Breech Flystrike Prevention Genetic R&D Review

Independent review of Australian Wool Innovation's Breech Flystrike Prevention Genetic Research, Development and Extension Program, June 2018

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Executive Summary

The 2018 review of the genetic components of AWI's Breech Strike R, D and E program was conducted by reference to research reports, extension materials and meeting catalogues and at a meeting with Australian Veterinary Association and AWI representatives in June 2018. The major breech flystrike selection flock experiments, commenced in 2005 at Mt Barker in WA and at Armidale in NSW, have been completed. The key findings are that genetic gains can be made in reducing susceptibility to breech flystrike by selecting sheep on scores for breech wrinkle, breech cover, dag and urine stain and that these gains can be made simultaneously with improving production traits, including fleece weight and fibre diameter. A study is currently under way to quantify the genetic gains possible across different Merino sheep types and production environments. Notwithstanding, considerable variation in flystrike susceptibility remains unexplained particularly in males, suggesting the importance of other, unidentified underlying factors. Early studies with sniffer dogs indicated that they could accurately distinguish wool samples from resistant and susceptible sheep, suggesting the importance of differences in sheep odours in attracting flies, but studies on the response of flies to sheep odours have been equivocal. A review of the results and other research to date is now underway to determine the future direction of this research. Ewes from the Mt Barker and Armidale flocks are being retained as a genetic resource to be used in these and other potential future studies.

The utilization of gene markers and genomic indices for selection offers major potential advantages by enabling selection in the absence of environmental challenge and at younger ages and further research, currently underway, which is utilising the available genetic material is strongly supported. Sequencing the sheep blowfly genome has been a further major advance and research currently underway to exploit this knowledge offers great promise for the future by assisting the development of new, rationally designed blowfly insecticides, flystrike vaccines and other novel methods directly targeting fly populations. This is frontier basic research, in an area of major potential impact for the wool industry, which directly targets the genetic code of the sheep blowfly. Continuing work in this area is strongly supported.

Australian Sheep Breeding Values for breech wrinkle, breech cover and dag scores, implemented since 2009 as part of the MerinoSelect service (provided by Sheep Genetics) are now available on 20-30% of recorded animals for use by ram breeders and wool producers. The scoring systems developed for flystrike resistance indicator characters also assist resistance selection on properties with more traditional breeding strategies. Further extension to encourage the recording of breech traits and the development of selection index options that incorporate them are supported.
A considerable extension effort has been made to make the research to date available to wool producers and facilitate its practical usefulness through grower training days and workshops and the provision of updated information via the AWI ‘Beyond the Bale’ newsletter and the FlyBoss, Sheep Genetics and AWI websites. Notable additions in this reporting period are the publications ‘Managing Breech Flystrike’ and ‘Planning for a Non-Mulesed Merino Enterprise’, downloadable from the AWI Website. These publications give guidelines and capture the experiences of woolgrowers attempting to phase out mulesing and will be valuable to others seeking to reduce reliance on mulesing for breech strike control.

We commend the considerable effort that has been expended to date to develop improved methods for selecting sheep for breech strike resistance, adapting these to practical recommendations for woolgrowers and in ensuring the information is readily available to wool producers, ram breeders and other industry stakeholders. Detailed recommendations from the 2018 review are provided in the body of the report. A detailed summary of progress is contained in a table in the appendix, which follows the framework of the Research, Development and Extension matrix used by AWI.

**Breeding and selection research**

General consensus within the wool industry is that breeding more resistant sheep will be critical and over the long-term the most important and sustainable tool to control breech strike in non-mulesed flocks. To identify the most effective breeding strategies AWI, in partnership with wool producers, national and State research providers and other industry stakeholders has undertaken a comprehensive research, development and extension program to develop and advance the implementation of optimal breeding programs for breech strike resistance.

In 2005, AWI commenced a major research project (EC940 Breeding for breech strike resistance) conducted in two major sheep production zones, the summer rainfall zone of New South Wales and the Mediterranean climate of south-western Western Australia. These two areas have dissimilar environments and different management systems and are representative of a significant proportion of sheep production in Australia. Final reports of Phase 1 of the project were provided in 2010. Phase 1 confirmed the presence of significant genetic variability amongst sheep in susceptibility to breech strike, identified breech wrinkle, dags, breech wool cover and urine stain as indirect characters for flystrike resistance and provided preliminary genetic parameters for flystrike resistance. The scoring systems developed for these indicator characters have found industry acceptance and are aiding selection for breech strike resistance in both performance and traditional selection programs. Phase 2, conducted from 2010 to 2016 collected further data and significantly improved the precision of genetic parameter estimates for resistance to breech flystrike and its relationship with other traits. In addition, in Phase 2 management of the WA selection flock was modified to include a winter crutch, a commonly used management practice in Mediterranean environments. This reduced the overwhelming effect of dags in Phase 1 at the WA site and provided valuable insight into the relative value of indirect traits and rates of gain in flystrike resistance that can be made under both crutched and un-crutched management systems. A large amount of extra data was contributed to the Sheep Genetics data base, significantly adding to the accuracy and precision of Australian Sheep Breeding Values (ASBVs) for flystrike resistance available to sheep producers.
One of the findings from this research was that despite the identification of key factors responsible for differences in resistance of sheep to flystrike (breech wrinkle, dags, breech cover and urine stain), a large proportion of the total variation in susceptibility remained unexplained, particularly for males. Studies indicated that carefully trained sniffer dogs could accurately distinguish wool samples from resistant and susceptible sheep. AWI Project ON-00169, aimed at identifying other factors underlying the unexplained variation in susceptibility to flystrike, commenced in 2012. Early studies examining differences in odour, fleece microflora and responses elicited from fly antennae were extremely encouraging, but some, more recent aspects have been more equivocal. A review of the outcomes from the project and the results of other research has been initiated to determine the future direction of this work. Ewes from the Mt Barker and Armidale selection flocks are currently being retained as a genetic resource to be used in these and other potential future studies and we consider maintenance of this critical resource a significant priority.

**Genomic breeding values and indexes**

Directly assessing the genes responsible for variation in flystrike susceptibility is a further means of increasing the accuracy of selection and rate of improvement in breech strike resistance and could provide an alternative to the use of traits such as dags and urine stain, which can be difficult to assess in practice. Gene markers and genomic breeding values offer advantages by enabling selection at younger ages and in the absence of environmental challenge, reducing the substantial labour input involved in scoring phenotypic characters, and potentially increasing rates of genetic gain in breech strike resistance. Project WP550 identified substantial numbers of genomic (SNP) markers for breech flystrike resistance and breech indicator traits for the sheep bred in Phase 1. However the sizes of the individual SNP effects were found to be small and unlikely to be useful in selection programs. Difficulty in identifying individual genes or loci for factors determined by interactions of many genes, as is likely to be the case with flystrike susceptibility, is not uncommon. This has led to increasing use of genomic indexes incorporating many genes, which has generally given much higher levels of accuracy. Sheep in Phases 1 and 2 of the project were accurately phenotyped for flystrike resistance, have good pedigree data and have had wool, skin and blood samples collected. Sheep bred in Phase 2 have a greater diversity of resistant and susceptible animals and have also had DNA samples collected. Analysis of these samples to investigate the potential development of genomic associations with flystrike resistance, as well as with dags and intestinal worms is now underway and is strongly supported.

**Sheep blowfly genetics**

Mapping of the sheep blowfly genome (AWI project ON-00217) has to date identified some 14,544 protein coding genes, with more than 2000 of them sheep blowfly specific, which offers wide ranging prospects for the development of new controls in the future, including new generation vaccines and insecticides and directly targeting blowfly populations in area wide approaches. Work based on this substantial achievement is already being utilized towards the development of new blowfly-specific insecticides (AWI project ON-00110) and AWI Project ON-00315 is adapting the ground-breaking new CRISPR/Cas9 gene editing technology for potential use in directly knocking out critical sheep blowfly genes. Although adapting the technology from use in *Drosophila* to use in sheep blowflies has been more challenging than expected, significant progress has been
achieved with successful deletion of the ‘Orco’ gene, which is thought to be important in odour detection, a critical step for the establishment of strikes by sheep blowflies. Given the large amount of transcriptome data being analysed, there are likely to be many other genes of interest that could targeted in control programs. This ground-breaking work offers exciting prospects for the development of novel flystrike controls in the longer term and is also strongly supported.

Grower, industry and domestic stakeholder extension, training and communications

Analysis of all data collected in Mediterranean and summer rainfall climates in Phases 1 and 2 of the breech strike resistance program has enabled translation of the research outcomes to optimal recommendations for wool producers and ram breeders. Australian Sheep Breeding Values (ASBV$s$) are available through the MerinoSelect service for the principal indicators of breech strike resistance, breech wrinkle, breech cover and dag scores. Extensive submission of industry data has enabled ASBV$s$ for breech wrinkle and breech cover to be issued with acceptable levels of accuracy and broad applicability to the range of Merino strains and types run in different parts of Australia. These ASBV$s$ are facilitating the design of effective breeding programs for Merino breeders and commercial wool producers to improve breech strike resistance and increasing confidence that genetic improvement of sheep will be able to provide the basis for wool producers to cease mulesing. The availability of ASBV$s$ for dag score whilst less widely measured than that for breech wrinkle and breech cover scores, provides some limited coverage of the diversity of Merino types in areas where dag prevalence is high. However management practices can affect expression of this trait, accurate assessment can be difficult and there is understandable industry resistance to allowing young sale rams and young ewes to become exposed to significant levels of dags. ASBV$s$ are presently not available for urine stain for similar reasons and accurate assessment of urine score is even more difficult than for dags. Research Flocks and progeny testing sites run by the Australian Merino Sire Evaluation Association (AMSEA) are very important industry resources where dag and urine stain scores are currently being collected. Industry should be urged to obtain dag and urine stain data from mixed aged breeding ewes where possible, as well as research flocks and sire evaluation sites when the opportunity arises, to assist in overcoming these deficiencies. Wrinkle scores on the neck and body have been found to be genetically correlated with those on the breech and could be used for estimating breech wrinkle ASBV$s$. Where direct assessment of breech wrinkle is difficult. Notably, in the recent AWI Merino Husbandry Survey, breeding less wrinkled sheep was the second most common reason given by wool producers who have ceased mulesing, noting a high awareness amongst producers of the importance of this approach.

A considerable effort has been made to make the results of research from the AWI Breech Strike Research and Development Program available to wool producers and facilitate their use in practice in both traditional and performance breeding flocks. Vehicles for this include 7 breech strike newsletters from each of the 2 sites during Phases 1 and 2 of the project, the AWI ‘Beyond the Bale’ newsletter and the FlyBoss, Sheep Genetics and Australian Wool Innovation websites. Notable additions in this reporting period are the publications ‘Managing Breech Flystrike’ and ‘Planning for a Non-Mulesed Merino Enterprise’, downloadable from the AWI Website. A major emphasis over the last 4-5 years has been on breeding and selection strategies and the optimal use of breech strike trait scoring systems and breeding tools, including flystrike-related ASBV$s$, to achieve most rapid rates of genetic improvement in breech strike resistance. Although ASBV$s$ are available for breech traits from the
MerinoSelect website, they are not currently available as part of any index aimed at increasing flystrike resistance and have to be used independently. The Ram Select mobile phone App has increased ready accessibility to sheep ASBVs, but in its current form, because flystrike resistance is not included in any formal index, has limited practical usefulness for strike resistance breeding programs. Incorporation as part of formal index options, provided from the website, would greatly assist the adoption and optimal use of breech traits in breeding programs.

The FlyBoss website presents a dynamic and accessible platform for rapid dissemination of research outcomes and new information on best practice strategies and FlyBoss usage has continued to grow in the last year with user numbers increasing by 142% from 2014/15 to 2015/16 a further 26% in 2016/17 and 24.9% to 24,406 unique users in 2017/18. The ParaBoss News e-newsletter is now sent to 3181 subscribers, twice per month. The ParaBoss Facebook page, started in mid-2016, has 1034 likes, while the ParaBoss Technical Forum (a web forum for professional advisors) has 150 members. Further innovations currently under development in FlyBoss are a ‘roadmap’ to assist woolgrowers wishing to move to management of flystrike in flocks without the use of mulesing and a more ‘responsive’ web format which will make FlyBoss information more accessible from mobile phones. Communication activities to other wool industry stakeholders during the reporting period include meetings with broker, processor and retail organisations during September and October 2017.

Concluding comments and recommendations

- The breech flystrike selection flocks have provided a critical source of accurately phenotyped animals for use in a number of facets of breech strike research and provide a critical resource for future studies. Sheep from these flocks should be retained if possible and wool and genetic material should be collected from all study animals for use in potential future research.
- The further recording of dag score in industry flocks and of urine score in areas of low dag prevalence should be encouraged. Where dag prevalence is low, the collection of faecal consistency scores and the development of a breeding value for the trait are supported.
- The availability of ASBVs for neck and body wrinkle scores is encouraged, for situations where breech scores are difficult to record.
- To facilitate more widespread adoption and efficient selection of sheep for lower susceptibility to breech strike and to improve genetic gains, there is a need to formally include breech strike resistance in breeding objectives, preferably as options in MerinoSelect, but also as customized breeding objectives for individual breeders.
- The potential of progeny testing elite sires for estimation of more accurate ASBVs for flystrike resistance should be investigated.
- The use of genomic indicators and indexes for breech strike resistance could significantly improve the ease of selection and rates of gain in breech strike resistance and further research now underway, utilizing the phenotypic data and tissue material collected during Phases 1 and 2 of the breech strike selection project and recently improved analysis techniques, is strongly supported.
• Strong support is given for the updating of predictions of the rates of genetic gain in improving resistance to breech strike in different environments, management systems and Merino sheep types to provide realistic assessments to growers and other stakeholders of the likely time necessary to genetically change flocks to a point where surgical mulesing and undue reliance on chemical treatments is unnecessary.

• Investigations of sheep blowfly biology and host-parasite interactions to clarify factors underlying unexplained variation in flystrike resistance may lead to the identification of new criteria to improve rates of gain in selecting flystrike resistance. A review of research in this area is currently underway to recommend future directions for this research.

• Recent mapping of the sheep blowfly genome is an exciting achievement which, together with availability of the ground-breaking CAS/CRI SPR gene editing technology, offers the possibility of directly targeting essential sheep blowfly genes and the development of innovative new controls for flystrike. Continued investment in this area is strongly supported.

• The provision of genetic trends for breech traits by Merino sheep type on the MerinoSelect/Sheep Genetics website should be encouraged, as it would allow ram breeders, commercial wool producers and other wool industry stakeholders to more readily assess industry progress towards breeding sheep less prone to breech flystrike.

• Research toward new and better methods of control has been accompanied by a comprehensive extension campaign to stakeholders and we congratulate the numerous organisations and individuals involved in the design and delivery of these programs. Extension designed to assist rapid genetic gains in breech strike resistance in both performance and traditionally bred flocks should continue to be a priority and support of the excellent web based information resources and tools and programs of meetings and forums to aid practical implementation will be critical to maximizing industry adoption.
## Appendix - Detailed response to AWI's Research, Development and Communication Strategy Framework

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<th>Areas of Investigation</th>
<th>Objective</th>
<th>Current Status/Progress Made</th>
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<td><strong>Breeding &amp; Selection</strong></td>
<td>Identify the incidence of flystrike resistance in the current population</td>
<td>Project EC940 (Phase 1, 2005-10) found that 10% of sires produced progeny that could be considered resistant to flystrike without mulesing under most conditions and could be reliably used to increase genetic resistance to breech flystrike. Un-mulesed progeny of the most resistant sire with no preventative treatments had a strike rate of only 2.4% under moderate to heavy challenge. In comparison, in the same environment at Mt Barker WA the average breech strike rate from birth to hogget shearing was 23% for unmulesed males and 31% for females. Project WP468 (Phase 2, 2010-2012) confirmed repeatability of resistance status under different climatic conditions and different levels of strike challenge and improved the accuracy with which the sheep industry can best identify resistant animals under commercial management conditions. Project ON00169 (Phase 2, 2012-2016) demonstrated a lower breech strike incidence in unmulesed animals at Mt Barker WA of 4% to 9.5% when they were crutched as yearlings in early winter to reduce dags during the spring fly season. Phase 2 (2010-2012) provided further data confirming the consistent importance of breech wrinkle, dags and breech cover as indicators of breech flystrike under different seasonal conditions, but also suggested the importance of urine stain, scored strategically, as an indicative criterion in the Mt Barker WA flock but not the Armidale NSW flock. Management of the WA selection flocks was modified in 2012, to include a pre-winter crutch of yearling sheep, and has provided better definition of the role of urine stain and factors related to odour in differences between resistant and susceptible sheep. Change in crutching time led to reductions in the recorded incidence of breech flystrike and dags in WA and has</td>
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<td>Task</td>
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<td>Identify breech trait heritability and correlations with other important traits to be used in the creation of Australian Sheep Breeding Values (ASBVs)</td>
<td>Heritabilities for breech traits and correlations of these with production traits were estimated with good precision in Phase 1 and 2, aided by the collection of industry data. This is facilitating the design of more accurate breeding programs to improve breech strike resistance while maintaining or improving production. No strongly antagonistic correlations have been found that would prevent simultaneous improvement of breech strike resistance and production traits. From 2012, unmulesed animals were crutched at yearling age at the Mt Barker site to reduce dag accumulation. This has markedly decreased the observed variance and estimated heritability of breech strike incidence in the Mt Barker WA flock to levels similar to the Armidale NSW flock.</td>
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| Produce and extend research prototype breeding values and ASBVs for main breech traits | Over the last 5 years ended June 2017, recording of breech wrinkle and cover and availability of ASBVs for these traits has averaged 30% and 25% respectively, of all animals recorded, with a slight downward trend over that period. Over the same period, recording of dags has remained steady at 19% of all animals recorded and may partly reflect that the trait is not easy or worthwhile recording in areas of low dag prevalence. Whilst there continues to be good coverage of different strains of Merinos with breech wrinkle and breech cover records (and to a lesser extent, dag score), run in different parts of the country, further emphasis of the importance of ongoing collection of data for breech traits and particularly urine stain scores will be required to achieve maximum rates of gain in flystrike resistance.

Further R&D is needed to encourage breeders of very low wrinkle sheep to collect data on more wrinkle traits (neck and body wrinkle), as there can... |

provided refinement of genetic parameter estimates. Under these conditions wrinkle was the most important indicator factor of breech strike resistance. A substantial proportion of the variation between animals in susceptibility remains unexplained and a review to investigate potential directions of research to clarify the source of this variation is currently underway.
be technical difficulties in providing ASBVs for breech wrinkle in sheep with very low variability. Use of scores for neck and body wrinkle to estimate breech wrinkle ASBV may provide a solution.

The collection of industry records on urine stain (a further indicator of breech strike) is encouraged, but as yet no records have been submitted. Sire evaluation sites and the Merino Lifetime Productivity Project are important sources for obtaining data and if sufficient industry data becomes available, a Research Breeding Value (RBV) for urine stain will be developed. It is considered highly desirable to have ASBVs for urine stain provided in Sheep Genetics reports.

### Development of further breeding tools or options for identifying unknown causes of flystrike

Effective indicator traits have been identified and can be used to obtain worthwhile genetic gains in breech strike resistance. However, substantial variation in flystrike incidence remains unexplained for males. A number of other traits have been tested, but provide little advantage over the four key indicator traits. Neck wrinkle is highly correlated with breech wrinkle and is often more accurately and easily assessed. Selecting on the basis of ASBVs for neck wrinkle can rapidly reduce breech wrinkle. Wool colour has been related to breech strike susceptibility in some instances and fleece rot is an important indicator of body strike susceptibility. ASBVs have recently been made available for these two traits through Sheep Genetics.

The cause and roles of differences in odour between resistant and susceptible animals and differences in microbial communities in the fleece have been investigated in a series of experiments in Project ON-00169. An association has been found but results to date have been too variable for practical usefulness. A review of the potential of odour and other possible risk factors underlying unexplained variation amongst sheep in flystrike susceptibility to determine possible future directions for this work is currently underway.

### Identify genomic associations with flystrike resistant traits

A total of 612 Single Nucleotide Polymorphisms (SNPs) for breech flystrike were identified in AWI supported project WP550, however, none of these, taken individually, showed a statistically significant association.
with susceptibility. To date only the first 5 drops of the Breech Strike Selection Flocks have been tested with the remaining 5 drops of more diversely resistant and susceptible sheep yet to be tested. This will now occur as part of a further AWI supported project.

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<th><strong>Grower, industry and domestic stakeholder extension, training and communications</strong></th>
<th>Investigate and monitor changes in on-farm management strategies for breech flystrike control</th>
<th>Responses have been collated from sheep producers surveyed for parasite control practices, including the use of genetic selection strategies for breech strike resistance, in AWI/MLA project ‘Benchmarking Australian Sheep For Parasite Control’. Surveys were mailed to 6,361 producers with a 36% total reply rate. The proportion of producers using some sort of visual selection for blowfly related traits was 61% for ewes and 45% for rams while the proportion using ASBVs for blowfly related traits was relatively low (5% for ewes and 10% for rams). Indicator traits (both breeding values and phenotypic scores) used for selection were breech wrinkle (65% in rams and 51% in ewes), low dag score (31% in rams and 27% in ewes) and bare breech (28% in rams and 19% in ewes). This survey will provide a baseline for monitoring future change in flystrike control practices, including breeding for breech strike resistance. A further survey which will provide trends in these figures will be conducted later in 2018.</th>
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<td>Provide woolgrowers, the domestic wool industry and relevant stakeholders updates of the RD&amp;E outcomes and welfare advances to manage breech flystrike prevention and improve animal welfare</td>
<td>Outcomes of research have been well publicised to woolgrowers and other industry stakeholders by production of 14 ‘Breech Strike Genetics’ newsletters in WA &amp; NSW and regularly in AWI ‘Beyond the Bale’ articles. Scoring systems for indirect selection traits, developed to provide practical tools for use in breech strike resistance selection programs, have been widely promoted and made freely available to sheep owners in print form and on the AWI, FlyBoss and Sheep Genetics websites. These systems form the basis for breech trait ASBVs available from the Sheep Genetics website and can also be used in traditional breeding programs. Further funding for ‘ParaBoss’, until 2020 has been confirmed by AWI. This project manages the FlyBoss site and provides a readily accessible</td>
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resource for wool growers on all aspects of flystrike control. The breeding section is updated in light of most recent research outcomes, links to material available on the AWI and Sheep Genetics websites and is facilitating the rapid communication of latest research to ram breeders and woolgrowers. Use of the FlyBoss site has continued to increase with the number of visits in January to June 2017, increasing by 25% on the corresponding period in 2016. A further innovation, a ‘roadmap’ for sheep producers aiming to phase out mulesing is currently under development and will be added to FlyBoss in the next 6 months.

A suite of resource material has been developed for trainers, and Breech Strike Management Workshops have been delivered by the Sheep CRC in partnership with AWI, MLA and a range of woolgrower organisations, State agencies and private consultants. The major focus in recent years has been on promoting breeding technologies and the use of ASBVs for breech and flystrike related traits. The total combined attendance at these workshops was just over 7,000 people. The Sheep CRC RamSelect App, released in 2015, that runs on smart phones and tablets, as well as computers, will assist easy use of ASBVs for breech traits in breeding programs, particularly for breeders and wool producers without a detailed knowledge of ASBVs. Notwithstanding, there is a continuing need for practical extension activities on using genetic tools to aid in breeding sheep for less susceptibility to flystrike.

Communication of progress and advances to animal welfare organisations has continued at AWI’s Animal Welfare Forum and on a regular basis to major retailers associations in Europe and North America.

Another biennial AWI Breech Strike R & D technical update forum is scheduled to be held in July 2018 in Sydney, with stakeholders invited from a variety of sectors of the wool and sheep industries and the presentations to be uploaded on AWI’s website.