2022 FLYSTRIKE RD&E

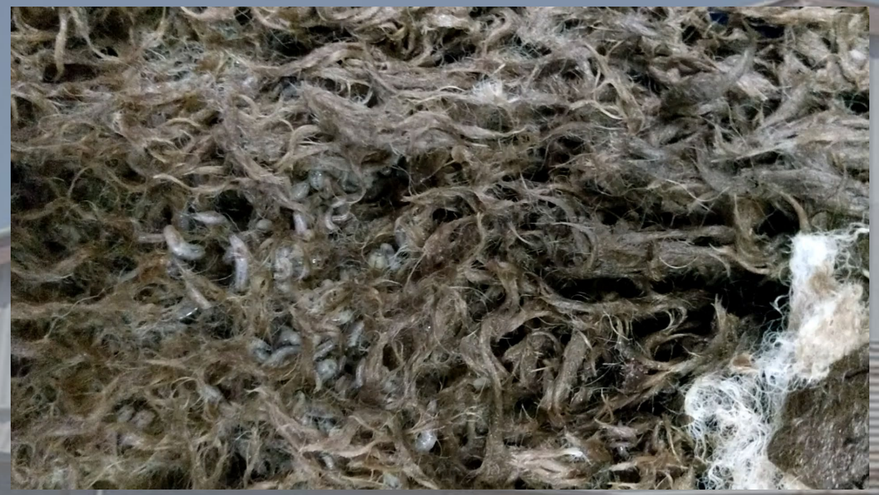
TECHNICAL FORUM

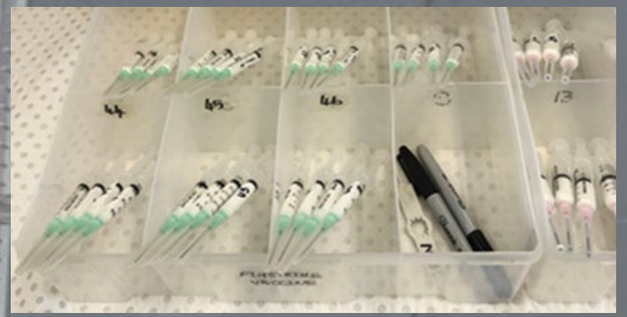
Development of a Flystrike Vaccine

Tony Vuocolo – CSIRO

10 August 2022

Striking back at Flystrike
Development of a Flystrike Vaccine
(research update)





Prototype ‘N’ Vaccine -

Prototype ‘R’ Vaccine -

No Vaccine -

The journey so far.....

20xx

2022

2021

2020

2019

2018

2017

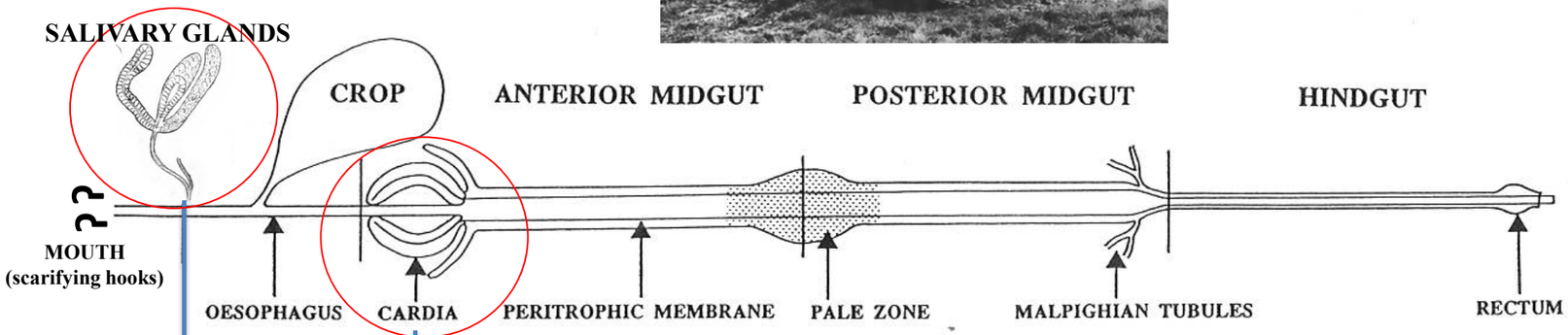
2003

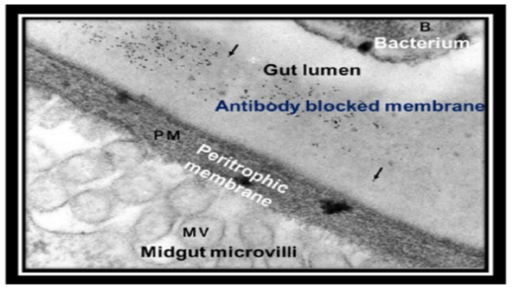
1996

1990

1986

Sheep blowfly vaccine targets



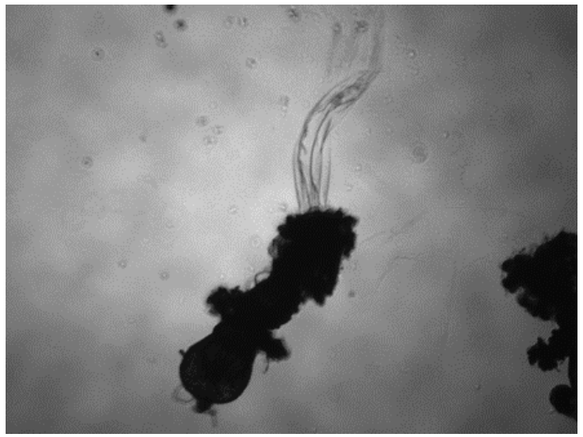


Cuticle lined
(robust/protected)

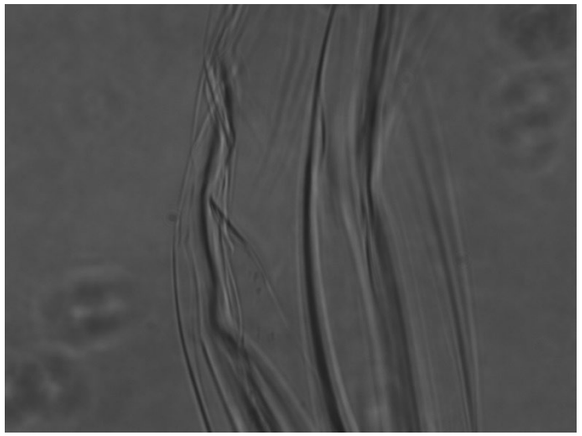
Cardia and Anterior Midgut
- Peritrophic membrane (produced by specialised cells)
  - Lines gut
  - Protective and digestive function
  - Amenable to immunological targeting
  - Function knockout potentially lethal

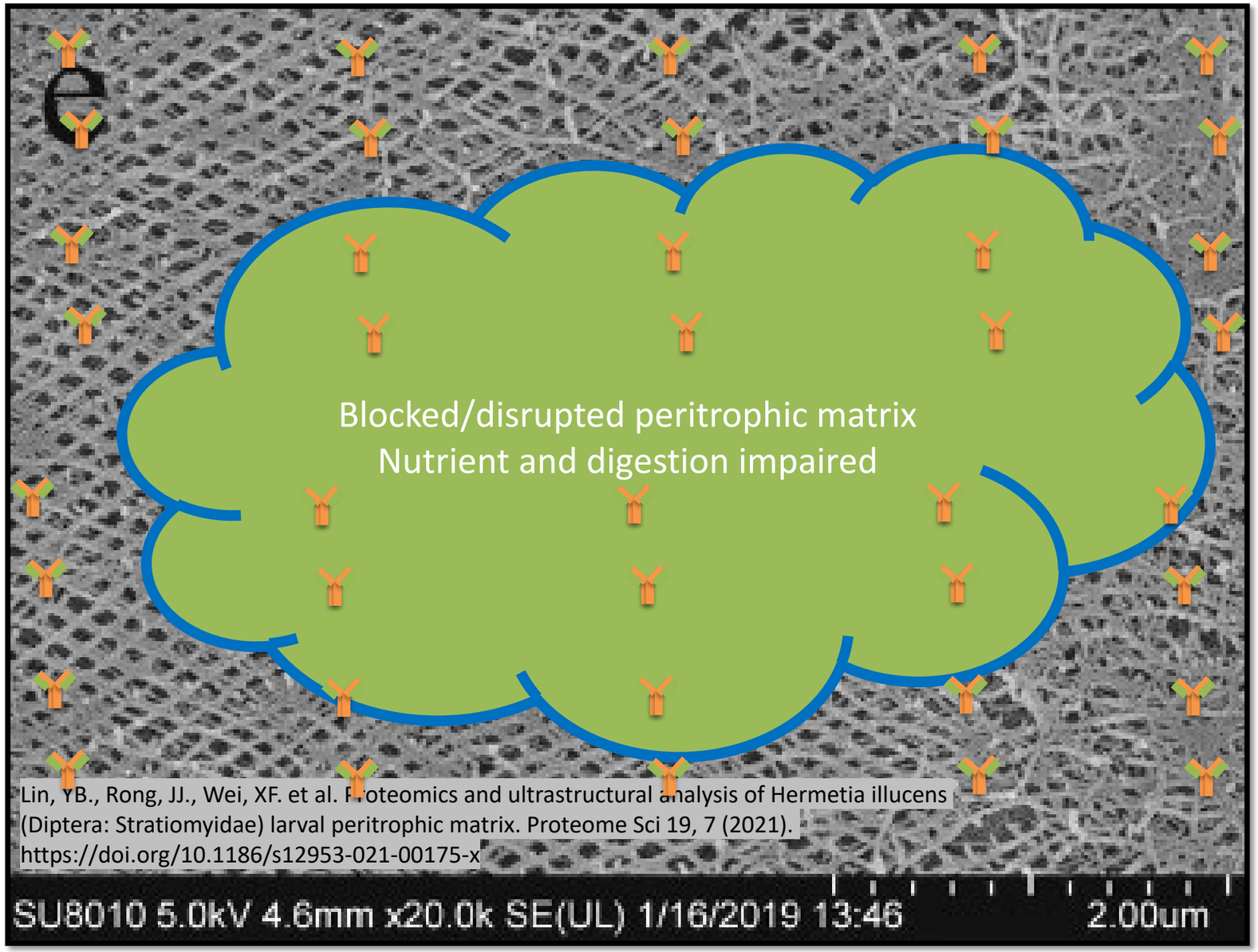
Salivary Gland
- Secretory proteases
  - critical in strike establishment
  - ~ 1000 predicted proteins
  - ~ 80 larval specific

Vaccine Primary Target



Sheep Blowfly larval midgut and PM



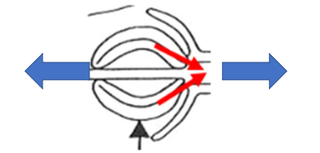


Peritrophic matrix (PM) is a meshwork of highly associated proteins and chitin

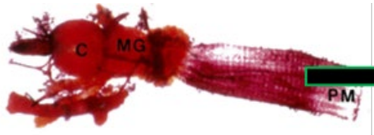
The native antigen approach (Type N)

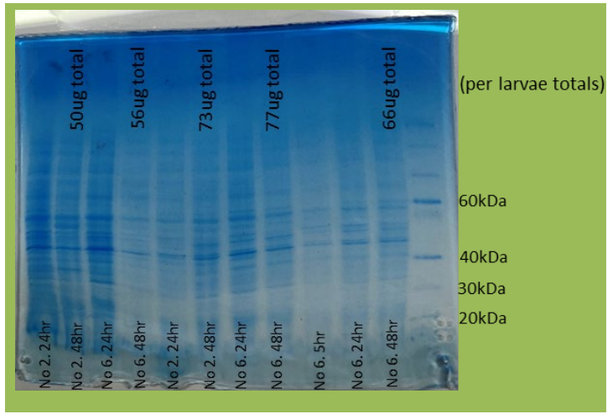
a) Using larvae against larvae for vaccine production

Native antigen vaccine

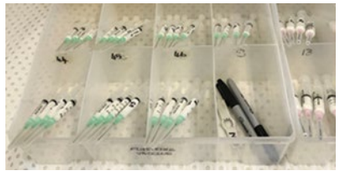


Peritrophic Matrix
Made by specialised cells
In cardia and anterior mid-gut

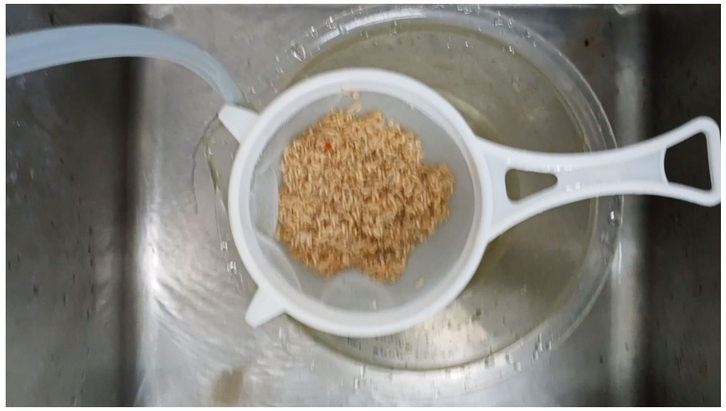




Native protein mix from Peritrophic matrix



Type “N” Vaccine



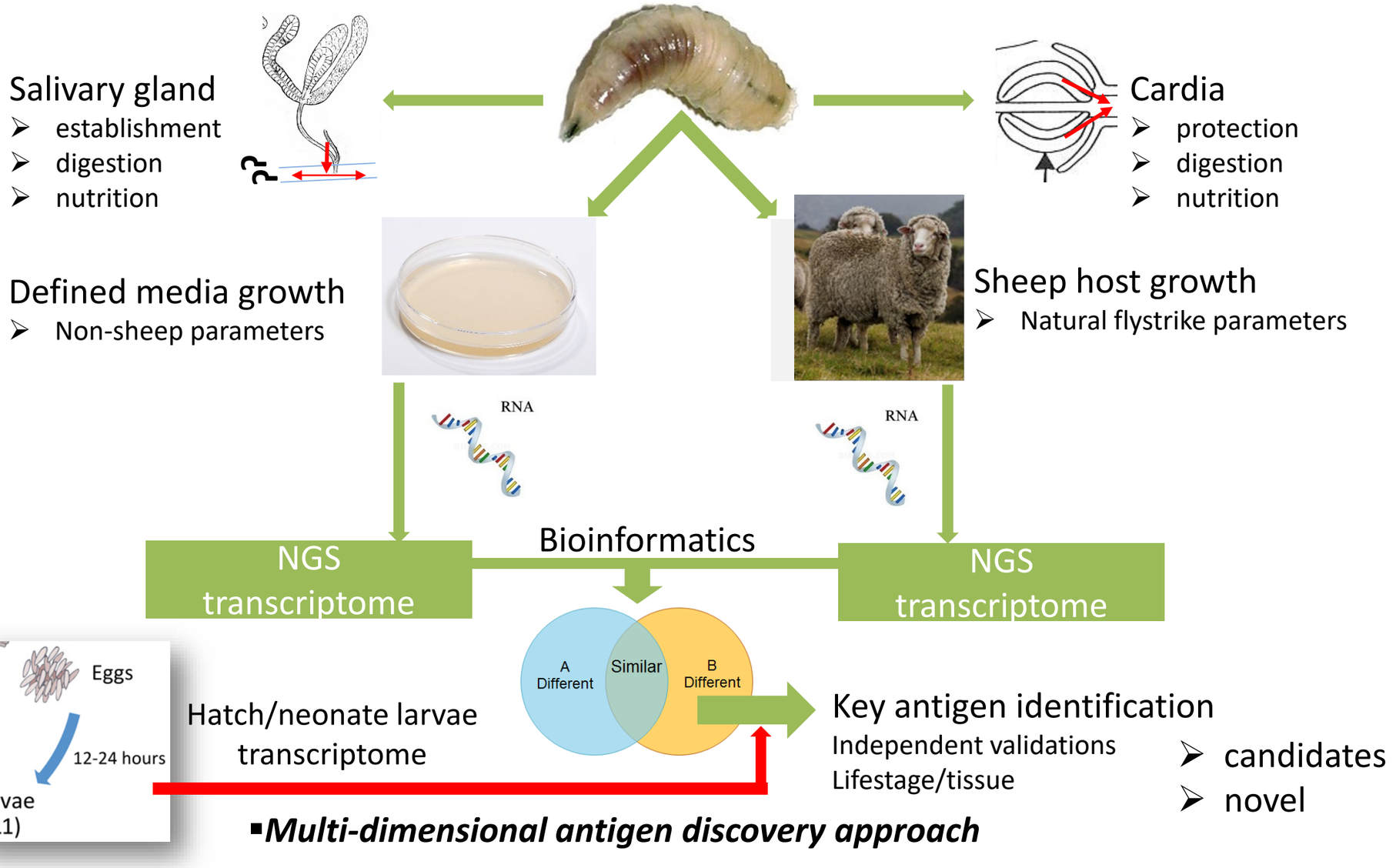
Larvae in lab culture

Dissected larval anterior mid-gut

Specific Antigen identification strategy

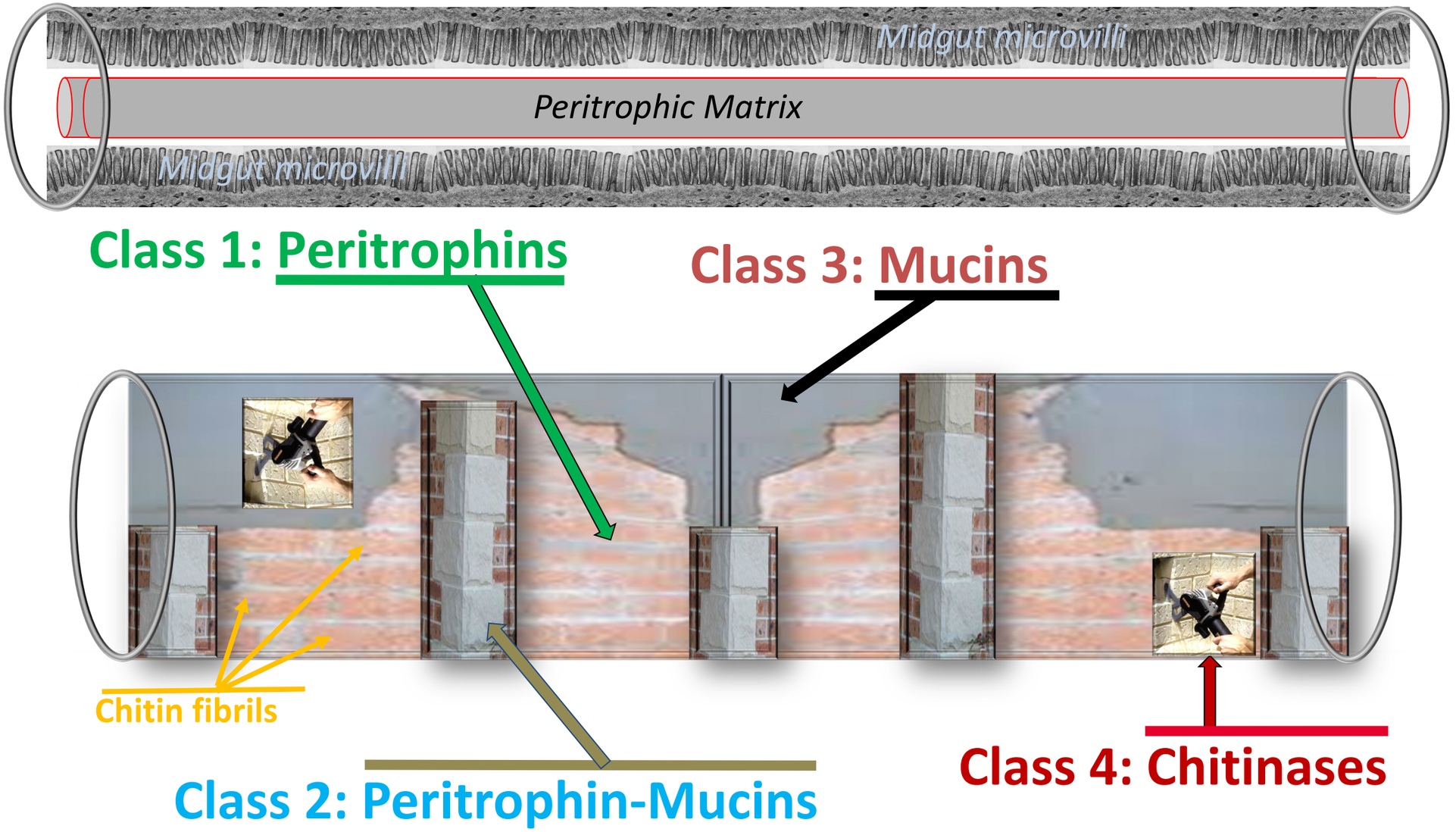
b) Recombinant antigen approach (Type R)

- Key: Vaccine target antigens involved in larval establishment and early growth
- Multi-dimensional antigen discovery process
- Extend and refine foundation knowledge of host-parasite interaction



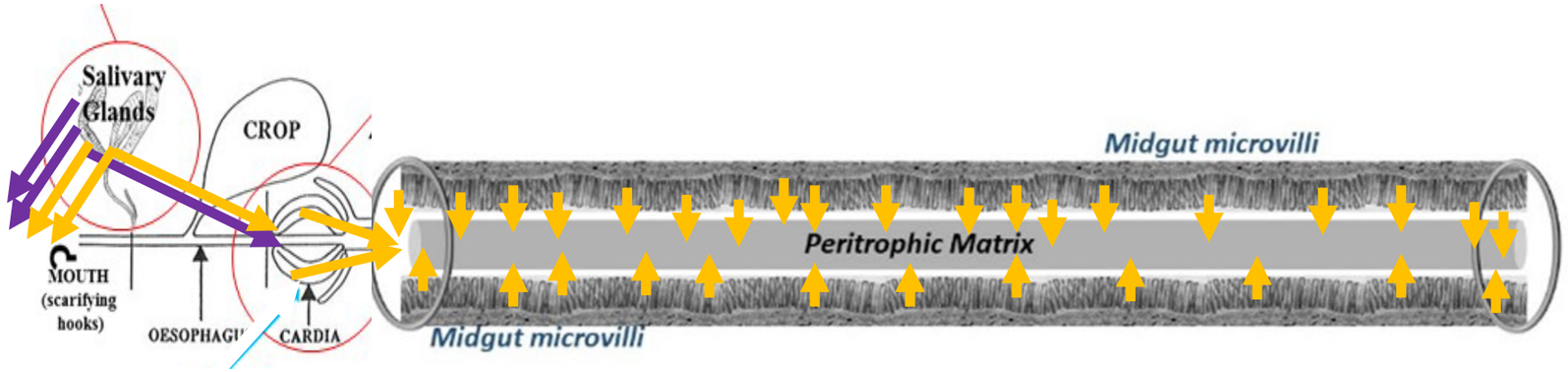
▪ ***Multi-dimensional antigen discovery approach***

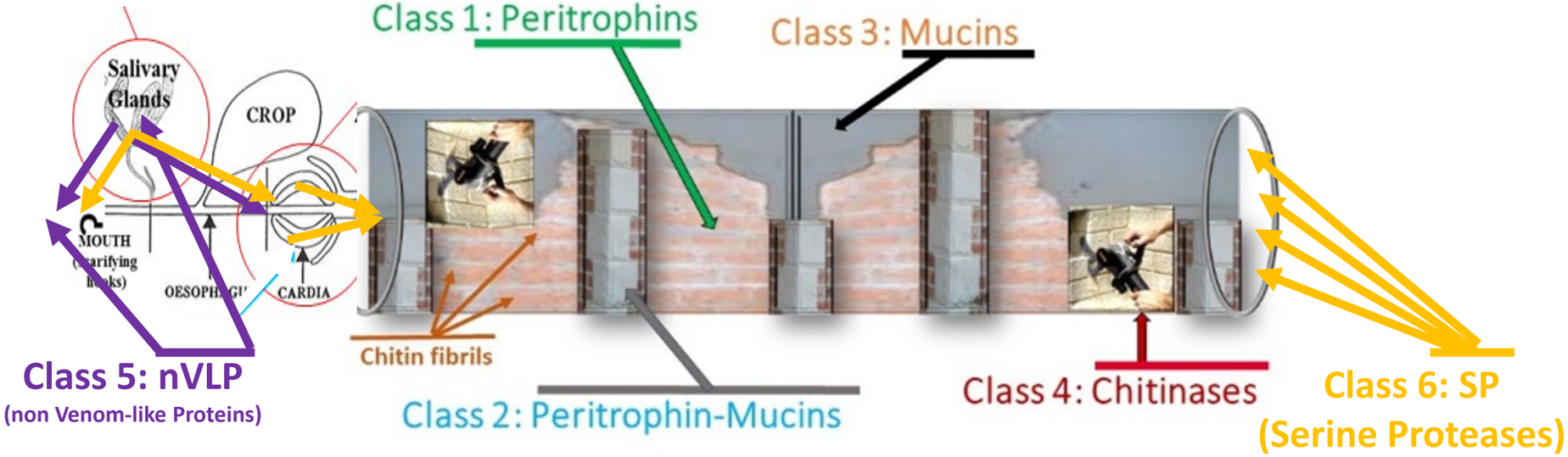
Vaccine Approach: Antigen Classes under Investigation (Type R)



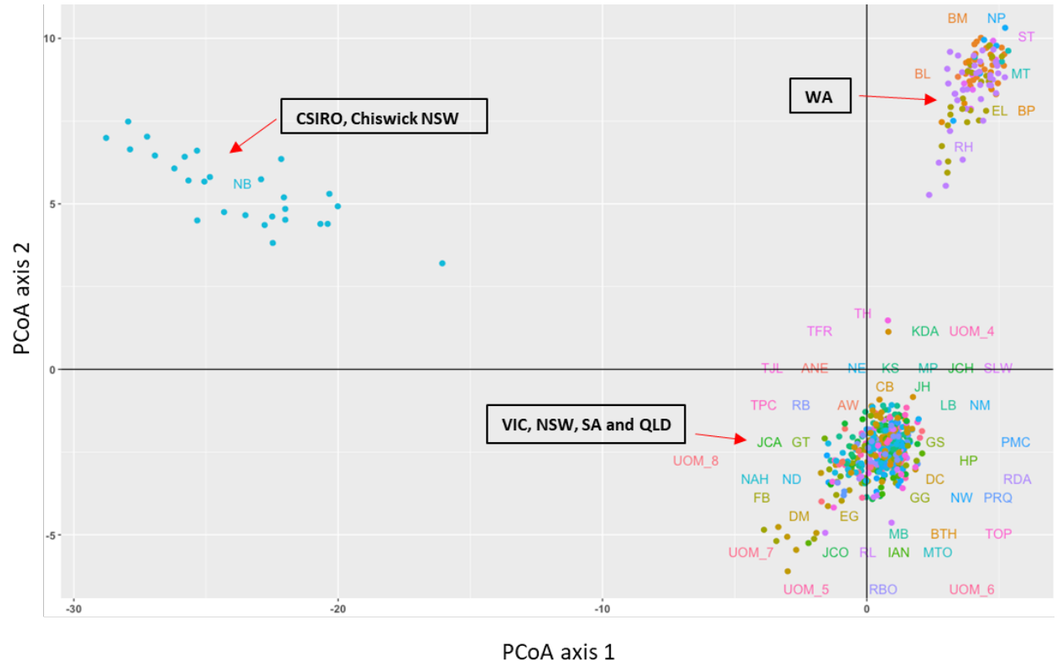
……..Block/disrupt/remodel/perforate/compromise…….

Vaccine Approach: Other Antigen Classes under Investigation (Type R)



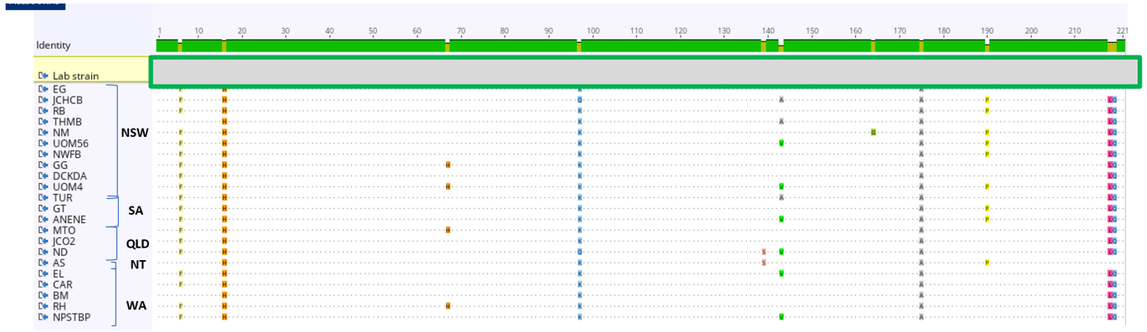


Population studies on blowfly populations help inform for strain variation implications to vaccine design



Trent Perry
Clare Anstead
Vern Bowles
Shilpa Kapoor

Dots reflect protein differences between populations



From alignments presented in last meeting (August 2021): The NB strain has 5 polymorphisms to the reference lab strain out of the 12 polymorphic sites identified as present in more than one sequenced populations.

We are using Insect cells as factories for vaccine production (Type R)

Candidate discovery

gccctatgtataagcggctataaataaggctttttaatttgcttaa
ATGCAGTGATGAGTTATGTAGCCCCTTAGTTAATTTATGGCTAGTGA
ACCTTGAGGAGTTAGTTAATTTATGGGATGAGTTATGTTAAGCGGAT
CTTTATTATGGCATTACTGATCTAGTTTAAATTGGGCCCTAATATCGTA
CGTAGTGTATATGGGGAAAACCCTTGGAAGTATATCCAGTTACTGG
AAGGAGTTAGTTAATTTATGGGATGAGTTATGAACCCTTGGAAGACC
AGGAGTTAGTTAATTTAACCCTTGGAAGTATAtaaataaataaattta
ataataaataaaaaaaaaaa...*HHHHHH*

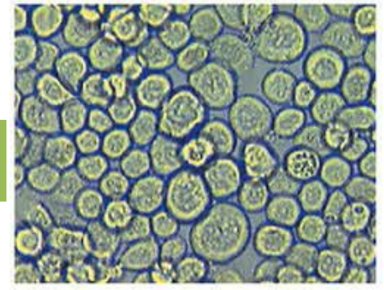
Protein-encoding gene sequence engineered for recombinant production

Lepidopteran / Dipteran

In collaboration with

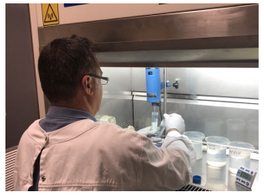
THE UNIVERSITY OF QUEENSLAND
AUSTRALIA

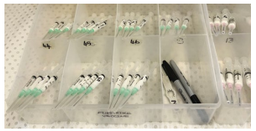
PEF
PROTEIN EXPRESSION FACILITY



Insect cells in culture

Purified recombinant protein

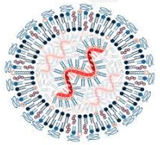




Formulate

Prototype vaccine tested in sheep

Bacterial *E.coli* culture

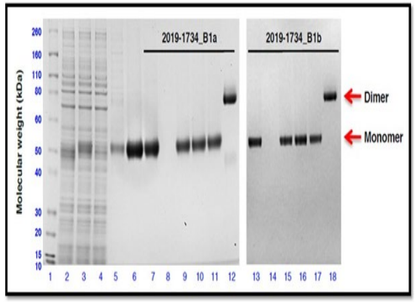


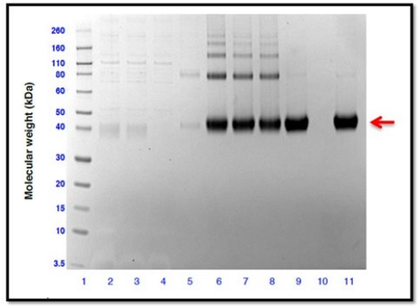
mRNA approach

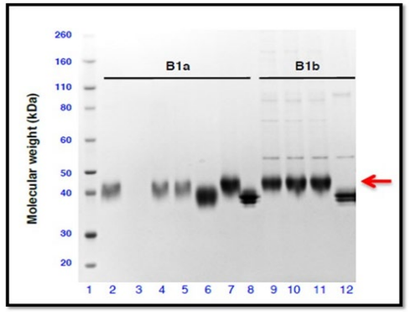
Not suitable for our complex antigens

Vaccination trial : In vitro larval feeding bioassay (Models 1, 2 and 3)

Recombinant protein

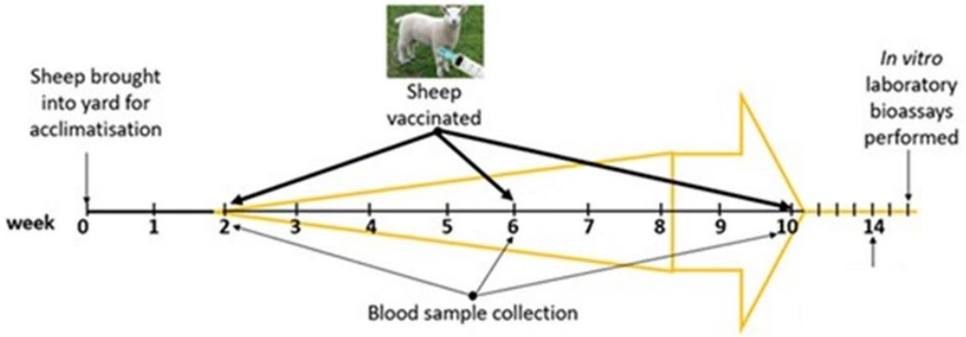




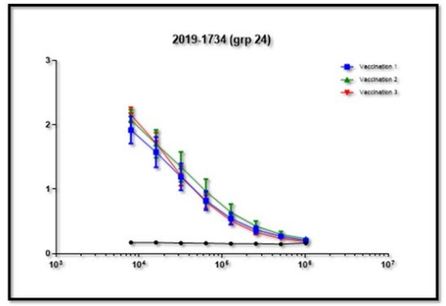


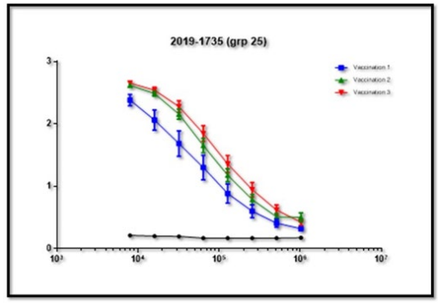
Vaccination trial

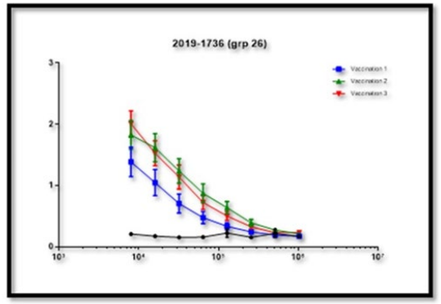
Flystrike prototype vaccine studies



Antibody assessment

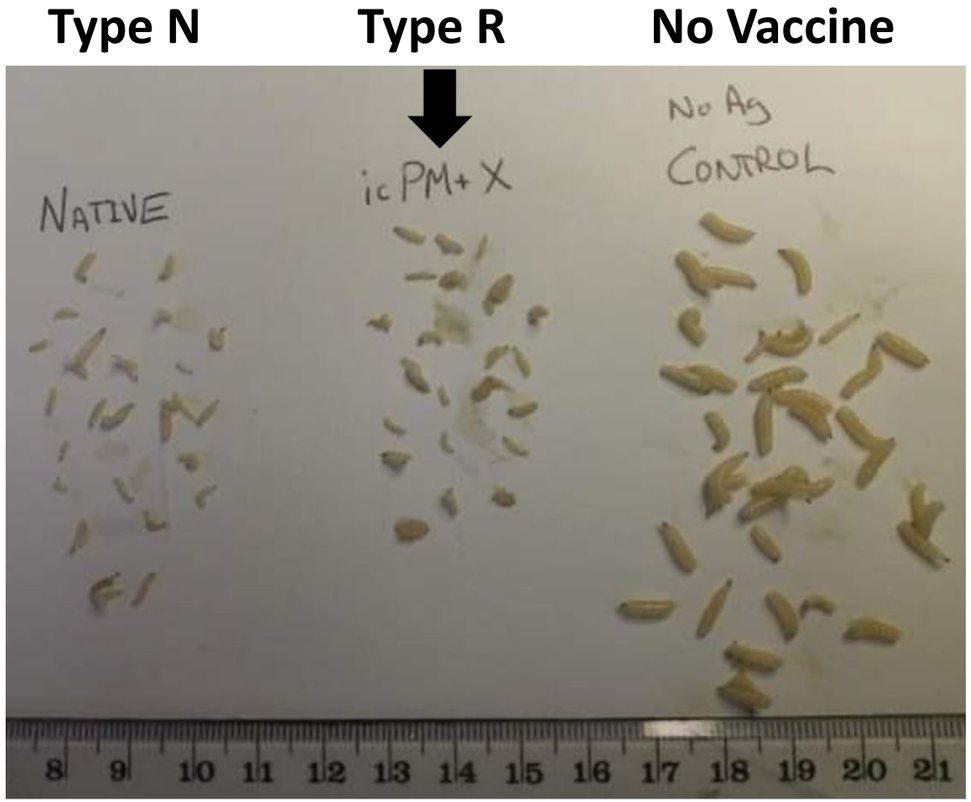




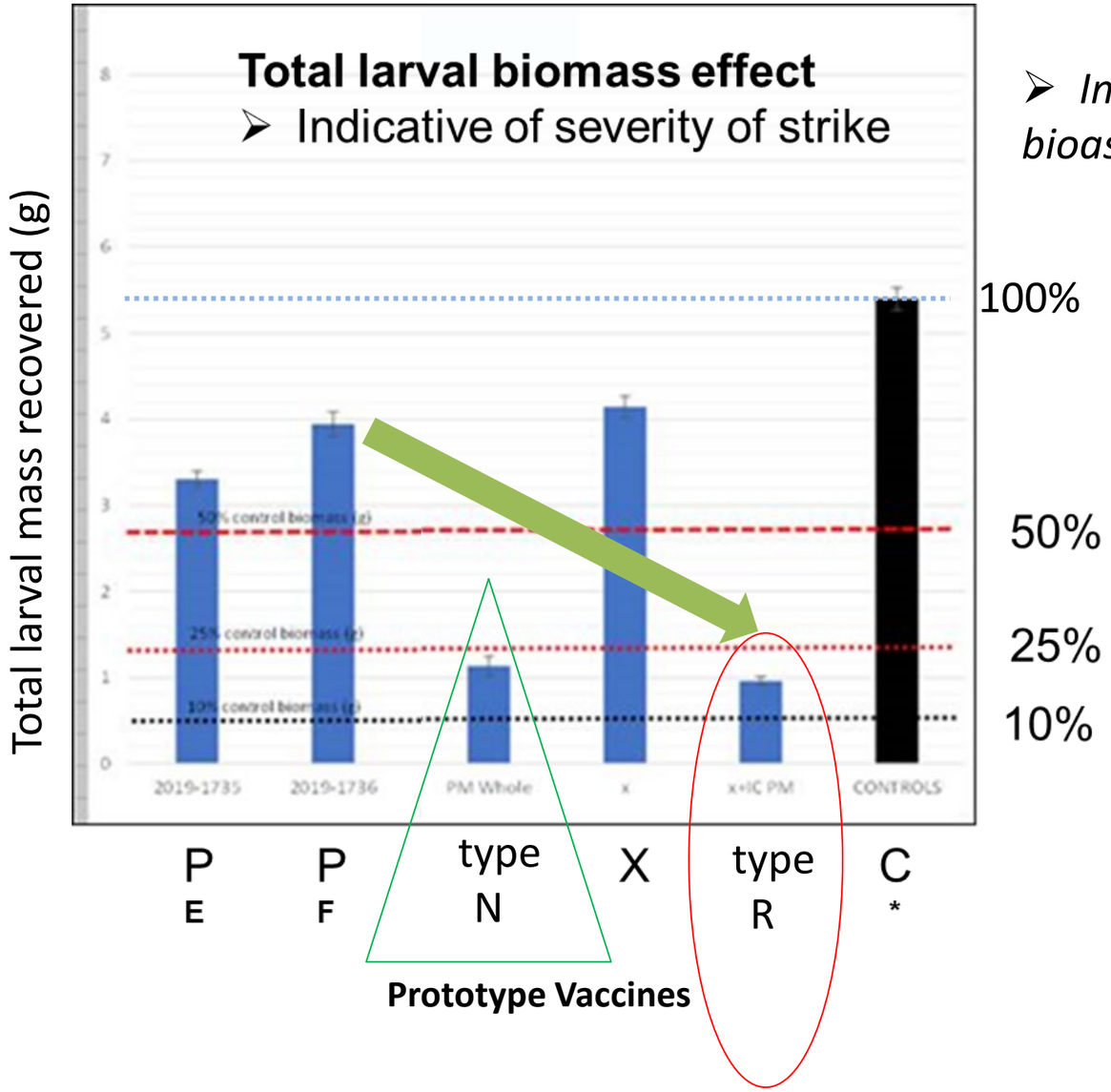


In vitro bioassays (skin/wool/biomatrix)

A vaccine could stop or reduce severity of flystrike?



72 hour growth in vitro/skin model/immune sera



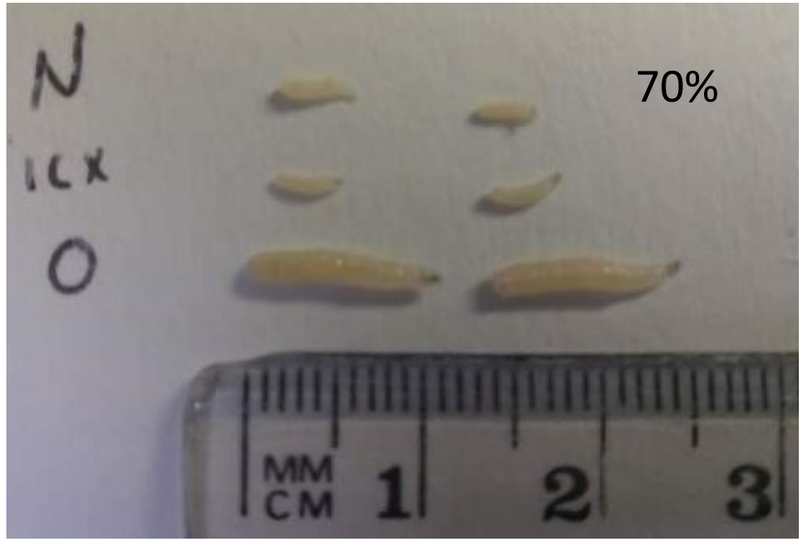
- *In vitro larval feeding bioassays*

Flystrike Vaccine – what next

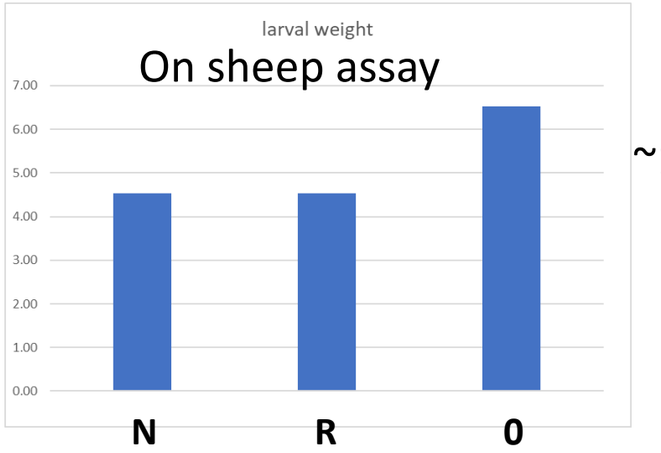
Type N

Type R

No Vaccine



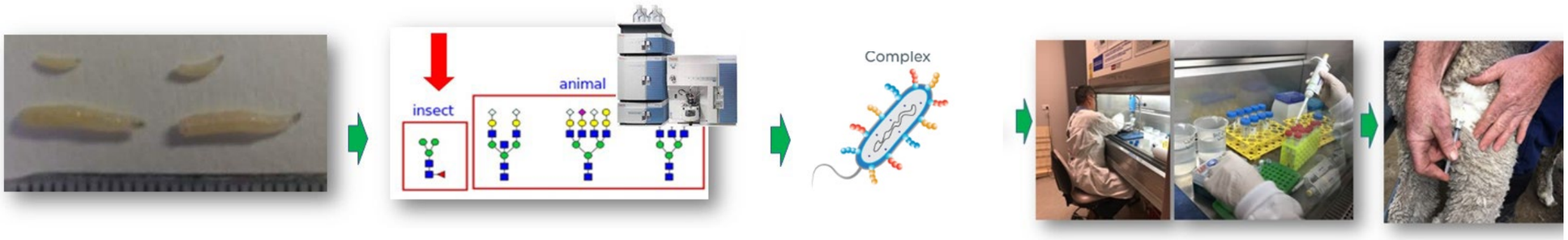
- Translate promising preliminary results to on-sheep effects
- Significant challenge



~15% weight reduction

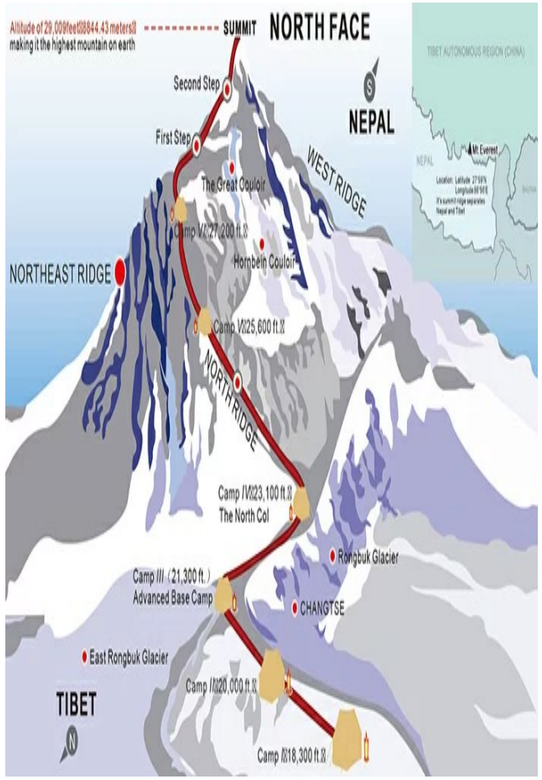
Future Science …….

- ✓ ***Exploring and enhancing skin immune response to the Flystrike Vaccine***
- ✓ ***Adjuvant technology / Delivery mechanism***
- ✓ ***Applying the science of Glycomics to vaccine development***
- ✓ ***CSIRO supporting a Future Science Platform initiative in novel parasite vaccines***



Summary – take home points

- ✓ Step 1: Used foundation knowledge to expand potential of vaccine development
- ✓ Step 2: Identified and characterise potential vaccine antigen targets
- ✓ Step 3: Engineered and produce prototype vaccines (>50 prototypes tested in sheep)
- ✓ Step 4: Assessed immune response in sheep and tested efficacy using a range of laboratory bioassays. (1) native and a (2) recombinant cocktail vaccine confer up to 80% growth inhibition of larval growth *in vitro*.
- ✓ Step 5: The two current lead vaccines have been tested in sheep and assayed for on-sheep protection. On sheep effects translate to 10-20% growth inhibition of larvae. A lot to be done to improve this on-sheep efficacy.



Step 6: Optimise vaccine: key antigen formulation, adjuvant and delivery method, assessment of period of protection studies are currently underway.

Step 7: CSIRO Novel vaccine platform initiative underway.

Step 8: Partner with Veterinary Pharmaceutical company to commercially develop and deliver to the sheep industry

Flystrike Vaccine – acknowledgements

Australian Wool Innovation Limited

Bridget Peachey
Jane Littlejohn

THE UNIVERSITY OF MELBOURNE

Trent Perry
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Vern Bowles
Shilpa Kapoor

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PEF PROTEIN EXPRESSION FACILITY

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Balaji Somasundaram
and Team

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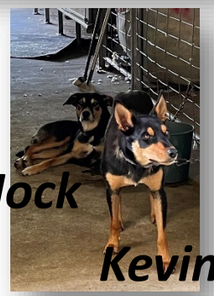
Suzie Briscoe

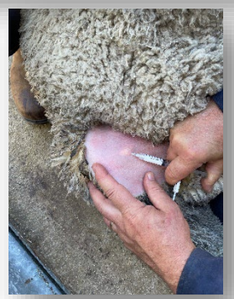
Jody McNally

Graham (Wom) Acton

Dan Driscoll

Jock and Kevin





Thank-you

CSIRO
Australian Wool Innovation Limited

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