCA assay.
    Aliquot L3 antigen prep and store at -80^{o}C

*: approximately 1 mL was added for about 320k Teladorsagia larvae. About 0.2 mL of the solution was used to rinse the filter, giving the final protein concentration approximately 2.5 mg/mL.

Solutions Required

PBS/Antibiotic

Mix 1 mL of 5 mg/mL Streptomicin/5,000 i.u. Penicillin (Sigma, Cat no. P4458), 50 μL of 10 mg/mL Gentamycin (Sigma, Cat no. G1272), and 0.5 mL of 250 μg/mL Amphotericin B (Sigma, Cat no. A2942), make up to 50 mL with sterile PBS pH 7.4.

Tris Poisons in 10 mM Tris pH 8.3

Make the following stock solutions.

* EDTA & EGTA require heated stirring and pH 8.3 to dissolve. Pepstatin, NEM, TPCK require heating in 60°C H_2O bath to dissolve.

EDTA	**2.92g in 20 mL H2O**	**(0.5 M) (NaOH pelleted added to raise pH to 8.3 and also for dissolving) ***
EGTA	3.80g in 20 mL H_2O	(0.5 M) *
NEM	0.125g in 10 mL H_2O	(10 mL of 0.1 M) *
Pepstatin	6.85mg in 10 mL EtOH	(10 ml of 1 mM) *
PMSF	581mg in 10 ml EtOH	(10 ml of 0.33 M)
TPCK	352mg in 10 ml EtOH	(10 ml of 0.1 M) *

*Mix 2 mL 0.5M EDTA solution, 2 mL 0.5M EGTA solution. This is designated PI-A (aqueous). Store at -20°C.

Mix 3 mL of 0.33 M PMSF with 1 mL 0.1 M TPCK then add 1 mL of 1 mM pepstatin. Warm in 65°C water bath to dissolve. This is designated PI-B (organic).

Mix 31.52 g Tris in 200 mL H_2O and pH to 8.3 to give a 1M solution of Tris-HCl.
(NB: requires large volume NaOH to raise pH therefore add 31.52 g Tris powder to 100 mL H_20 – stir to dissolve. Then add NaOH to raise pH 8.3 and finally make up to 200 mL with H_2O.)

Mix 2ml Tris-HCl, 1 mL PI-A, 1 mL PBI and 2 mL 0.1M NEM solution,
then make up to 200 mL using H_2O. This gives the Tris Poisons solution.

For Tris Poisons + DOC repeat the above but add 2 g Sodium Deoxycholate.

Protease inhibitors

EGTA = Ethylene Glycol-bis(β-Aminoethylether)-N,N,N',N'-Tetraacetic Acid (Sigma, Cat no. E0396)

EDTA = Ethylene Diamine Tetraacetic Acid (Sigma, Cat no. E9884)

NEM = N-Ethylmaleimide (Sigma, Cat no. E3876)

Pepstatin A = (Isovaleryl-Val-Val-Sta-Ala-Sta) Sta=statine=(3S,4S)-4-Amino-3-hydroxy-6-methylheptanoic Acid (Sigma, Cat no. P4265)

PMSF = Phenyl Methyl Sulfonyl Flouride (Sigma, Cat no. P7626)

TPCK = N-Tosyl-L-Phenylalanine Chloromethyl Ketone (Sigma, Cat no. T4376)

ELISA for Detection of Sheep IgA in Serum
modified based on Mike Stear's assay (Mar 2016)

1) Coat a microtitre plate[1] with 100 µL of parasite antigen (5 µg/mL in 0.06M bicarbonate buffer pH 9.6) and leave at 4°C overnight.

2) Discard antigen and incubate the plate with the 100 µL blocking buffer at 37°C for 1 hour and keep shaking consistently at 600 rpm.

3) Wash the plate 5 times with PBS-T.

4) Add 100 µL of the serum samples in duplicate diluted 1:20 in PBS-T and incubate at 37°C for 60 mins and keep shaking consistently at 600 rpm. Also need positive and negative (PBS-T) controls in duplicate for each plate.

5) Wash the plate 5 times with PBS-T.

6) Add 100 µL of Rabbit anti-ovine IgA-HRP[2] diluted 1:100,000 in PBS-T and incubate at 37°C for 60 mins with shaking consistently at 600 rpm.

7) Take HRP substrate[3] out of fridge to warm to room temperature.

8) Wash the plate 6 times with PBS-T.

9) Add 100 µL of substrate (ready to use) and incubate at room temperature for 10 mins.

10) Add 100 µL of stop solution.

11) Read plate at 450nm.

Method for making 0.06M carbonate buffer pH 9.6.

Stock solutions:
1M $NaHCO_3$: 84g in 1L H_2O
1M Na_2CO_3: 106g in 1L H_2O

Mix 45.3 mL 1M NaHCO3 with 18.2 mL 1M Na2CO3.

Make up to 1 L with H_2O.
Check the pH and adjust to 9.6 by adding HCl or NaOH.

Method for making PBS-Tween solution (0.02 M sodium phosphate buffer, 0.15 M sodium chloride, 0.5% Tween 20, pH 7.4)

a) Add 3.073 g sodium dihydrogen phosphate ($NaH_2PO_4.2H_2O$, MW156.01)
b) Add 11.399 g disodium hydrogen phosphate (Na_2HPO_4, MW141.96)
c) Add 44.00 g sodium chloride (NaCl, MW 58.44)
d) Fill with water to the 5L maker on the bottle
e) Add 25 mL Tween 20
f) Check and adjust pH with NaOH or HCl if necessary.

The final concentration of washing PBS solution is 0.01M, pH 7.4.

Method for making blocking buffer

Dissolve 10g of bovine serum albumin (Sigma, fraction V, Prod No A9418. Obtained from Dr Dieter Palmer, DAFWA) in 100 mL of PBS-Tween to make a 10% solution.

Method for making stop solution (0.5M H_2SO_4).
Slowly add 5 mL 95% H_2SO_4 to 175 mL dH_2O.

[1]Corning, Costar® 96 well clear flat bottom polystyrene high bind Microplate, cat no. 9018. Replaced with Greiner Bio-one, ELISA microplate 655061

[2]AbD Serotec, Rabbit anti-ovine IgA-HRP, cat no. AHP949P

[3]Fisher Scientific, TMB substrate kit, cat no. 10076433. Replaced with ELISA Systerm, TMB, ready to use substrate, Prod No ESKE1000, lot No. 150815, obtained from Dr Dieter Palmer.

Between batches variation was 12.9%, based on a serum sample measured in 13 batches (or plates. Mean = 15.62%, STD = 2.02, CV = 12.9%).
Within batch variation = 4.83%.
The threshold of duplicate variation was set <20%. Otherwise sample was repeated for assay.

ELISA for Detection of Sheep IgE in Serum

1. Mix 80% saturated NH_4SO_4 with plasma sample at 1:1 in 96-well plate, shaking for 15 min (on a shaker), then stop the plate over night at 4°C. This procedure is adopted from Dr Richard Shaw, AgResearch.

2. Centrifuge the plate using plate-centrifuge at 3,214×g for 30min.

3. Coat a microtitre plate1 with 100 µL of parasite antigen (~5 µg/mL in 0.06M bicarbonate buffer pH 9.6) and leave at 4°C overnight.

4. Discard antigen and incubate the plate with 100 µL blocking buffer at 37°C for 1 hours.

5. Wash the plate 5 times with PBS-T.

6. Add 100 µL of the serum NH_4SO_4-supernatants in duplicate diluted 1:10 in PBS-T and incubate at 37°C for 1 hour. Also need positive and negative controls in duplicate for each plate.

7. Wash the plate 5 times with PBS-T.

8. Add 100 µL of mouse anti-sheep IgE monoclonal2 (2FI, originated from CSIRO Armidale, obtained from Dr Dieter Palmer) diluted 1:200 in PBS-T and incubate at 37°C for 1 hour.

9. Wash the plate 5 times with PBS-T.

10. Add 100 µL of sheep anti-mouse immunoglobulins/HRP conjugate3, diluted 1:200 in PBS-T and incubate at 37°C for 1 hour.

11. Take HRP substrate4 out of fridge to warm to room temperature.

12. Wash the plate 6 times with PBS-T (carefully check on existence of air bubbles).

13. Add 100 µL of substrate and incubate at room temperature for 5 mins (time may be shorter since blue colour develops very rapidly once the substrate for the peroxidase was added).

14. Add 100 µL of stop solution.

15. Read plate at 450nm.

Method for making 0.06M carbonate buffer pH 9.6.
Stock solutions:
1M $NaHCO_3$: 84g in 1L H_2O
1M Na_2CO_3: 106g in 1L H_2O

Mix 45.3 mL 1M NaHCO3 with 18.2 mL 1M Na2CO3.
Make up to 1L with H_2O.
Check the pH and adjust to 9.6 by adding HCl or NaOH.

Method for making TBS-Tween solution (TBS-T was replaced with PBS-T, no influence on the assay but less cost of the chemicals).

To make 1M Tris buffer pH 8.0
To a short, wide mouthed 100 mL Erlenmeyer flask:

a) Add 12.11 g Tris base (Tris(hydroxymethyl)-aminomethane, Sigma T-1503) and dissolve in 80 mL of RO water on the magnetic stirrer
b) When dissolved check pH with the probe in the solution
c) Add about 4.2 mL of concentrated HCl to bring the pH down to 8.0
d) Make up to 100 mL

To make 0.05M Tris buffer pH 8.0:
100 mL of 1M Tris buffer, dilute 1:20, adjust pH at 8.0. Final concentration is 0.05M.
Add 1 mL of Tween 20 (Sigma, cat no. P7949) to every 1L TBS.

Method for making blocking buffer.
Dissolve 1 g of dried bovine serum albumin (Sigma, fraction V, Prod No A9418) in 100 mL of PBS-Tween to make a 1% solution.

Method for making stop solution (0.5M H_2SO_4).
Slowly add 14 mL 95% H_2SO_4 to 250 mL dH_2O.
Make up to 500 mL with dH_2O.

[1]Corning, Costar® 96 well clear flat bottom polystyrene high bind Microplate, cat no. 9018. Replaced with Greiner Bio-one, ELISA microplate 655061

[2]Mouse anti-sheep IgE monoclonal (2F1) antibody, gift from CSIRO Armidale, obtained from Dr Dieter Palmer

[3]Sheep anti-mouse immunoglobulins/HRP antibody (CHEMICON Australia, Cat No AP326P. Catch No. C107C, gamma and light chain specific. Obtained from Dr Dieter Palmer

[4]Fisher Scientific, TMB substrate kit, cat no. 10076433. Replaced with ELISA Systerm, TMB, ready to use substrate, Prod No ESKE1000, lot No. 150815, obtained from Dr Dieter Palmer.

Updated 4-April-2016

Enzyme immunoassay for Total Ovine Serum IgE

Assay has been validated on Nunc MaxiSorp plates (Cat No. 430341).
M:/IgE/Total/IGEMET3DOC26/08/20
(Dr Richard Shaw, AgResearch, NZ)

EIA method

1. Add 50 μL/well of YD3 mAb at 2.0 μg/mL to MaxiSorp 96-well disposable microtiter plates. Add to rows B-G, columns 2-11. Incubate for 2 hr at room temperature.
2. Wash 6 times with PBST (2x-2x-2x).
3. Block plates with Blotto (5% skim milk powder) at 220 μL/well. Incubate for 1 hr at room temperature.
4. Wash 3 times with PBST (2x-1x).
5. Add samples, standards, internal controls, and blanks at 50 μL/well in duplicate. Incubate for 2 hr at RT then over-night at 4ºC. Add serum samples at 1/25 dilution, IgE standards over range 0.8-0.05 Units/mL and internal control samples at 1/25. Dilute in dilution buffer.
6. Wash 6 times with PBST (2x-2x-2x).
7. Add 50 μL/well of pooled biotinylated XB6 mAb at 1.2 μg/mL to plates. Incubate for 2 hr at RT.
8. Wash 6 times with PBST (2x-2x-2x).
9. Add 50 μL/well of Streptavidin peroxidase at 1/2,000 to plates. Incubate for 1.5 hr at RT.
10. Wash 6 times with PBST (2x-2x-2x - 5 min each minimum).
11. Add 100 μL/well TMB substrate. Incubate for 30 min at room temperature.
12. Stop reaction by adding 50 μL of 1 M H_2SO_4.
13. Read plates on ELISA plate reader using 450 nm filter.

Data manipulation.

Open ELISA plate reader file in Excel and copy to - LogLog.xls. This program allows the accurate data analysis of the Sheep Total IgE capture assay using a Log-Log plot of Reference serum.

This program allows the accurate data analysis of the Sheep Total IgE capture assay using a Log-Log plot of Reference serum. The crude data is copied to each respective plate worksheet. The duplicates are averaged, minus background then Log transformed. Graph (Scatter plot) Log Absorbance (Y-axis) versus Log Concentration (X-axis) of Reference serum standard. Click mouse on graph, the right mouse button click, click Add trend line (linear) and click Display Equation and Display R^2 value Copy formula and R^2 value to A30 and manually insert Y-Intercept and Slope values. The real values for Reference serum, Internal standard (1/25 dilution) and unknown values (at 1/25 dilution) are calculated. This in then converted by Power base 10 to a real number and multiplied by the dilution factor to get real values. As the Reference serum has a nominal value of 100 Units/ml the 5 standard dilution should calculate out to ~100.
The MDV is the minimum value of IgE in serum diluted to 1/25 that the assay will detect (lowest standard).

See J.H. Peterman, 1991, In Immunochemistry of Solid-Phase Immunoassay. Ch3 pg. 47-65.
M:/total/Igemet3.doc6/20/2016 3:10:00PM 26/08/2020 2:56:00 PM

Solutions for Total Serum IgE EIA

1. YD3 mAb - 2.0 μg/ml in PBS using 530 μg/ml solution made 13/9/01

YD3 stock at 530 μg/ml and **3500 μl** of diluted mAb required per plate.
>> 2.0 μg/ml * 3500 μl = 530 μg/ml *? >> ? = 13.2 μl in 3.5 ml

1 plate = 13.2 μl/3.5 ml
2 plates = 26.4 μl/7.0 ml
3 plates = 39.6 μl/10.5 ml
9 plates = 118.9 μl/35.0 ml

2a. Washing buffer - PBST: 0.15 M NaCl, 10 mM phosphate pH 7.4, 0.05% Tween 20.

Final Volume	1 litre	2 litres	3 litres	4 litres
NaCl (grams)	8.76	17.53	26.28	35.06
0.2 M Phosphate buffer (ml)	50	100	150	200
10 % w/v Tween 20 (ml)	5	10	15	20
Distilled water (ml)	945	1890	2845	3780

2b. Phosphate buffer 0.2 M, pH 7.2, 2litres.

47.7 g/2l Na_2HPO_4 anhydrous (di-Sodium hydrogen phosphate MW 141.96)
~19.2 g/2l $NaH_2PO_4.2H_2O$ (Sodium dihydrogen orthophosphate MW 156.01)
Titrate with $NaH_2PO_4.2H_2O$ to get pH 7.2

2c. Stock Tween 20 - 10% (w/v) in PBS.

Dissolve 50 g of Tween 20, 50ml 10x TBS to 500 ml MilliQ water.

3. Blocking solution - Blotto.

5% skim milk powder in Blotto buffer. 5 g powder in 100 ml Blotto buffer.
250 μl/well, 20 ml/plate, 100 ml/5 plates, 150 ml/9 plates (7.5g).

4. Serum and antibody dilution buffer - 0.5% BSA in PBS plus 0.1% Tween 20.

Dissolve **0.5 g** BSA (Gibco) in 100 ml of PBS plus **0.1%** Tween 20.

5. IgE standards.

Dilute Reference serum to 1/125 then serially dilute this in tubes.
Dilute 400 µl of Reference serum in 49.60 ml of dilution buffer. MIX WELL.
Dilute 25.0 ml into 25.0 ml dilution buffer and mix well. Repeat to dilution 1/2000 - (5 tubes).
Dispense into microtitre dilution tubes – 1,000 µl/tube and freeze.
Internal standards are 6468-K (H), 5046-K (M) & 4109-K (L).
Alternatively use one sheep and dilute so that you have a high, medium & low standard.

6. Serum sample dilution.

Dilute serums 1/50 (1/25) in dilution buffer. (40 µl plus 960 µl). Serum containing high IgE levels (absorbance above that of 0.8 Units/ml IgE reference serum) are assayed at 1/50, **1/100**, 1/200 or 1/400. Automatic diluter (in RSG lab) has programs for diluting 1/25, 1/100, 1/200 and 1/400, IGE-25, IGE-100, IGE-200, and IGE-400, respectively.

7. Biotinylated XB6 (B-XB6) at 1.2 µg/ml in dilution buffer. (See IgE99-7.xls)

B-XB6 stock at 1560 µg/ml (21/1/99) and 3.5 ml of diluted biotinylated mAb required per plate.
>> 1.2 µg/ml * 3500 µl = 1560 µg/ml *? >> ? = 2.69 µl in 3.5 ml.
1 plate = 2.69 µl/3.5 ml
2 plates = 5.38 µl/7.0 ml
9 plates = 26.9 µl/35ml

8. Streptavidin Peroxidase (Pierce #21126) at 1/2000.

Dilute 12.5 µl in 25 ml of dilution buffer (6 plates).
i.e. 2.5 µl in 5 ml.
6 plates = 12.5 µl/25 ml
9 plates 15 µl/30 ml

9. TMB solution.

~ 8 ml/plate

	6 plates	8 plates	12 plates
TMB	6.5 mg	8.67 mg	13.0 mg
DMSO	500 µl	700 µl	1000 µl
TMB buffer	50 ml	70 ml	100 ml
Conc H_2O_2 (30%)	50 µl	70 µl	100 µl

Warm TMB to RT then weigh out. Disperse TMB powder and add DMSO. Pre-warm TMB buffer to RT.
Just before use add TMB/DMSO to TMB buffer and add H_2O_2.
Add 100 µl per well. Stop reaction after 30 min with 50 µl of 1M Sulphuric acid.

APPENDIX 5 GLOSSARY OF TERMS OF TRAITS SCORED ON SHEEP

Trait	Definition
BBDWR	Body wrinkle at birth
BIRTHCOAT	Birth coat score
BIRTHWT	Birth weight (kg)
E1BCOV	Breech cover at 1st post-weaning classing
E1BDWR	Body wrinkle at 1st post-weaning classing
E1BECOV	Belly cover at 1st post-weaning classing
E1BFLUF	Fluff score at 1st post-weaning classing
E1BRWR	Breech wrinkle at 1st post-weaning classing
E1CCOV	Crutch cover at 1st post-weaning classing
E1CS	Condition score at 1st post-weaning classing
E1DAG	Dag score at 1st post-weaning classing
E1DAGDM	Dag moisture at 1st post-weaning classing
E1FACE	Face cover at 1st post-weaning classing
E1NKWR	Neck wrinkle at 1st post-weaning classing
E1SC	Scrotal circumference (cm) at 1st post-weaning classing
E1TALE	Measured tail length (cm) at 1st post-weaning classing
E1TAWDTH	Tail length (cm) at 1st post-weaning classing
E1TAWR	Tail width (cm) at 1st post-weaning classing
E1TOES	Toe score at 1st post-weaning classing
E1URINE	Urine score at 1st post-weaning classing
E1WAX	Wax at 1st post-weaning classing
E1WCOL	Wool colour at 1st post-weaning classing
E1WT	Body weight (kg) at 1st post-weaning classing
E2BCOV	Breech cover at 2nd post-weaning classing
E2BDWR	Body wrinkle at 2nd post-weaning classing
E2BRWR	Breech wrinkle at 2nd post-weaning classing
E2CS	Condition score at 2nd post-weaning classing
E2DAG	Dag score at 2nd post-weaning classing
E2DAGDM	Dag moisture at 2nd post-weaning classing
E2FACE	Face cover at 2nd post-weaning classing
E2NKWR	Neck wrinkle at 2nd post-weaning classing
E2TAWR	Tail wrinkle at 2nd post-weaning classing
E2WT	Body weight (kg) at 2nd post-weaning classing
E3CS	Condition score at 2nd post-weaning classing
E3DAG	Dag score at 3rd post-weaning classing
E3DAGDM	Dag moisture at 3rd post-weaning classing
E3WT	Body weight (kg) at 3rd post-weaning classing
EBRSTRWEAN	Total breech strike up to weaner shearing
H10Dust_ConWt	Dust index
H10Suint_ConWt	Suint index
H10Water_ConWt	Water moisture index
H10Wax_ConWt	Wax index
H13TALE	Tail length (cm) post hogget shearing
H13TAWDTH	Tail width (cm) at post hogget shearing
H1CS	Condition score at 1st hogget measurement

H1DAG	Dag score at 1st hogget measurement
H1DAGDM	Dag moisture score at 1st hogget measurement
H1URINE	Urine score at 1st hogget measurement
H1WT	Body weight (kg) at 1st hogget measurement
H2CS	Condition score at 2nd hogget measurement
H2DAG	Dag score at 2nd hogget measurement
H2DAGDM	Dag moisture score at 2nd hogget measurement
H2WT	Body weight (kg) at 2nd hogget measurement
H3BCOV	Breech cover score at 3rd hogget measurement
H3BDWR	Body wrinkle score at 3rd hogget measurement
H3BECOV	Belly cover score at 3rd hogget measurement
H3BEPLUC	Belly pluck score at 3rd hogget measurement
H3BFLUF	Belly fluff score at 3rd hogget measurement
H3BLK	Black colour score at 3rd hogget measurement
H3BRWR	Breech wrinkle score at 3rd hogget measurement
H3CCOV	Crutch cover score at 3rd hogget measurement
H3CHAR	Wool Character score at 3rd hogget measurement
H3COL	Wool colour score at 3rd hogget measurement
H3CS	Condition score at 3rd hogget measurement
H3DAG	Dag score at 3rd hogget measurement
H3DAGDM	Dag moisture score at 3rd hogget measurement
H3DERMO	Dermatophilosis score at 3rd hogget measurement
H3DUST	Dust score at 3rd hogget measurement
H3FACE	Face cover score at 3rd hogget measurement
H3FLROT	Fleece rot score at 3rd hogget measurement
H3NKWR	Neck wrinkle score at 3rd hogget measurement
H3SHLDR	Shoulder score at 3rd hogget measurement
H3SPOT	Black colour spot score at 3rd hogget measurement
H3SSTRC	Staple structure score at 3rd hogget measurement
H3TAWR	Tail wrinkle score at 3rd hogget measurement
H3TOES	Toe score at 3rd hogget measurement
H3URINE	Urine score at 3rd hogget measurement
H3WAX	Wool wax score at 3rd hogget measurement
H3WEATH	Wool weathering score at 3rd hogget measurement
H3WT	Body weight (kg) at 3rd recording at hogget measurement
H4Belly_Wt	Belly wool weight (g) at hogget shearing
H4BULK	Wool bulk (grams/sq cm) at hogget shearing
H4CEM	Coarse Edge Micron - (micron) - Distance in Micron from the centre of the histogram to a point at the start where the highest 5% of fibres start. (coarse tail)
H4CFW	Clean fleece weight (kg) at hogget shearing
H4CURV	Fibre curvature (degree) at hogget shearing
H4CURVESD	Standard deviation of fibre curvature (degree) at hogget shearing
H4FD	Fibre diameter (micron) at hogget shearing
H4FD15	Proportion of fibres below 15 micron at hogget shearing
H4FD30	Proportion of fibres above 30 micron at hogget shearing
H4FDCE	Fibre diameter (micron) of the tail of the fibre diameter distribution with broadest 5% of fibres
H4FDCV	Coefficient of variation (%) of fibre diameter at hogget shearing

H4FDSD	Standard deviation of fibre diameter at hogget shearing (micron)
H4FDSF	Spinning fineness
H4FEM	Fibre diameter (micron) of the tail of the fibre diameter distribution with the finest 5% of fibres
H4FFC	Fibre fabric comfort
H4GFW	Greasy fleece weight at hogget shearing
H4GFW_belly	Greasy belly wool weight at hogget shearing
H4pRtoC	Resistance to compression of wool at hogget shearing
H4SL	Staple length (mm) of wool at hogget shearing
H4SS	Staple strength (N/Ktex) of wool at hogget shearing
H4YLD	Clean yield (%) of wool at hogget shearing
H7BCOV	Breech cover score at pre hogget shearing
H7BDWR	Body wrinkle score at pre-hogget shearing
H7BECOV	Belly cover score at pre-hogget shearing
H7BFLUF	Belly fluff score at pre-hogget shearing
H7BRWR	Breech wrinkle score at pre-hogget shearing
H7CCOV	Crutch cover score at pre-hogget shearing
H7COL	Wool colour score at pre-hogget shearing
H7CS	Condition score at pre-hogget shearing
H7FACE	Face cover score at pre-hogget shearing
H7HORN	Horn score at pre-hogget shearing
H7NKWR	Neck wrinkle score at pre-hogget shearing
H7SC	Scrotal circumference (cm) at pre-hogget shearing
H7SHLDR	Shoulder score at pre-hogget shearing
H7TAWR	Tail wrinkle score at pre-hogget shearing
H7TOES	Toes score at pre-hogget shearing
H7WT	Body weight (kg) at pre-hogget shearing
H8FEC	Faecal worm egg count at pre-hogget shearing
H8FMOIST	Faecal moisture content at pre-hogget shearing
HBRSTRHOG	Number of breech strikes between weaner and hogget shearing
MANBALE	Length (cm) of the bare skin area from the anus to the wool are in the crutch
MANBAWD	Width (cm) of bare skin area across the anus
MBCOV	Breech cover score at marking
MBDWR	Body wrinkle score at marking
MBFLUF	Belly fluff score at marking
MBRWR	Breech wrinkle score at marking
MCCOV	Crutch cover score at marking
MCOL	Wool colour score at marking
MDAG	Dag score at marking
MDAGDM	Dag moisture score at marking
MFACE	Face cover score at marking
MHAIR	Hairiness score at marking
MNKWR	Neck wrinkle score at marking
MTABALE	Bare area under the tail score at marking
MTABAWD	Bare area width score of the tail at marking
MTALE	Tail length at marking
MTALESC	Score for the length of the tail relative to the cannon bone at marking
MTAWDTH	Tail width score at marking

MTAWR	Tail wrinkle score at marking
MURINE	Urine score at marking
P1CS	Condition score at 1st post-weaning classing
P1DAG	Dag score at 1st post-weaning classing
P1DAGDM	Dag moisture score at 1st post-weaning classing
P1URINE	Urine score at 1st post-weaning classing
P1WT	Body weight at 1st post-weaning classing
P2CS	Condition score at 2nd post weaning
P2DAG	Dag score at 2nd post-weaning classing
P2DAGDM	Dag moisture score at 2nd post-weaning classing
P2URINE	Urine score at 2nd post-weaning classing
P2WT	Body weight score at 2nd post-weaning classing
P3CS	Condition score at 3rd post-weaning classing
P3DAG	Dag score at 3rd post-weaning classing
P3DAGDM	Dag moisture score at 3rd post-weaning classing
P3FACE	Face cover score at 3rd post-weaning classing
P3URINE	Urine score at 3rd post-weaning classing
P3WT	Body weight score at 3rd post-weaning classing
P4BCOV	Breech cover score at 4th post-weaning classing
P4BDWR	Body wrinkle score at 4th post-weaning classing
P4BECOV	Belly cover score at 4th post-weaning classing
P4BEPLUC	Belly pluck score at 4th post-weaning classing
P4BFLUF	Belly fluff score at 4th post-weaning classing
P4BRWR	Breech wrinkle score at 4th post-weaning classing
P4CCOV	Crutch cover score at 4th post-weaning classing
P4CHAR	Wool character score at 4th post-weaning classing
P4COL	Wool colour score at 4th post-weaning classing
P4DAG	Dag score at 4th post-weaning classing
P4DAGDM	Dag moisture score at 4th post-weaning classing
P4DERMO	Dermatophilosis score at 4th post-weaning classing
P4DUST	Dust penetration score at 4th post-weaning classing
P4FLROT	Fleece rot score at 4th post-weaning classing
P4NKWR	Neck wrinkle score at 4th post-weaning classing
P4SHLDR	Shoulder score at 4th post-weaning classing
P4SSTRC	Staple structure score at 4th post-weaning classing
P4TAWR	Tail wrinkle score at 4th post-weaning classing
P4TOES	Toe score at 4th post-weaning classing
P4URINE	Urine score at 4th post-weaning classing
P4URINEDM	Urine moisture score of the stain at 4th post-weaning classing
P4WAX	Wool wax score at 4th post-weaning classing
pH9CS	Condition score at scanning post hogget shearing
pH9EMD	Eye muscle depth (mm) at scanning post hogget shearing
pH9FAT	Subcutaneous fat depth (mm) at scanning post hogget shearing
pH9WT	Body weight at scanning post hogget shearing
W1FEC	Faecal worm egg count at weaning
W2BCOV	Breech cover score at 2nd weaning
W2BDWR	Body wrinkle score at 2nd weaning
W2BECOV	Belly cover score at 2nd weaning

W2BRWR	Breech wrinkle score at 2nd weaning
W2CCOV	Crutch cover score at 2nd weaning
W2CHAR	Wool character score at 2nd weaning
W2COL	Wool colour score at 2nd weaning
W2CS	Condition score at 2nd weaning
W2DAG	Dag score at 2nd weaning
W2DAGSDM	Dag moisture score at 2nd weaning
W2DUST	Dust penetration score at 2nd weaning
W2FACE	Face cover score at 2nd weaning
W2FLROT	Fleece rot score at 2nd weaning
W2NKWR	Neck wrinkle score at 2nd weaning
W2SHLDR	Shoulder score at 2nd weaning
W2SSTRC	Staple structure score at 2nd weaning
W2TAWR	Tail wrinkle score at 2nd weaning
W2TOES	Toes score at 2nd weaning
W2URINE	Urine score at 2nd weaning
W2WAX	Wool wax score at 2nd weaning
W2WT	Body weight at 2nd weaning
W3CS	Condition score at 3rd recording at weaning
W3DAGDM	Dag moisture score at 3rd recording at weaning
W3DAGS	Dag score at 2nd weaning
W3WT	Body weight at 3rd recording at weaning
WFMOIST	Faecal moisture content at weaning
Y1CS	Condition score at 1st yearling
Y1DAG	Dag score at 1st yearling
Y1DAGDM	Dag moisture score at 1st yearling
Y1URINE	Urine score at 1st yearling
Y1WT	Body weight (kg) at 1st yearling
Y2BCOV	Breech cover score at 1st yearling
Y2BRWR	Breech wrinkle score at 1st yearling
Y2CS	Condition score at 2nd yearling
Y2DAG	Dag score at 2nd yearling
Y2DAGDM	Dag moisture score at 2nd yearling
Y2DERMO	Dermatophilosis score at 2nd yearling
Y2TAWR	Tail wrinkle score at 2nd yearling
Y2URINE	Urine score at 2nd yearling
Y2WT	Body weight (kg) at 2nd yearling
Y3CS	Condition score at 3rd recording at yearling
Y3DAG	Dag score at 3rd recording at yearling
Y3DAGDM	Dag moisture score at 3rd recording at yearling
Y3URINE	Urine score at 3rd recording at yearling
Y3WT	Body weight (kg) at 3rd recording at yearling