

2020 FLYSTRIKE PREVENTION RD&E PROGRAM PROJECT SUMMARY REPORT



AWI PROJECT NO: ON-00619

VACCINE FOR CONTROL OF FLYSTRIKE

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SUMMARY

CSIRO with support from AWI has just completed its first 12 months of research in developing a vaccine to control flystrike. During this initial period, specific classes of antigens have been identified and 26 individual antigens produced as recombinant proteins in bacterial or insect cell expression systems. These prototype vaccines are currently undergoing testing in vaccine trials in sheep and being assessed for immunological response and efficacy in controlling flystrike. There are more antigens in the pipeline that are being developed and novel approaches using synthetic biology being investigated. The next 12 months will provide important information derived from these vaccine trials and identify lead priorities for the next step in this technically challenging initiative.

CSIRO acknowledges its productive collaborations with the University of Melbourne, Griffith University and the University of Queensland Protein Expression Facility in this research endeavour.

PROJECT BACKGROUND

CSIRO is proud to partner with AWI in a project aiming to develop a Flystrike Vaccine for the Australian sheep and wool industries. Flystrike, and its control, costs the industry dearly each year (>\$170m p.a.) and the industry is facing mounting hurdles in the effective and socially acceptable process of combating this pest. The industry needs an effective, welfare-friendly alternative flystrike control and prevention technology. CSIRO has a long history in ectoparasite vaccine research, developing a commercial cattle tick vaccine and undertaking foundation research in buffalo fly, screwworm fly and sheep blowfly vaccine research in the 1990's to early 2000's. The strategic investment by AWI and research undertaken by the University of Melbourne in sequencing the sheep blowfly genome has created new opportunities in better understanding the biology of this pest. With the advent of new technologies and the genome information, significant new opportunities now exist that is allowing CSIRO to build on its foundation flystrike vaccine knowledge and helping make the development of a flystrike vaccine a real possibility.

Development of a Flystrike Vaccine is a high-risk venture with complex technical hurdles to overcome, however it offers the real potential of a new paradigm for flystrike control for the producer that will garner the support of consumers, retailers and animal welfare advocate organisations, thereby contributing to the future success, profitability and sustainability of the sheep wool and meat industry. In addition, a flystrike vaccine will fulfil a wide range of requirements by providing whole animal protection against body and breech-strike, reducing the use and reliance on chemical insecticides and overcome the need for the practice of breech modification. The other advantage a vaccine will provide is that it can also be used as part of an integrated pest management approach that will allow reduced insecticide use and help prolong the utility of this control measure.

Since the start of the project, 14 months ago, CSIRO has been working in collaboration with the University of Melbourne (Perry and Anstead Group), Griffith University (Kolarich Glycomics Group) and the University of Queensland Protein Expression Facility utilising their respective expertise to assist in the development of prototype vaccines for testing in sheep for flystrike control. Vaccine trials in sheep are currently underway to test these prototype vaccines with more research and testing still to be done. The following is a brief description of what has and will happen with the research project over its initial 3-year period.

GOALS AND OBJECTIVES OF THE PROJECT

Key project objectives are to:

- Identify a pipeline for antigen identification and production.
- Produce prioritised vaccine antigens as recombinant proteins in bacteria, yeast or insect cell systems or by chemical synthesis.
- Test recombinant protein antigens in prototype vaccines in sheep trials.
- Identify vaccine antigens that induce strong immune responses in sheep and produce inhibition of *Lucilia cuprina* larval growth *in vitro*.
- Identify lead vaccine antigens to progress to testing and commercial development of a flystrike vaccine with an Animal Health Veterinary Pharmaceutical partner.

WHAT HAS BEEN ACHIEVED BY THE PROJECT TO DATE?

Candidate vaccine antigen identification

Sheep blowfly larvae are incredibly tough and resilient organisms and there is limited opportunity to target the larvae through an immunological approach using a vaccine. The larvae are coated in an incredibly tough waxy cuticle whilst the foregut and hindgut are lined by a tough impermeable polysaccharide polymer called chitin. The most susceptible region of the larvae is the midgut that is devoid of this chitin lining but lined instead by a secreted semi-permeable membranous matrix called the peritrophic matrix, a stocking-like structure. CSIRO established that certain classes of protein that help make up this membrane, when used as antigens in a vaccine, produce an immune response in sheep that significantly inhibits larval growth. The discerned mode of action of this vaccine approach is directing antibodies to key proteins contained within the peritrophic matrix and blocking it. This blocking mechanism restricts the secretion of proteases into the midgut lumen, inhibits the passage of nutrients to the underlying midgut microvilli, thereby starving the larvae and inhibiting their growth and viability. Additionally, targeting excretory and secretory proteases, enzymes that are crucial to larval food digestion and establishment on the skin of the sheep may have potential as vaccine antigens and help promote the growth inhibition described.

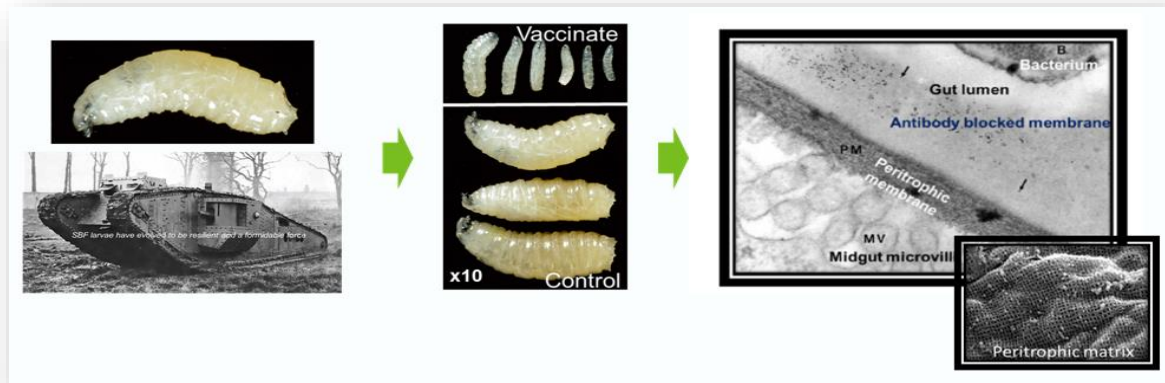


Figure 1. Sheep blowfly larvae are tough and resilient organisms and finding a susceptible region in their armour is key to vaccine development. Key midgut associated proteins have been used and demonstrated to be good targets for vaccine development. Vaccination with these proteins as antigens has resulted in larval growth inhibition by immunological blocking and interference of the peritrophic matrix lining the midgut.

Using Next Generation Sequencing technology and the blowfly genome sequence, a sequencing approach has been used to identify all the genes that are active in the blowfly larval lifestages and in the key larval tissues, the cardia and the salivary gland. This process has enabled identification of the complete repertoire of the cardia expressed peritrophic matrix associated proteins. The salivary gland gene expression analysis has also provided information on excretory and secretory proteins that are produced by larvae. Together with blowfly lifestage gene expression analysis, candidate protein-encoding genes have been identified that are now being investigated as vaccine antigens.

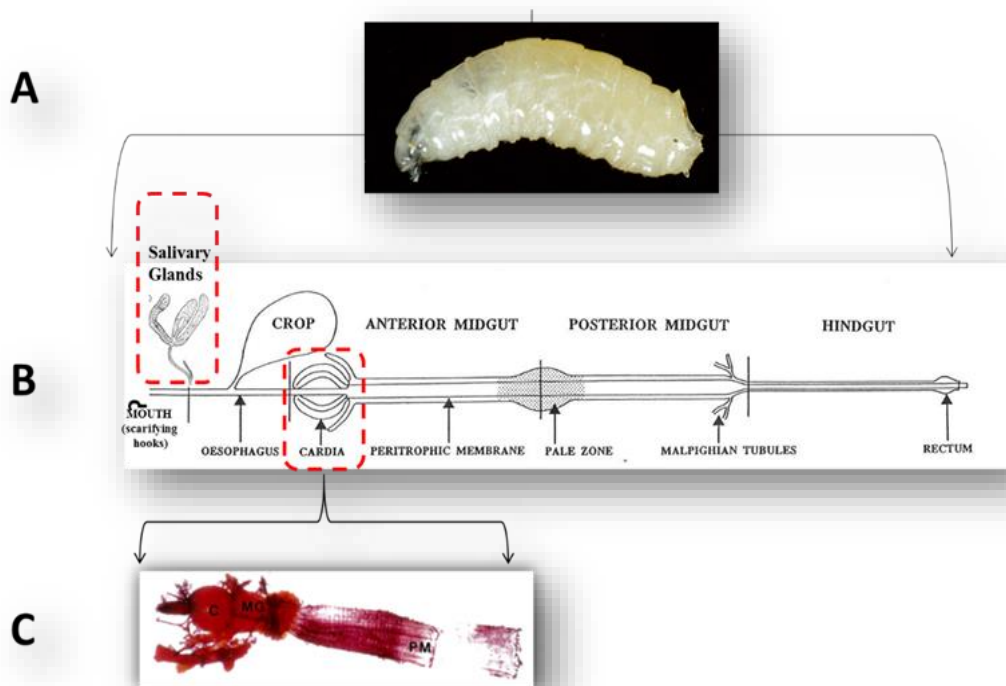


Figure 2. (A and B) Blowfly lifestages and key tissues (salivary gland, cardia and midgut) have been sequenced to identifying activated genes encoding proteins that are secreted and potentially accessible to immunological targeting by sheep antibodies when larvae start to feed on sheep. (C) Cardia (C) and anterior midgut (MG) cultured in the lab showing the peritrophic matrix (PM) that they produce. The PM represents a key target for immunological targeting a potential source of vaccine antigens.

Candidate vaccine antigen production

Lead candidate antigens representing key protein classes identified from the tissue and lifestage gene expression analyses are being tested as prototype vaccines. These antigens have been engineered and produced as proteins using recombinant molecular technologies utilising either Bacteria or Insect Cells as “protein production factories”. Twenty-six recombinant antigens have been engineered, cloned, expressed, purified and formulated with adjuvants to produce prototype vaccines and are at different stages of testing and validation as prototype vaccines tested in sheep. During and at conclusion of the trial, blood is collected and sera assayed for immunological response and ability to confer protection from flystrike initiation and larval growth.



Figure 3. Key steps in the process of engineering and formulation of candidate antigens for prototype vaccine production and testing. Steps involve engineering the candidate gene encoding the protein target of the vaccine, cloning it into bacteria or insect cells, culturing the cells to produce the recombinant protein/antigen, purifying the antigen, formulating it with an adjuvant and administering it to sheep which are then assessed for antibody titre response to the vaccine and larval growth effects.

Sheep immunological studies have also been undertaken to investigate immunological response of sheep to control antigens simulating a flystrike vaccine. This is informing the effect of vaccine dose and longevity of the immunological response and assisting with formulation of the trial flystrike vaccines. In addition, studies in collaboration with Griffith University Glycomics Institute are underway to investigate the chemical synthesis and modification of vaccine antigens to modulate and improve the immune response of sheep to the vaccines.



Figure 4. CSIRO research flock at the Chiswick research laboratory and farm are used for vaccine testing.



Figure 5. CSIRO research team undertaking tasks associated with flystrike vaccine development.

WHAT DO WE ENVISAGE THE PROJECT WILL BE UNDERTAKING IN THE NEAR TO MID FUTURE?

Stage two of the vaccine project involves further refining and testing of lead vaccine antigens with the aim to deliver a validated short-list of candidate antigens that when formulated into prototype vaccines prevent or inhibit flystrike establishment and larval growth. Information garnered from this project will help inform future research into flystrike vaccine development. CSIRO will continue to work together with collaborators at University of Melbourne and further refine candidate antigen selection.

Engineering and producing highly effective Flystrike Vaccine antigens will require refining the protein production system or developing new protein production tools to achieve this critical need. This rationale is core to the collaboration CSIRO is forging with Griffith University researchers in developing synthetic biology approaches to vaccine development and exploring novel cell lines for production of recombinant antigens.

CSIRO aims to deliver by the completion of Stage Two, refined and validated lead vaccine antigens that when formulated into prototype vaccines prevent or inhibit flystrike establishment and larval growth. We have strong interest in this project from VetPharma companies and once a validated short-list of candidate antigens is identified, discussions with a commercial partner will be progressed with the key aim of commercial development of a flystrike vaccine.

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