AWI PROJECT NO: ON-00510

A REVIEW OF PREDISPOSING FACTORS FOR BREECH FLYSTRIKE

AUTHORS

Peter James[1], Forbes Brien[2] and Alisha Anderson[3]

[1]QAAFI, University of Queensland, St Lucia, Qld 4072
[2]School of Animal and Veterinary Sciences, University of Adelaide Roseworthy, SA 5371
[3]Division of Health and Biosecurity, CSIRO, Black Mountain, ACT 2601

PROJECT SUMMARY

Research over many years has focussed on the development of better methods of breech strike control. As a result, efficient fly control consists of an integrated approach which may include breeding for resistance, strategic crutching and shearing, prevention of scouring, strategic use of flytraps, paddock management to minimise strike risk and tail docking. However effective control of breech flystrike often relies primarily on mulesing and the prophylactic use of chemical insecticides with breeding for strike resistance a key element in an increasing number of flocks. Mulesing is exceptionally effective in preventing breech strike and has been the keystone procedure in integrated programs for flystrike control over many years but is increasingly untenable because of animal welfare concerns. In addition, resistance has emerged to the major chemical groups currently used for flystrike control, although the extent of this resistance amongst flocks is currently unclear. In this project the potential for new approaches to control was assessed.

This project, completed in 2019, was to undertake a comprehensive review of the predisposing risk factors for breech flystrike and report on recommendations for further flystrike research. The project:

- Reviewed Ausvet report documenting outcomes from a Flystrike Risk Factors Review Planning Meeting to summarise risk factors for flystrike and identify areas of knowledge deficit (www.wool.com/flystrikecausalweb)
- Assisted in the planning, conduct and assessment of outcomes from a Breech Flystrike Review Workshop (4th December 2018, Stamford Plaza Sydney Airport) to identify areas of research and development priority
- Reviewed AWI research conducted to date towards the identification of sheep risk factors for breech strike
- Reviewed the literature on the role of odour in the development of breech strike and determining differences in strike susceptibility amongst sheep
- Assessed a number of areas of interest flagged at the Flystrike Review Workshop, but not addressed in the main workshop recommendations.

RECOMMENDATIONS FROM THE REVIEW

Recommendations made from the different processes have been summarised below under three main headings: a) Breeding for resistance, (where a number of the areas were identified as high priority at the Breech Flystrike Review Workshop) b) Better understanding of the fundamental biology of *Lucilia cuprina* and strike development (also an outcome from the Flystrike Workshop); c) Other means of control. For a detailed description of the outcomes from this project, including a response to the recommendations from AWI, please see the project final report, available at www.wool.com/flystrikelatest. A number of these research areas are now currently being further addressed by AWI funded research programs or more detailed reviews to explore the opportunities in particular areas. Some of these are noted below

Breeding for resistance

- *Increase the collection of phenotypic data from industry flocks (and other research flocks where relevant) with a view to the development of a breech strike index/indexes)*
  Encouragement of much more widespread phenotyping for flystrike traits is required to provide more robust and widely applicable estimates in Merino Genetics. This is particularly so for urine stain, which currently does not have a breeding value available in MERINOSELECT, and for scouring/dags. To this end there is a need to facilitate easier methods of measurement of 'difficult' traits such as urine stain and scouring/dags. This could be easier methods of assessing them, or perhaps indirect methods of estimating urine stain/risk of urine stain. The recording of alternative more readily measured estimates for the main flystrike traits e.g. faecal consistency for scouring, face cover for bare area, neck and body wrinkle for breech wrinkle for recording in MERINOSELECT and presentation of ASBVs for these traits should also be considered. Progeny testing of elite sires directly for breech strike incidence would provide an avenue for increased accuracy and maximising industry genetic gain in flystrike resistance.

- *Development of breech strike / welfare indexes.*
  There is a need to facilitate practical 'useability' of breech strike traits for sheep breeders in MERINOSELECT. Breeding indices incorporating breech strike resistance while maximising genetic gains for other traits are needed for a range of different environments and sheep types. Optimal incorporation of breech strike resistance will require the derivation of an economic value(s) for breech strike resistance that are specific to these different situations.

- *Better understand the unexplained variation in the occurrence of strike in resistant and susceptible sheep and the effect of management regime on this*
  The amount of variation in breech strike susceptibility not explained by the major indicator characters will be key to a consideration of the need for new or better indirect selection criteria. There is little unexplained variation in some data sets (e.g. crutched ewes in WA breech strike resource flocks where only 9.38% of the variation remains unexplained) and dags and skin wrinkles explain most of the phenotypic variation, as opposed to the NSW flocks and unmulesed, uncrutched flocks in WA where approximately 50% of the variation remains unexplained.

- *Invest in a genomics reference flock to generate genomics breeding values*
  This was considered a high priority, with a high cost, but high potential reward by Workshop participants. Genomic methods have major potential benefits for selecting flystrike resistance because sheep do not need to be exposed to strike or subject to the predisposing conditions for flystrike to enable selection and detailed and difficult phenotyping is not required. Rather, the genotype is estimated from a small blood sample. Furthermore, a genetic value can be attributed to all animals in all years and all environments, regardless of level of flystrike challenge. The establishment of a Genomics Implementation Working Group to determine the best path forward, with regard to available resources /resource constraints was recommended. This panel should include high-level specialist expertise in sheep/animal genomics, sound industry reference and representation from Sheep Genetics to facilitate implementation.

- *Support continuation of the breech strike resource flocks.* The two flocks provide a source of very accurately pedigreed and phenotyped animals and are in completely different environments with different flystrike profiles. The depth of phenotyping for flystrike incidence in the flystrike selection lines in WA (now at Katanning) and NSW (Chiswick) makes these flocks an important core resource for genomic studies, a prime resource for identifying and testing new indicator characters and valuable for obtaining more precise genetic parameters for the development of more accurate selection and breeding programs. The flocks would also be an important resource for research in other areas, for example investigating the role of microbiome profiles in strike etiology and susceptibility, testing the efficacy of new vaccine technologies and resistant phenotypes, and the future development of welfare indices (that incorporate resistance to breech strike) and breeding values.

- *Development of a detailed business case for investing in genetic improvement of sheep resistant to breech strike*
  To understand if further investment into breeding programs focussed on reducing breech flystrike is worthwhile, and to underpin promotion to woolgrowers about the application of genetic technologies or other approaches, an understanding of the size and scale of potential benefits is required – i.e. a value proposition/business case. A component of this work, for example, would be a benefit cost analysis for establishing genomic evaluation of flystrike'. This would also inform the feasibility/attractiveness of different approaches by quantifying the size of trade-offs that growers are willing to make.

Biology of *Lucilia cuprina* and strike development

- *Increase understanding of the fundamental biology of the Australian sheep blowfly*.
  In particular, to understand the genes that operate at different times in key developmental processes and in host detection, the location of suitable sites for oviposition and at different stages in the establishment of strikes by first instar larvae. This may assist in the development of improved bait or deterrent options and identify targets for new families of blowfly strike insecticides and vaccines. These studies need to be carefully targeted to provide knowledge with specific endpoints towards improving control efficacy and will be facilitated by the recent mapping of the sheep blowfly genome and advances in molecular technology. The need for a careful review of the abundant work already undertaken in this area before commencing new research was emphasised. The research needs to be targeted to specific outcomes in order to ensure efficiency and value of the investment. (Work in this area supported by AWI is underway, Project ON-00624 - Informed Development of a Blowfly Vaccine).

- *Understand the fleece / dag microbiome, and its role in breech strike susceptibility*
  It is well established that bacterial growth is important at various stages in the development of body strike, for example in providing odour cues for attraction and oviposition, causing skin scalding and extravasation which provides protein for the development of 1st instar larvae, and by providing a focus for skin invasion by newly hatched blowfly maggots. Microbial odours, particularly in association with urine or other decomposing organic matter have also been shown to be important in the attraction of other livestock ectoparasites to their hosts and bacteria often also provide critical nutritive factors for larval development of some livestock-associated flies.

  There has been much less study of the importance of the breech fleece microbiome and interactions with urine stain and scouring or of the importance of bacteria in the development of breech strike. However, there is indication that bacterial growth could be similarly important in determining breech strike susceptibility. The microbiome could also influence skin proteomic/metabolomic profiles and associated studies of the fleece/skin proteomics and metabolomics may yield additional important information towards the development of new approaches to control, for example vaccination against key bacteria, blocking bacterial odours, the use of bactericides or biological methods to control critical bacteria.

- *Better understand the role of attractants/odour in sheep susceptibility and the genesis of strike*
  Odour is involved at a number of stages during development of strike. In particular, the location of sheep, the identification of susceptible sites on sheep for oviposition and stimulating egg laying by flies. In sheep, odours associated with bacterial growth, particularly when in association with urine staining, scouring and diseases such as fleece rot and lumpy wool, are critical in determining susceptibility to strike. Many of the main odours involved at different stages appear to be bacterially and environmentally mediated and there is little evidence that innate (genetically controlled) odour differences between sheep influence fly attraction or are related to susceptibility. In addition, any innate differences in sheep odour are likely to be overwhelmed by the effects of bacterial odours during strike waves.

  However, bacterial odours and other volatiles associated with predisposing causes of flystrike, such as urine and faecal staining, are critical to the initiation of strike and methods that interfere with the perception of odour by the flies, for example by targeting critical olfactory genes or processes, or the identification of strongly repellent molecules may lead to novel control approaches. Studies in this area should take account that odour could be operating at a number of stages in strike development in addition to attraction (for example acting as an arrestant or egg laying stimulant) and design experimental tests accordingly. Clarification of olfactory mechanisms in *L. cuprina* and the genetic basis underlying these may lead to the identification of new insecticide or vaccine targets, novel compounds with persistent repellent modes of action or which work by disrupting egg laying by the flies.

Other means of control

- *Manage insecticide resistance and maintain the efficacy of available flystrike control products*
  The availability of effective flystrike protection and treatment chemicals remains critical to effective management of flystrike in Australian flocks, particularly in non-mulesed flocks. There is a long history of resistance development to flystrike control chemicals in *L. cuprina* populations and the recent emergence of resistance to the keystone control products, dicyclanil and cyromazine is a major threat to sustainability of wool production. (See AWI project ON-00491 Sheep Blowfly Resistance Update). This will be particularly important in unmulesed flocks, highly susceptible flocks and flocks in high flystrike risk regions. It is currently unclear whether or not resistance is widespread and how many flocks are affected.

  There has been limited detailed consideration of the optimal design for long term resistance management programs. A project to investigate different resistance management approaches is required. The possibility of developing products based on chemical mixtures, a strategy widely used for combating resistance to gastrointestinal parasites but not currently used for flystrike control should be considered.

- *Develop new insecticidal actives for flystrike control*
  With increasing costs of development and registration, the rate of new sheep blowfly insecticides coming onto the market has "slowed to a trickle". The sheep parasiticide market is relatively small in the world context and this is particularly relevant as most of the major pharmaceutical companies that conduct research in this area have a multinational focus. Research in this area, desirably in partnership with commercial AgPharma companies, will assist the continued availability of effective flystrike preventatives for use by Australian woolgrowers. The availability of the *L. cuprina* genome provides the possibility of new insecticidal targets and AWI is currently funding a project in this area (ON-00454 New Chemicals for Flystrike Control). There may also be an opportunity to consider other previously tested compounds known to be effective against *L. cuprina* but not developed for this use to date because of prevailing market circumstances.

- *Development of flystrike vaccines*
  AWI projects towards the development of a flystrike vaccine are currently underway (AWI Project ON-00619 Vaccine for Control of Flystrike). This is a high risk, but potentially very high reward area which will be facilitated by the recent availability of the *L. cuprina* genome. A vaccine directed against fleece rot bacteria, critical in susceptibility to bodystrike was previously developed and patented, but never commercialised (Burrell 1985). This vaccine gave extended protection against fleece rot and bodystrike. As preliminary evidence suggests that many of the same bacteria may be important in susceptibility to breech strike, investigation of the potential of this vaccine for use in reducing susceptibility to breech strike may be worthwhile. AWI Project ON-00723 is currently reviewing the feasibility of fleece rot and lumpy wool vaccines.

- *Reducing the incidence of scouring*
  Scouring (diarrhoea) and resultant dags in the breech wool of sheep are major predisposing causes for breech strike in the southern sheep production areas of Australia. Dags are also a major management issue in their own right in these areas. Methods to reduce the incidence of scouring and dags would have a major impact in reducing breech strike incidence. Recommendations towards the reduction of dags have been provided to AWI in a previous project (AWI Project WP520 and subsequently updated (AWI Project ON-00610 Minimising Dags in Sheep). A fact sheet (www.wool.com/dag-factsheet) and advisor manual (www.wool.com/dag-manual) on managing dag have recently been published.

- *Biological control of sheep blowflies*
  Biological control could be either classical biological control, which consists of the release of specialist natural enemies that are expected to persist in blowfly populations keeping fly populations low (classical biological control), or innundative biological control (biopesticides) where large numbers of pathogenic organisms (fungi, bacteria, viruses), parasites or predators are released, or used to treat sheep, as 'biological pesticides'. *L. cuprina* occurs at low population density at most times and flystrike is episodic with fly populations building rapidly when conditions become suitable. The rate of spread of pathogens and parasites is almost invariably density-dependent. This factor and the lag time generally experienced between a pest outbreak and a corresponding increase in numbers of biocontrol agents would seem to present difficulties for classical biocontrol agents to persist and impact on *L. cuprina* populations, or more particularly, to reduce strike incidence.

  Biopesticides such as *Bacillus thuringiensis* and some entomopathogenic fungi have shown short term protection when applied to sheep in experimental studies and suitable agents may have application as part of an integrated approach or in organic flocks. However, they are unlikely to provide a level or persistence of protection comparable with chemical pesticides, which limits their practicality in many situations. Pathogens that persist in the soil, such as some fungi or entomopathogenic nematodes, may have effect against soil stages of *L. cuprina* (prepupal larvae and pupae) particularly during the overwintering phase, better knowledge of the spatial and temporal ecology of the soil phases of *L. cuprina* will be required to assess whether sufficient mortality could be induced to significantly affect flystrike incidence. One particular type of soil fungus, *Tolypocladium cylindrosporum* was shown to cause high mortality in the soil stages of *L. cuprina* in NZ and the potential for biological control of *Lucilia spp*. using sheep blowfly pathogens was reviewed in more detail as part of AWI Project ON-00620 Review of Sheep Blowfly Pathogen Control. *Tolypocladium cylindrosporum's* prevalence in Australia is currently being investigated (ON-00721 Identifying cylindrosporum in Australia).

- *Area wide genetic controls for Lucilia cuprina*
  These methods seek to bring about suppression or eradication of the target pest by the release of insects of the same species that have been modified to confer sterility or cause genetic death in the pest population. This approach is also known as autocidal control and could be used in area wide strategies focussed on eradicating sheep blowflies from an area or suppressing fly abundance through ongoing releases of modified

flies. The most well-known method, the sterile insect technique (SIT) was successfully used to eradicate screwworm flies (a cattle pest with similar biology to sheep blowflies) from North and Central America, as well as to eradicate an exotic incursion of screwworm flies in Libya and which is also used for eradicating regional incursions of fruit flies in fruit fly-free areas of Australia.

The availability of gene editing technologies such as CAS CRISPR provide the potential for more elegant systems of genetic control such as RIDL (Release of Insects with Dominant Lethality) or potentially using gene drives to spread deleterious genes (often sex-linked or stage specific genes) through fly populations. The previously noted research currently being funded by AWI, to identify critical genes in *L. cuprina* may facilitate the design of genetically modified strains suitable for use in area wide autocidal approaches. Transgenic sexing "male only" strains have been developed in North American *L. cuprina* strains and consideration should be given to the feasibility of the future use of these strains in the design of area wide strategies in Australia.

FURTHER INFORMATION

For a detailed description of the outcomes from this project, including a response to the recommendations from AWI, please see the project final report, available at www.wool.com/flystrikelatest.

REFERENCE

Burrell, D. H. 1985. Immunization of sheep against experimental pseudomonas-aeruginosa dermatitis and fleece-rot associated body strike. *Australian Veterinary Journal* 62: 55-57.

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