Breech Flystrike Prevention Genetic R&D Review

Independent review of Australian Wool Innovation's Breech Flystrike Prevention Genetic Research, Development and Extension Program, June 2021

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Executive Summary

The breech strike selection flock projects are now complete with final reports from both flock sites finalised. This major program has defined the key factors underlying differences in susceptibility and provided critical genetic information to enable the development of robust and efficient breeding programs towards increased breech strike resistance.

Development activities are now building on these findings to facilitate phenotyping of animals on farm and to optimise the effectiveness of selection for breech strike resistance in an overall practical economic breeding context. Two major areas of development are noted; 1) Facilitating ease of selection through the use of easy-to-measure correlated characters, such as the use of neck and body wrinkle for breech wrinkle, the use of face cover for breech cover and the use of faecal consistency as an alternative to dag score. There is also the potential for the development of new automated methods of measuring breech traits. 2) Use of genetic parameter estimates from the breech strike flocks to provide information for Merino producers on the rates of gain and economic impacts of different breeding approaches to increasing breech strike resistance. The development of breeding indices that incorporate blowfly resistance within MERINOSELECT is signalled as a major priority and work towards this end is currently underway.

The use of gene markers or genomic methods, utilising knowledge of the very genes that underlie differences in resistance, has a number of major advantages. Sheep do not need to be exposed to strike, or predisposing conditions such as scouring or urine stain for a genetic evaluation to be made and a genetic value can be attributed to all animals, regardless of whether they are bred in high or low flystrike environments or whether it is a high or low flystrike risk year. Preliminary work done using data and genetic material collected from the breech strike selection flocks indicates that there appears to be no major gene markers for breech strike resistance, but that genomic breeding values (GEBVs) based on a large number of genes will be able to assist major gains in this area. The development of accurate GEBVs requires large numbers of accurately phenotyped and genotyped animals and can be an expensive exercise. The establishment of a virtual resource flock comprised of sheep from a range of sources such as research flocks, the Merino Lifetime Productivity project, sire evaluation sites and private flocks has been suggested and represents a pragmatic approach to achieving the required data base at reasonable cost.

The sequencing of a high quality sheep blowfly genome has been a significant development which is expected to underpin major advances in a number of areas and perhaps novel approaches to sheep flystrike control. New gene editing technologies (CAS/CRISPR) that have already contributed to major advances in other areas of science have been successfully adapted for use with *Lucila cuprina* and a number of edited strains of sheep blowflies developed (for example one strain with deletion of an odour receptor gene that may be important in fly to attraction to sheep). The work is being run in parallel with a flystrike vaccine project and aims to characterise maggot genes that are important in

the establishment of strikes on sheep, towards the identification of potential vaccine targets, new flystrike protectants or area wide approaches to the suppression or eradication of sheep blowfly populations. This work has the potential to provide exciting outcomes towards novel control technologies in the longer term.

There has been an exceptional and ongoing communication effort undertaken throughout the breech strike selection flock projects, relaying outcomes and implications to producers in an easily digestible format and timely fashion so that wool growers can make appropriate changes to their breeding and management programs in light of the information produced. A recently conducted survey of parasite control practices indicates that an increasing proportion of sheep producers use electronic sources to find their information and in the last reporting period, partly necessitated by the Covid crisis, there has been increasing development of excellent web based resources, most particularly on the AWI website and in the ParaBoss/Flyboss parasite control site. The survey also indicated ParaBoss as a major and growing source of information for sheep producers. ParaBoss is currently moving to a new management structure and continued timely modification of breeding material on this site and harmonisation with other sources of sheep breeding and selection information in light of new data should continue to be a priority.

1. Introduction

A multifaceted research program towards the development of new methods of flystrike control has been conducted over the last decade by Australian Wool Innovation with significant advances in many areas. It remains widely considered that breeding more resistant sheep will be the keystone strategy to control breech strike in non mulesed flocks as well as in reducing the risk of strike in mulesed sheep. A 12-year research program funded by AWI to increase genetic resistance is now complete. Results from analysis of the data from this work confirm that there is considerable genetic variation for breech flystrike and that with appropriately designed breeding programs, breeding sheep with enhanced resistance to breech strike is feasible with minimal reduction in production gains.

2. Breech strike selection flocks

Selection flocks to study the genetics of breech strike were established at Mt Barker in WA and at Armidale in NSW in 2005. Active data collection from these selection flocks is now complete with final project reports available on the AWI website (2 reports on Project ON-00169, one for the WA site and the other the NSW site). Results from analysing data from these flocks confirm that there is considerable genetic variation for breech flystrike resistance and that resistance was genetically associated with three main traits, breech wrinkle, breech and crutch cover and dags. ASBVs for these three traits were made available to industry in 2009. The project confirmed the overwhelming importance of breech wrinkle in susceptibility to breech strike in present day Merino types, both in the Mediterranean climate in WA and in the summer rainfall zone of NSW. Furthermore, the results indicated that the accuracy of selection and rates of gain can be increased by using the correlated and more readily assessed characters, neck wrinkle and body wrinkle. Dag was shown to be one of the most important predisposing characters for breech strike in more southerly environments such as SE Western Australia where scour worms predominate, but less important in areas where *Haemonchus contortus* is the major gastrointestinal parasite. The genetic correlation between dag score and breech strike was also high in some data sets from the summer rainfall environment but it was noted that in this latter area dag is a transient trait, which is most frequent in young sheep, that it can be attributable to any one of several causes, and that it is seldom a problem in adult sheep. The studies have confirmed that crutch and breech wool coverage assessed using Visual Sheep Scores developed

during this project and now used widely in industry for breech phenotyping are both heritable and genetically associated with breech strike resistance. Urine stain was also found to be associated in some data sets in both WA and NSW. However, its value as an indirect character was suggested to be limited because of difficulties in accurate assessment caused by the presence of dags and effects of time of shearing.

A wide range of other potential indirect characters for breech strike susceptibility were also assessed in the breech strike resource flock projects (WP468 and ON-00169) and the potential role of fleece/skin bacteria examined in a later project (WP639 and ON-00169). However, most of these characters provided little advantage over the four characters discussed above. One group of factors indicated from this project that may warrant further investigation are tail-associated characteristics such as tail wrinkle, tail width, and tail bare area length. Interestingly, tail characteristics were also flagged as potentially important in very early studies of breech strike susceptibility. In addition, urine stain showed association in some sheep, and is known to be an important predisposing character for flystrike. Difficulties in accurately scoring urine stain were identified and no ASBV for urine stain has been developed and further work in this area is required.

An immense amount of data was collected during this project and ongoing analysis continues to provide further insights. A new paper recently published from data collected in this project (Greeff et al. 2021), examining a potential role for differences in the fleece microbiome in susceptibility found that there was no effect, suggesting that further work in this area is not justified. An important finding in another recent paper was that in the WA flocks for breech wrinkle, as well as for dag and breech cover, there was a strong genetic correlation between values in unmulesed and crutched, unmulesed and uncrutched, and mulesed and crutched sheep, and that data collected for these traits under all of these management systems can be used to estimate breech strike breeding values (Greeff et al. 2019). In addition, genetic material has been preserved from all of the animals and in the breech strike flocks and has already provided an important resource for studies towards the development of genomic breeding values (see section 4).

3. Industry implementation of research findings

The recently completed survey of Australian sheep parasite control practices (Project ON-00540, Colvin et al. 2020) has provided a good snapshot of the use of breeding in the sheep industry. Results of the survey indicated 58% of Merino producers are currently using genetic selection as part of their blowfly control program. Of these producers 55.5% used visual traits on ewes to breed sheep that are less susceptible to flystrike, and 43.7% used visual traits for ram selection. Notably scoring for these traits is done using breech scores that have been made available to wool producers in the AWI and MLA Visual Sheep Scores Booklet and which are now widely used for assessing these traits in industry. The most common visual traits used were ‘breech wrinkle’ (22.1%), ‘cull sheep with breech strike’ (20.0%), ‘dag score’ (14.6%), ‘urine stain’ (12.1%) and ‘breech cover’ (12.1%). Breech wrinkle was the most commonly used ASBV (63.6%), with 52.3% using WEC 36.4% using scouring and dags and 36.4% using breech cover. Australian Sheep Breeding Values (ASBV) were used as traits in ram selection by 17.3% of those who answered questions on blowfly control.

The 2019 genetic review indicated that much more widespread phenotyping for flystrike traits (particularly for urine stain and dag score) was needed to provide more robust and widely applicable estimates in MERINOSELECT. This remains the case, as does the need to improve the ‘usability’ in a practical sense of flystrike traits in MERINOSELECT. The use of neck and body wrinkle rather than breech wrinkle in situations where direct scoring of breech wrinkle is difficult has been noted. Scoring

of face cover as an indicator for bare area and the use of faecal consistency as an indicator of dag score have also been suggested to facilitate scoring. The use of automated photography, image analysis and machine learning to assess breech characters has also been suggested and may warrant investigation for future use.

Sheep Genetics publishes annual genetic trends for traits evaluated in its genetic evaluation schemes. MERINOSELECT provides genetic trends for the key traits of breech wrinkle, breech cover and dag score. Examination of these trends indicates relatively modest improvement in breech wrinkle with minor or no improvement in bare area and dags. However, these data should be interpreted with caution because data sets can vary from year to year with the effects of additional data from new members potentially masking gains made by longer term users, the difference in effect of selection in the different merino types and the effect of improvement in production characters obscuring the impact of selection for breech characters. Importantly, sires have been identified that are in the top 1% for low breech wrinkle that are also in the top 1% for Adult Clean Fleece Weight and top 1% on Index, with a larger number of sires in the top 5% for these traits. The existence of such sires demonstrates that high levels of genetic resistance to breech strike can be compatible with high genetic levels of productivity. Further information is available at:

https://www.wool.com/globalassets/wool/sheep/research-publications/welfare/breeding/breeding-and-selection---industry-trends---geoff-lindon.pdf

As 58% of Merino producers stated they are currently using genetic selection as part of their blowfly control program in the recent survey (project ON-00540, discussed above), the genetic trends available from MERINOSELECT may not reflect the full extent of changes occurring in the genetic level of resistance to breech strike in Australian Merinos.

4. Translation/interpretation of breech strike data

One of the largest barriers to selection for breech strike resistance is undesirable correlation with production traits, in particular fleece weight. However, it has now been shown in a number of studies that if breech wrinkle is included in a carefully designed selection index, breech wrinkle can be reduced with little associated reduction in rates of gain in production traits. Two studies indicated that using index selection and depending on the emphasis placed on breech wrinkle in the index, reductions of 0.4 to 0.9 units in wrinkle could be achieved over a 10-year period while maintaining reasonable rates of genetic gain in production traits. These studies have recently been extended by including all three of the main indirect criteria for which ASBVs are available in MERINOSELECT (Project ON-00524) and predicting the rates of gain in flystrike resistance achievable by adding a flystrike trait to MERINOSELECT indices. Substantial genetic gains in flystrike resistance were predicted without unrealistically compromising rates of genetic improvement in the other production traits, with the project concluding that reduction of breech strike to levels similar to those achieved by mulesing is achievable after 10–20 years of index selection with relatively minor reductions in rates of gain in other traits.

Although ASBVs for wrinkle, dag and breech cover that allow breeders to select indirectly for breech strike resistance are now available from SHEEPGENETICS, there is a clear need for suitable selection indices in order to maximise rates of improvement in breech strike resistance and concurrent rates of gain in production traits. AWI have previously indicated support for this recommendation. A survey conducted by Sheep Genetics with 60 breeders and consultants provided an almost unanimous view that wrinkle score trait should be a part of all indexes as well as dag score, where records are available, such as in areas of high dag prevalence. This will be implemented soon in the MERINOSELECT Service,

likely using an informal method ('desired gains approach') to determine the selection emphasis to be placed on reducing wrinkle and dag scores relative to production traits. This is an excellent interim step to defining and incorporating a formal breech strike resistance trait into MERINOSELECT indexes, which will require the formal derivation of accurate economic values. This should remain a longer-term priority to enable the development of optimal indexes incorporating breech strike resistance.

5. Genomic selection

The potential advantages of using genomic selection for flystrike resistance are substantial as animals do not need to be exposed to strike, or predisposing conditions such as scouring or urine stain for a genetic evaluation to be made. In addition, a genetic value can be attributed to all animals, regardless of whether they are bred in high or low flystrike environments or whether it is a high or low flystrike risk year. It has been demonstrated that genetic progress in sheep breeding programs could be fast-tracked with the application of genomic breeding values. Furthermore, the major benefit of GEBVs is usually seen with traits that are difficult or expensive to measure, such as flystrike.

Genotyping of animals from the breech strike selection flocks has provided the basis for genomic studies (Projects WP550 and ON-00515) and 576 DNA samples from the WA and NSW Breech Flystrike Resource flocks were genotyped with the 50K Ovine SNP chip. The current data was combined with genotype and phenotype data from WP550 (2014). The previous data set included ~950 genotypes of 600K density. All of the current genotypes were imputed to 600K SNP density and all data analysed as one data set.

This study also demonstrated that currently there is no strong evidence that marker assisted selection would be a successful approach to breed for breech flystrike resistance and genomic breeding values for breech strike resistance were estimated with an accuracy of 33%. The project demonstrated that genomic selection based on genomic breeding values will be the most efficient approach to create benefit to industry within the next 5 years, but that for GEBVs to be a reliable selection tool, this accuracy would need to be improved significantly. Ideally this approach requires a large reference population of accurately phenotyped and genotyped animals. However, genomic-enhanced breeding values for flystrike could be potentially developed successfully with the collection of further data by setting up a reference population (series of linked resource flocks). Planning to implement a modest but cost-effective reference population is underway with potential sources of sheep including the MLA Resource Flock, Sheep CRC Info Nucleus, Breech Strike Selection Flocks, AMSEA Sire evaluation sites, the Merino Lifetime Productivity project and suitable private flocks.

6. Sheep blowfly genetics

The cloning of the sheep blowfly genome is an exciting development providing the possibility of a range of novel flystrike controls and facilitating new approaches to previously tested areas. The initially high quality genome (Project EC456) has been further improved using new sequencing technologies, significantly increasing its utility for the current project (Project ON-00217). New gene manipulating and editing technologies (for example CAS/CRISPR) have been adapted to efficiently edit the *L. cuprina* genome, facilitating the identification of genes that could be targeted in new control approaches. The potential of the technology for use with *L cuprina* has now been demonstrated in the development of strains of sheep blowflies with deletions affecting eye colour (white eyes) and an odour receptor that could be important in the attraction of flies to sheep Projects ON-00516, ON-00570. Other analyses are investigating genes that are important in the early development of larvae on sheep and assays using gene knockdown have highlighted the potential for some genes to halt

larval development. These studies will assist in the identification of vaccine targets in parallel projects (Projects ON-00619, ON-00624).

In a further, related project *L. cuprina* from different areas of Australia are being sampled to determine their genetic similarity and any critical differences between populations Projects ON-00373, ON-00624). This will allow clarification of the degree gene flow between different fly populations, assisting in the planning of programs to manage insecticide resistance, to assess the general applicability of different insecticide or vaccine targets and to assess the feasibility of different population control strategies for sheep blowflies. This work is already yielding important data, and some interesting findings, for example that *Lucilia sericata* may be more important as a strike fly in Tasmania than in the rest of Australia and that genetically distinct strains of *L. cuprina* may be present in some other parts of the country.

Better understanding of the 'genetic architecture' of Australian sheep blowfly populations and the genes that operate at different stages in strike establishment (host finding, oviposition, egg hatch and larval invasion), critical developmental genes and conditional survival genes will aid the development of blowfly strike vaccines and insecticides and facilitate development of other novel approaches such as area-wide genetic controls aimed at eradication or suppressing sheep blowfly populations. This work is likely to provide exciting outcomes towards new control technologies in the longer term.

7. Education, Extension and Promotion

A clear and concise overview of the AWI strategy for industry education, extension and promotion of its 2020 Flystrike Prevention RD&E Program, by Geoff Lindon, Bridget Peachey and Emily King is available on the AWI website.

AWI is in the process of revamping its flystrike extension package (Project ON-00765). During 2021, this will include developing an extension package on breeding for breech strike resistance, which is to be commended.

The flystrike RD&E technical update forum (Project ON-00651), normally held every 2 years, was cancelled due to COVID-19 restrictions. Instead, presentations from relevant projects and AWI staff were posted on the AWI website, which collectively provide a great resource of information on breech strike RD&E for stakeholders.

Other very useful presentations on breeding for improved resistance to breech strike and supporting RD&E are available on the AWI website, as follows:

- 'Breeding for Improved Fly Control – SheepConnect NSW Webinar 10th Sept 2020', by Geoff Lindon, available at: https://www.wool.com/sheep/welfare/breech-flystrike/breeding-for-breech-strike-resistance/
- 'Fly on the Run?' by Bridget Peachey, available at: https://sheepconnect.s3.amazonaws.com/media/resources_files/2/f5f9c1162183/Fly_on_the_Run_-_Bridget_Peachey.pdf
- 'It's Flytime' AWI https://www.wool.com/sheep/welfare/breech-flystrike/flystrikeresources/

ParaBoss, incorporating FlyBoss website presents a dynamic and readily available platform for rapid dissemination of research outcomes (Project ON-00382). The recent survey of sheep owner parasite control practices (Project 0N-00540) indicates that a steadily increasing proportion of sheep producers use electronic sources (and particularly the ParaBoss website) to access parasite control information, with ParaBoss coming second only to 'other farmer or member of my staff' in importance of sources

of parasite control information. Paraboss has been recently reformatted to increase accessibility from mobile phones, which will make FlyBoss easily accessible from field sites for purposes such as for accessing breech trait scores during sheep classing or making decisions about the application of flystrike treatments. The management structure for ParaBoss is about to change and priority should be given to ensuring that breeding recommendations for flystrike resistance continue to be updated in light of new information and harmonised with other sources of sheep breeding information.

8. Concluding comments

- The shape of the research input has changed with completion of the 12-year program of collection, analysis and interpretation of data from the breech strike selection flocks in WA and NSW. This program has represented a very significant and sustained investment of funds by AWI, but also by DPIRD and CSIRO. All involved are to be congratulated. Results from the program provide a strong base for breeding recommendations, and a large data base to underpin future research, development and extension activities.

- The main area of work within the flystrike genetics program has now shifted to development of economically optimal and practical breeding programs and the adaptation of the research findings for on-farm use to suit different flock contexts.

  Development and extension areas flowing from the breech strike project include:
  - Increasing on farm phenotyping for breech traits and submission to MERINOSELECT. Dags and urine score are particular areas where data is still relatively sparse.
  - Validating and facilitating less labour intensive and accurate measurement of key traits for example use of neck and body wrinkle for breech wrinkle, face cover for bare area, faecal consistency for scouring.
  - The development of optimal breeding indexes that incorporate breech traits in MERINOSELECT
  - Development of robust recommendations for incorporation of breech traits in traditional breeding programs

- Genomic breeding values are particularly valuable for traits such as flystrike resistance as sheep do not have to be exposed to strike for selection to occur. However development of accurate genomic breeding values requires a large database of accurately phenotyped and genotyped animals and can be expensive. The development of a virtual resource flock, drawn from a number of different research and commercial flocks and existing databases as currently proposed seems a pragmatic and cost-effective approach and is strongly supported.

- The availability of a high quality sheep blowfly genome and the successful adaptation of new genome editing technologies represents exciting possibilities for the development of novel controls such as flystrike vaccines, new flystrike protectants and area wide controls to directly target sheep blowfly populations. This research focusses on longer term outcomes, but is strongly supported.

- The volume, quality and timeliness of extension communication from the flystrike research program has been exemplary and appropriately targeted to wool producers and other industry stakeholders. Continued collaboration and consultation with practical woolgrowers, ram breeders, Sheep Genetics personnel and other providers of sheep breeding advice (including ParaBoss) towards the formulation and communication of optimal breech strike breeding programs and the development of breech strike indexes in MERINOSELECT is encouraged.

9. References

Colvin AF, Reeve I, Peachey B, Walkden-Brown SW (2021). Benchmarking Australian sheep parasite control practices: a national online survey. *Animal Production Science* **61,** 237-245. doi:10.1071/AN20171.

Greeff JC, Karlsson LJE, Schlink AC (2019) Are breech strike, dags and breech wrinkle genetically the same trait in crutched, uncrutched and mulesed Merino sheep? *Animal Production Science* **59,** 1777-1782. doi:10.1071/AN18461.

Greeff JC, Paz E, Munyard K, Schlink AC, Smith J, Karlsson LJE, Martin G (2021) Differences in the microbiomes of the breech between Merinos resistant or susceptible to breech strike. *Animal Production Science* (Accepted for Publication 14 April 2021).