LA TROBE UNIVERSITY

Apiam animal health

JAPANESE ENCEPHALITIS VACCINE UPDATE

AGRICULTURAL BIO-SOLUTIONS LAB
SPEAKER: GEMMA ZERNA

AGRICULTURAL BIO-SOLUTIONS

TB

TRAVIS BEDDOE LABORATORY

BENCH-TO-BARN RESEARCH

DETECTION:

- Pathogen surveillance
- Diagnostics via LAMP

PREVENTION:

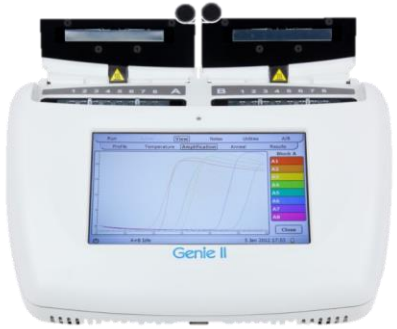
- Molecular understanding of disease and immunity
- Vaccine development

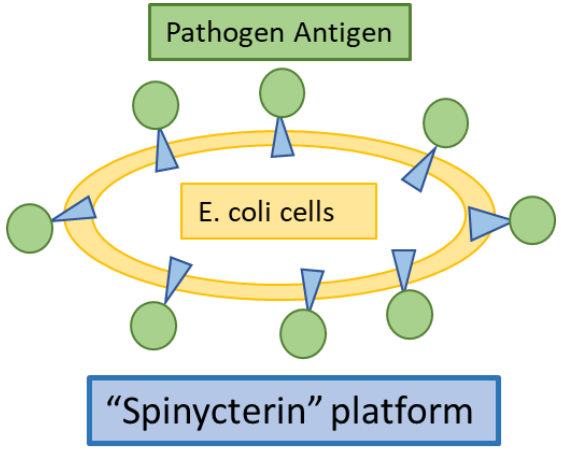
TRAVIS BEDDOE

IMPROVING AGRICULTURAL ANIMAL PRODUCTION THROUGH DISEASE DETECTION AND PREVENTION

Loop Mediated Isothermal Amplification:

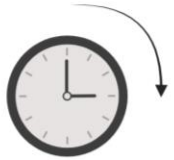
- Enteric redmouth (*Yersinia ruckeri*)
- *Vagococcus salmoninarum*
- Abalone herpesvirus-1
- American foulbrood (AFB)
- European foulbrood (EFB)
- Ross river virus
- Strangles (*Streptococcus equi subspecies equi*)
- Platypus (*Ornithorhynchus anatinus*)
- Human faecal material *(Bacteroides)*
- Liver fluke (*Fasciola hepatica*)
- *Austropeplea tomentosa* (Fluke snail)
- Sheep body louse (*Bovicola ovis*)
- Footrot (*Dichelobacter nodosus*)





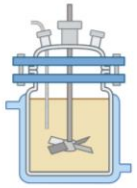
IDEAL BENCH-TO-BARN VACCINE PLATFORM

COMMERCIALLY FEASIBLE VACCINE DEVELOPMENT



Rapid

Antigen Swappable



Scalable

Low Cost

Reduced Cold-Chain

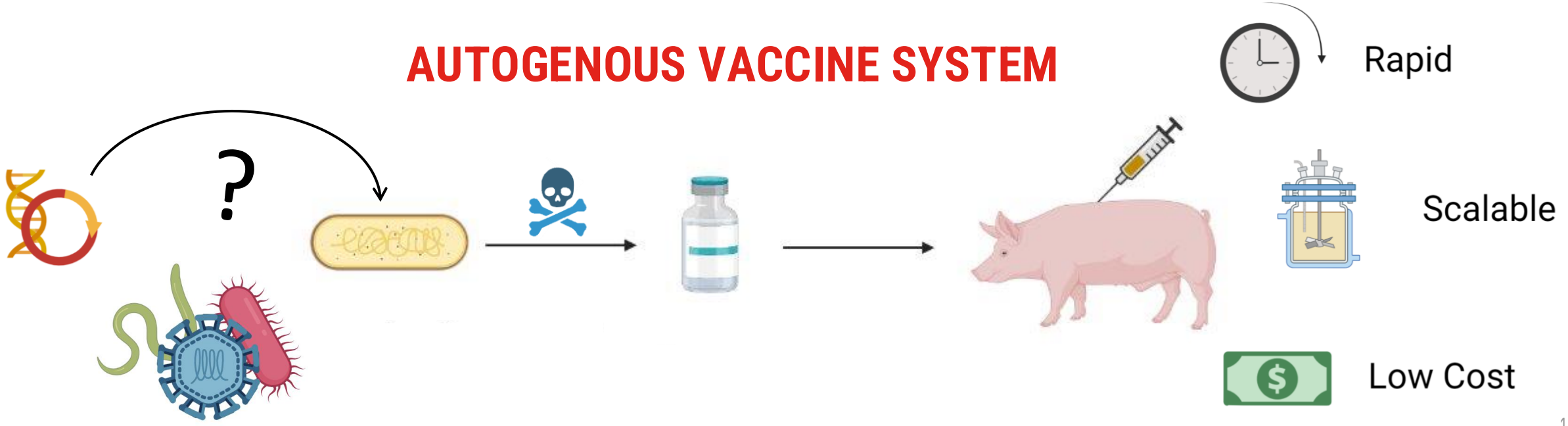
FARM-BASED VACCINE PLATFORM

DISEASE IDENTIFICATION

OR

OCCURRENCE OF A NEW STRAIN

AUTOGENOUS VACCINE SYSTEM



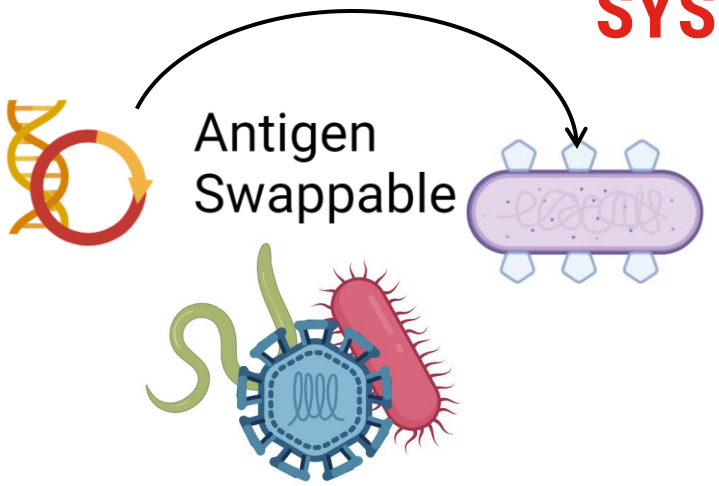
FARM-BASED VACCINE PLATFORM

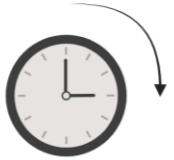
DISEASE IDENTIFICATION

OR

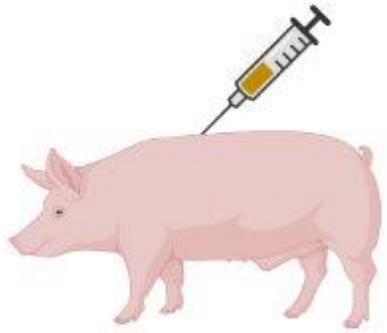
OCCURRENCE OF A NEW STRAIN

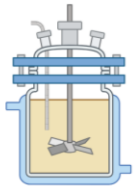
"SPINYCTERIN" VACCINE SYSTEM





Rapid

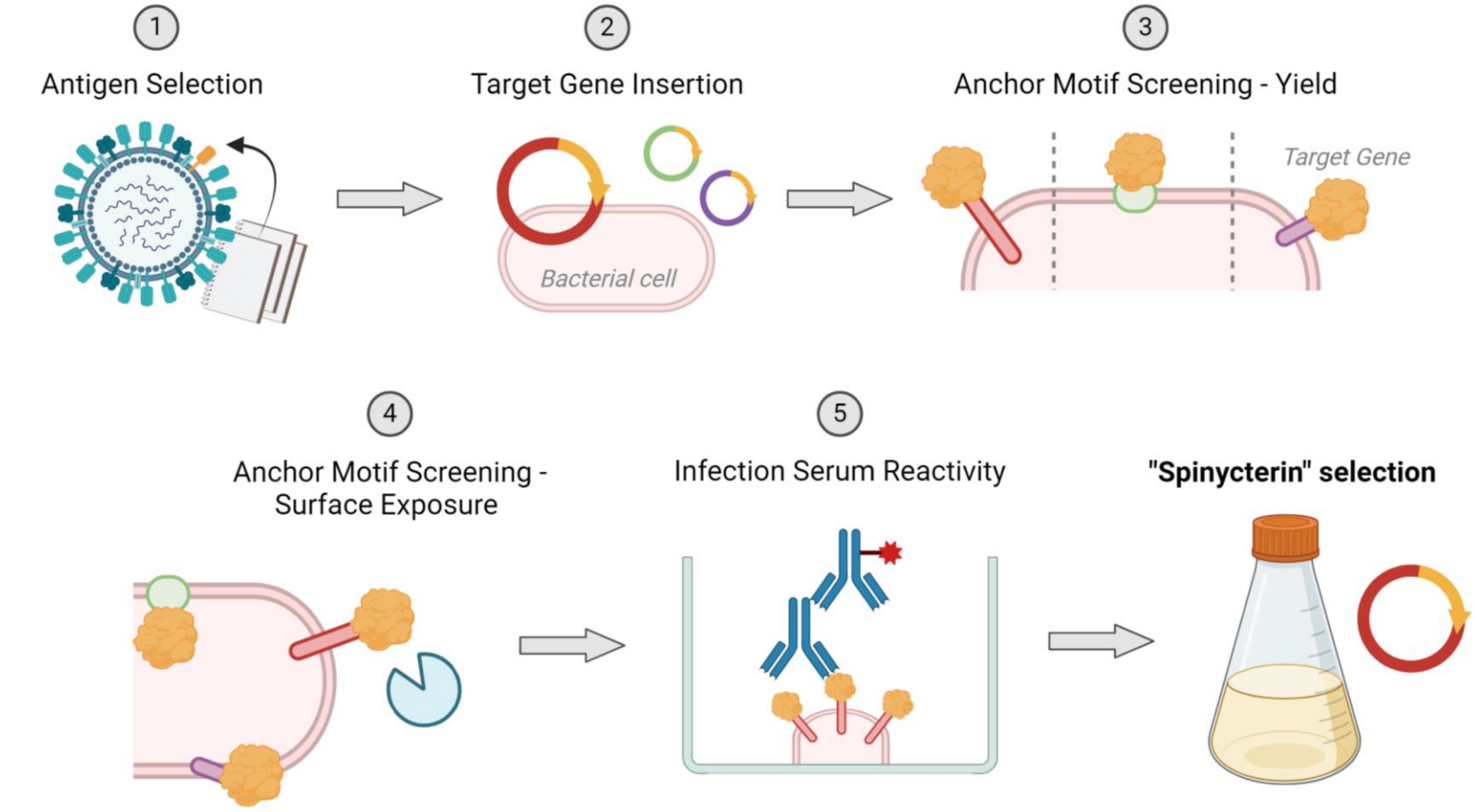




Scalable

Low Cost

“SPINYCTERIN” PLATFORM – DEVELOPMENT STAGE



DIFFERENT ANCHORS

- **MORE FLEXIBILITY FOR DIFFERENT ANTIGENS**
- **RAPID SCREENING**

JAPANESE ENCEPHALITIS

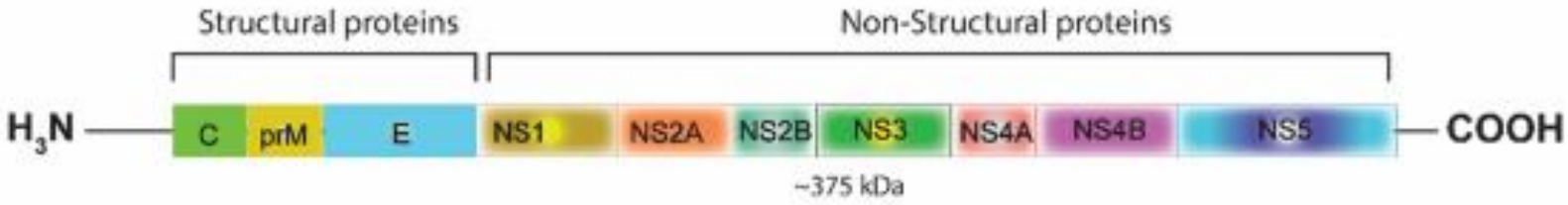
Flavivirus

ssRNA enveloped virus (11 kb)

<u>Antigen selection:</u>

- **Not hidden within the membrane**
- **Exposed to the immune system during the infection cycle**
- **Antigenic/Immunogenic**
- **Can produce a protective response**

ANTI-JEV ANTIGEN?



Non-Structural Protein 1 (NS1)

- Highly conserved in flavivirus

  (Homology: 76% KUN, 78% to MVE)
- Triggers a strong humoral response during infection
- Involved with viral replication/assembly, interacts with host proteins and modulates the host immune system for viral propagation
- Dimeric - ER & surface exposed
- Hexameric – secreted in the blood stream
- NS1-based vaccine shows protection in mice – Wan et al. 2021

JAPANESE ENCEPHALITIS

Antigen selection:

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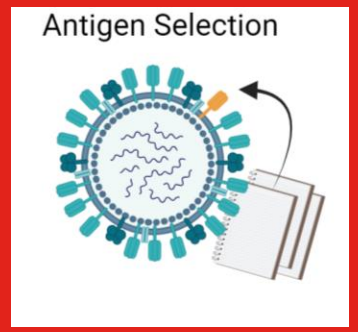


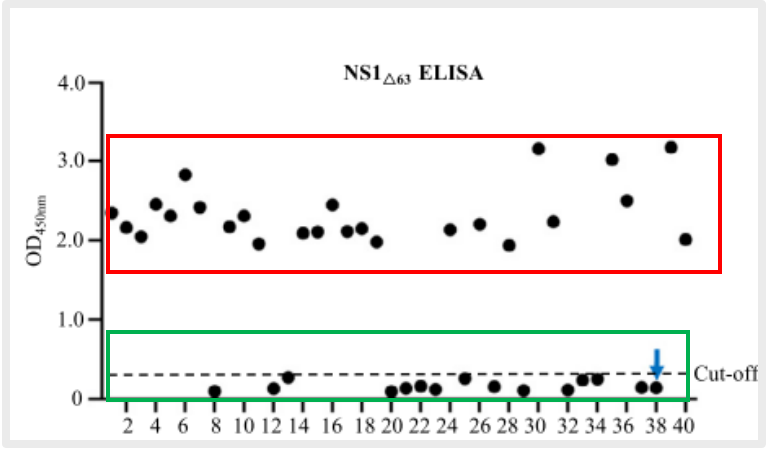
Research paper

Novel Japanese encephalitis virus NS1-based vaccine: Truncated NS1 fused with E. coli heat labile enterotoxin B subunit

Jiawu Wan[a,1], Ting Wang[a,1], Jing Xu[a], Tao Ouyang[a], Qianruo Wang[a], Yanni Zhang[b], Shiqi Weng[a], Yihan Li[a], Yu Wang[a], Xiu Xin[a], Xiaoling Wang[a], Sha Li[a,*], Lingbao Kong[a,*]

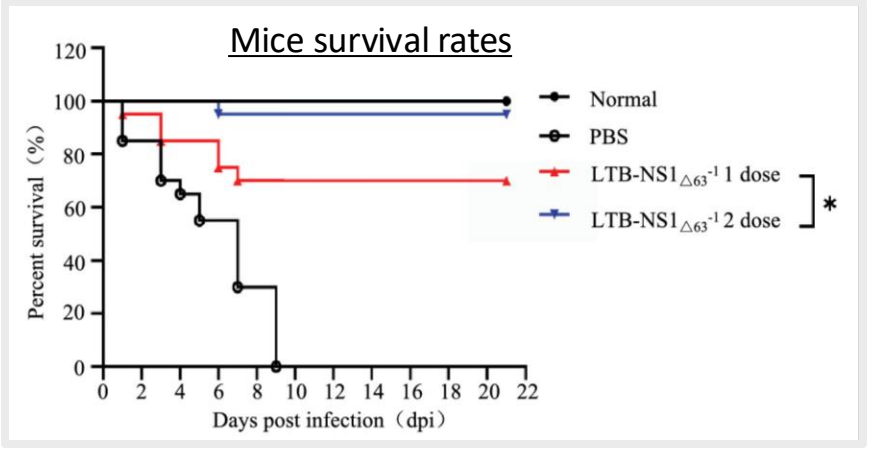
[a] Institute of Pathogenic Microorganism and College of Bioscience and Engineering, Jiangxi Agricultural University, Nanchang, Jiangxi, China
[b] Jiangxi Province Center for Disease Control and Prevention, Nanchang, Jiangxi, China

Truncated rNS1 is recognised by **infected** (and **non-infected**) pig serum



Infected and **non-infected** serum result are cross-validated with commercial JEV ELISA

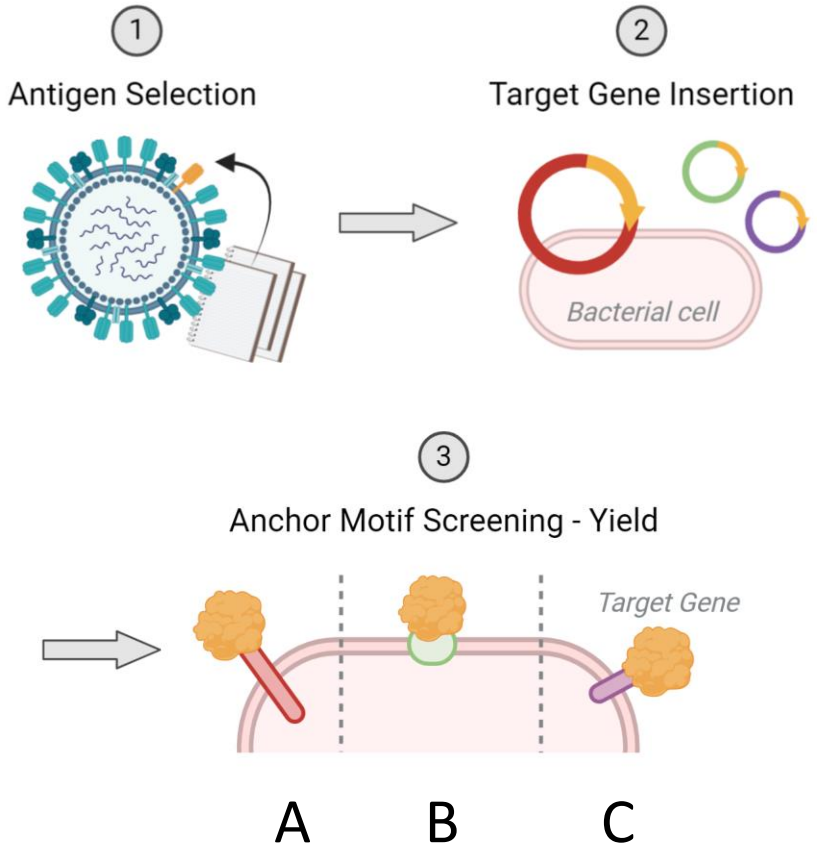
Vaccinated truncated NS1 linked to a protein adjuvant increases **mice** survival after infection



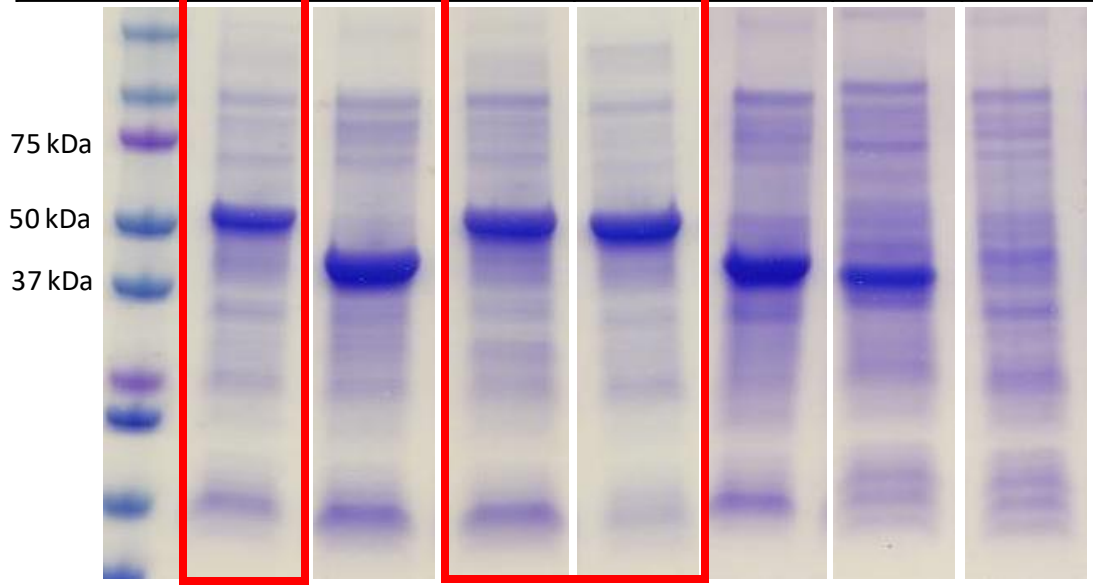
Both dose vaccine groups (**blue** and **red**) show increased survival compared to **PBS (open circles)** vaccination

“SPINYCTERIN” SCREENING PROCESS

AIM: HIGH EXPRESSION OF JEV NS1 ANTIGEN



Anchor	A	B	C	D	E	F	None
kDa	53	44	51	55	50	43	-



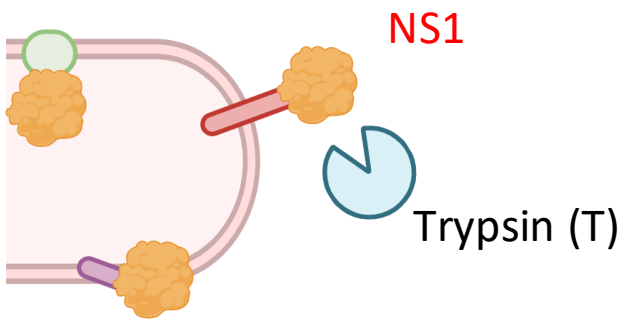
Significant overexpression of recombinant fusion proteins

“SPINYCTERIN” SCREENING PROCESS

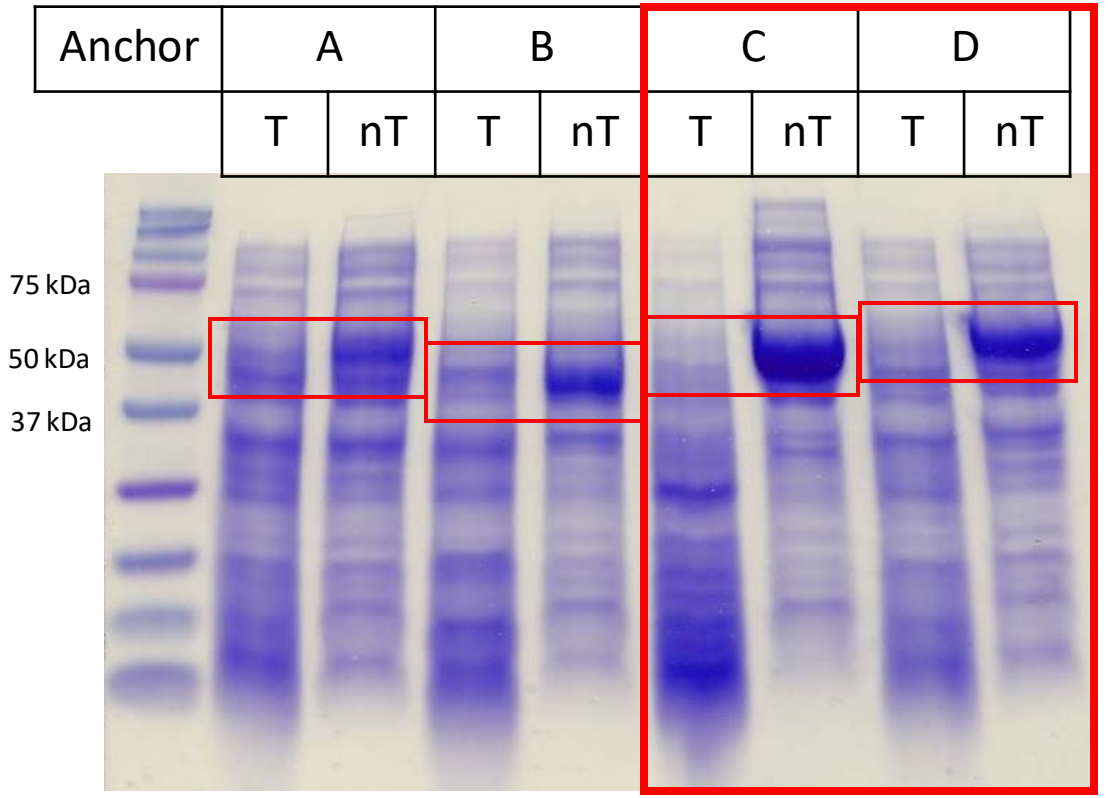
AIM: ANTIGEN IS LOCATED ON THE *E. COLI* SURFACE

4

Anchor Motif Screening - Surface Exposure



“Trypsin shaving”
Surface exposed antigens will be digested and disappear



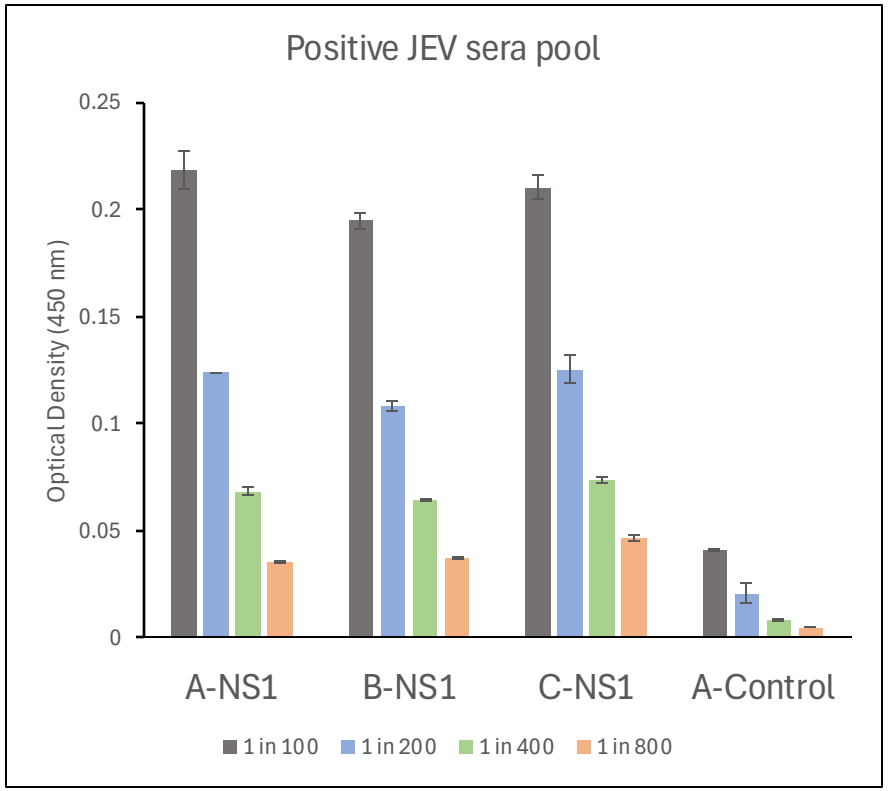
T = 0.5% Trypsin,

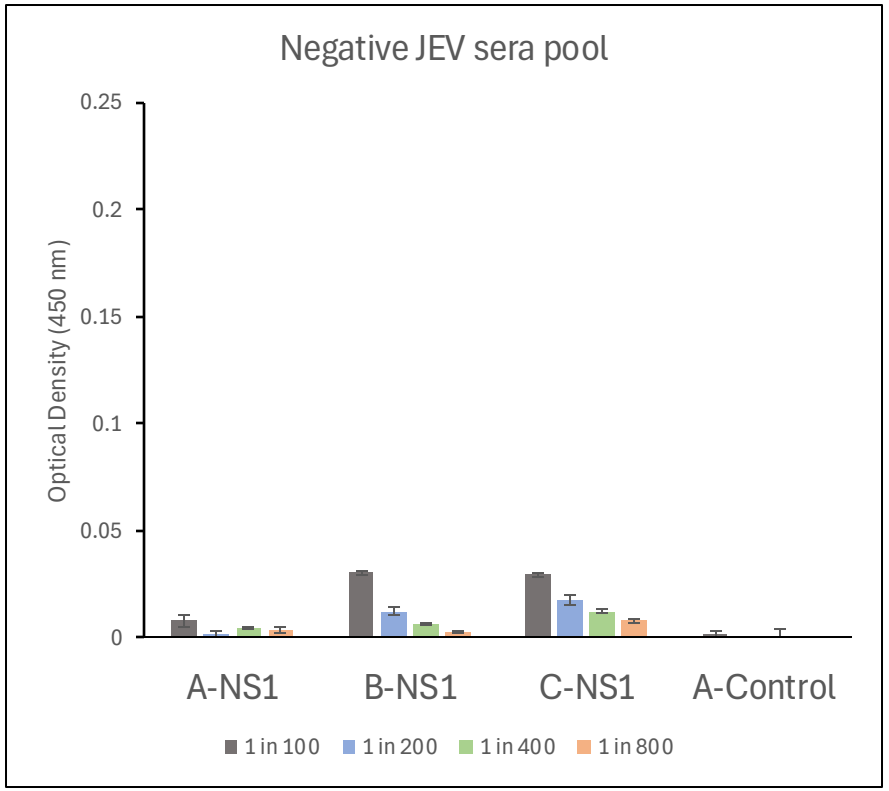
nT = no trypsin

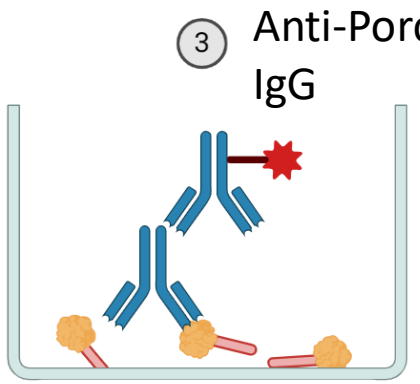
Strong decrease in band density = surface exposed

“SPINYCTERIN” SCREENING PROCESS

AIM: INFECTION SERUM RECOGNITION



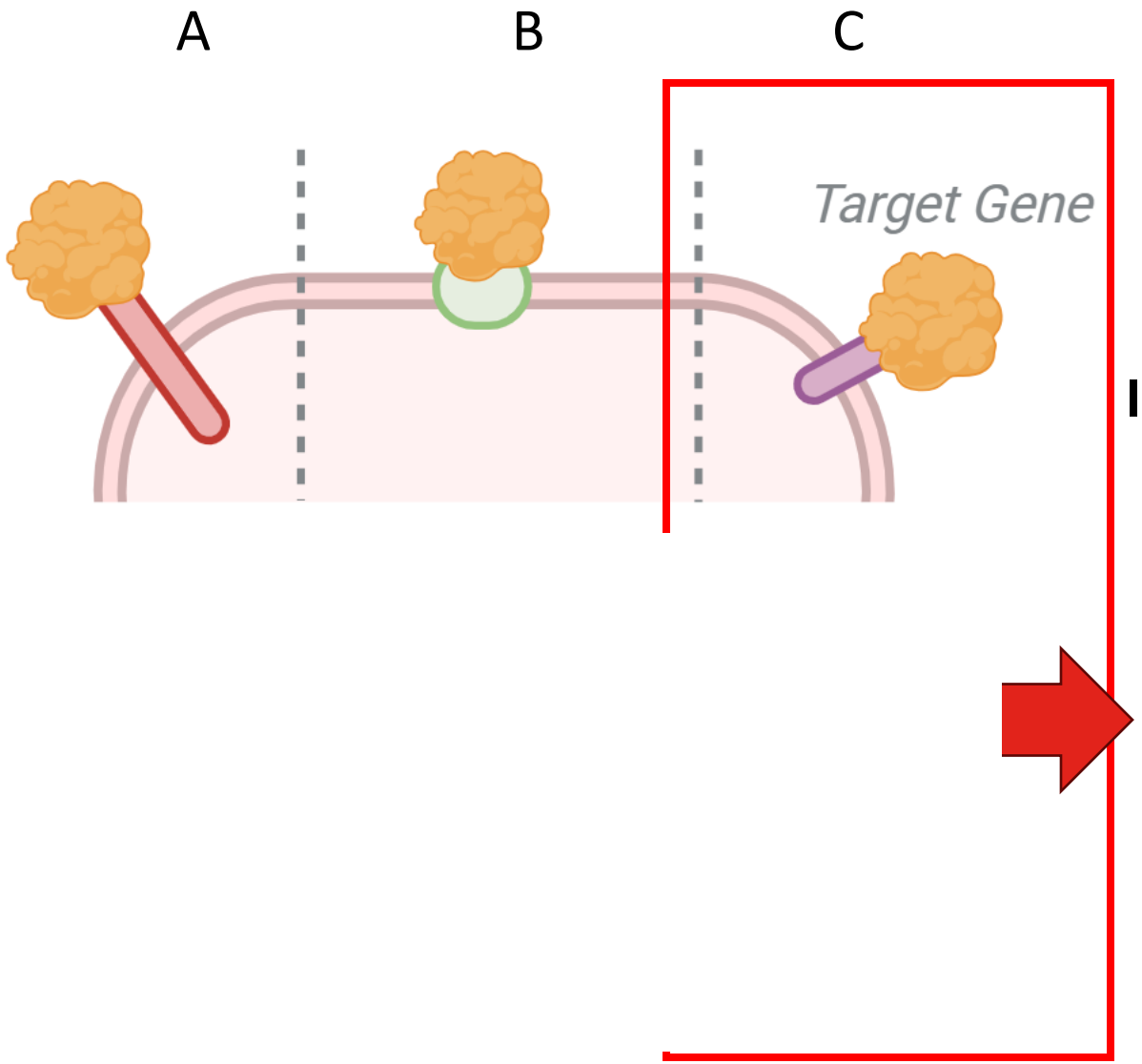




- Pig serum pools from confirmed JEV Positive and Negative samples
- AAHL, Geelong ACDP – Competitive ELISA antibody detection

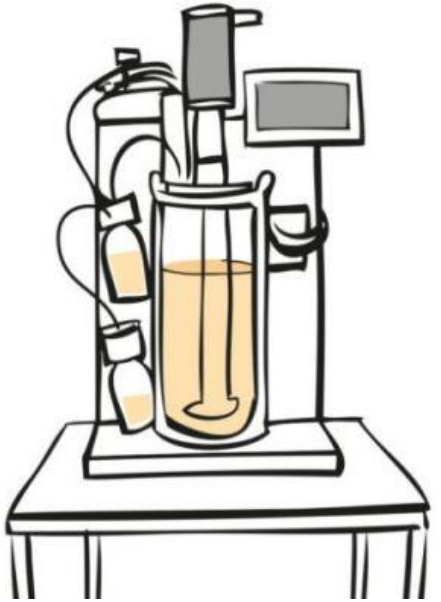
JEV-Antigen Retains Antigenicity = Potential Protection

“SPINYCTERIN” SCREENING PROCESS – SCORE CARD



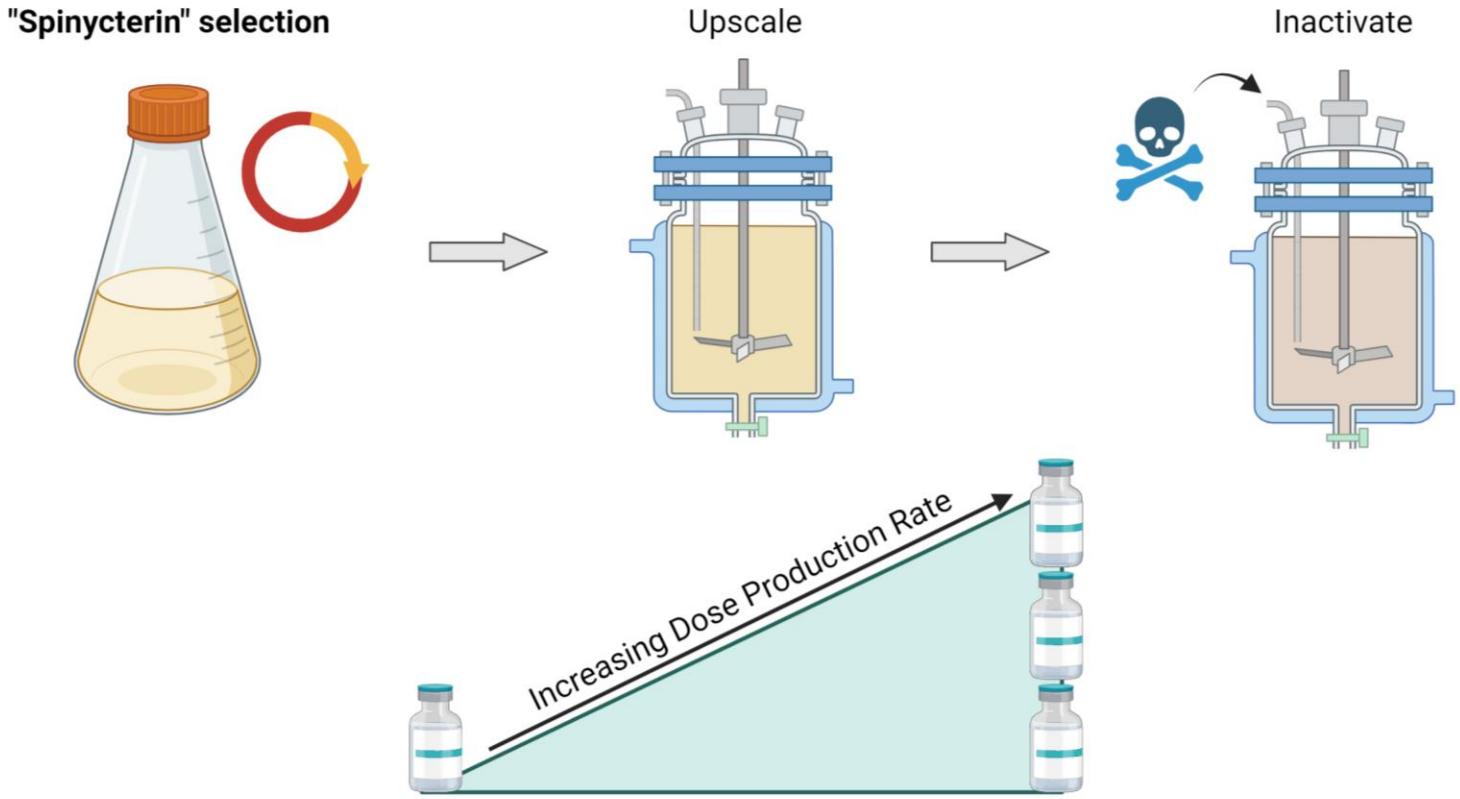
1. **HIGHLY EXPRESSED**
2. **SURFACE EXPOSED**
3. **ANTIGENIC**

INDUSTRY PARTNERS



UPSCALING AND TECH TRANSFER

STREAMLINED UPSCALING PROCESS FOR FUTURE APPLICATION



ACE LABORATORY SERVICES

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TRIAL LAYOUT

Saline Injection - Control Group (n=4)

4 ml of 150 µg/mL of NS1 Vax (n=4)

Week 0 *Week 3* *Week 6*

First Dose

Second Dose

Week 0 *Week 3* *Week 6* *Week 9* *Week 12* *Week 15*

Initial Safety Assessment

Gilts - 9 week pregnant @ commencement

Saline Injection - Control Group (n=8)

150 µg of NS1 Vax - Low Dose Group (n=8)

300 µg of NS1 Vax- High Dose Group (n=8)

15 weeks old @ commencement

SAFETY TESTING

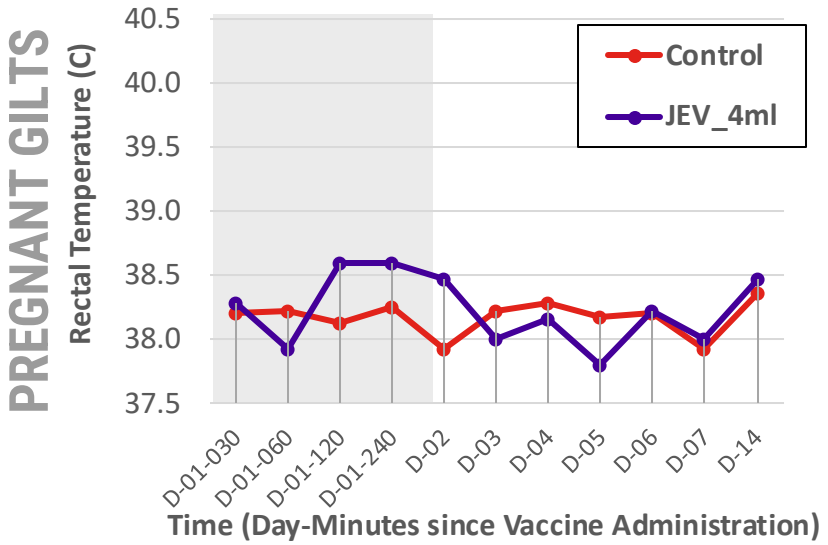
POST VACCINATION MONITORING (ALL GROUPS):

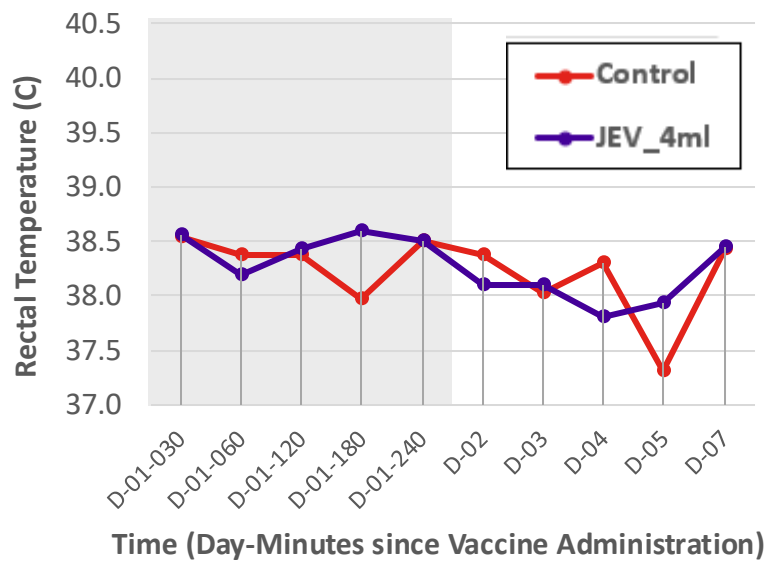
- Rectal temperature
- Demeanour (Alertness)
- Injection site (Swelling)
- Weight (Flank-to-flank)

POST-1ST DOSE

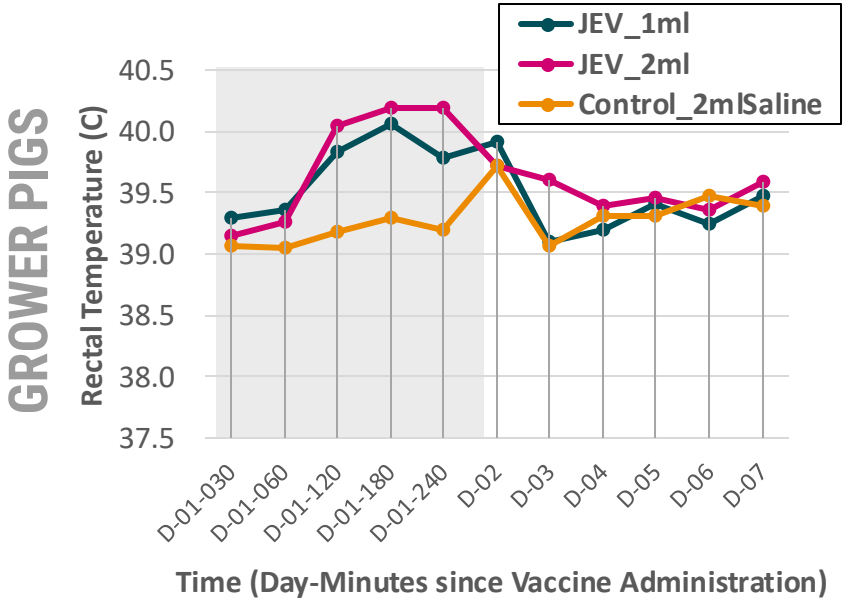
POST-2ND DOSE

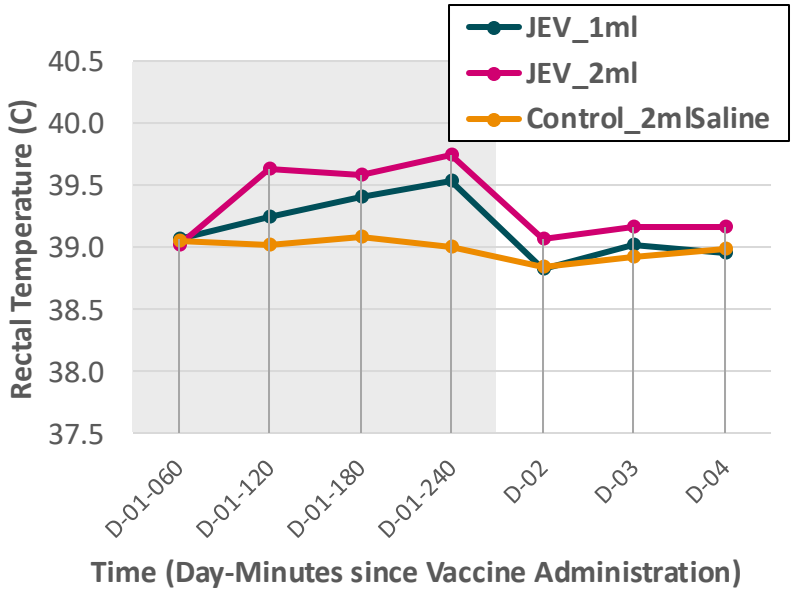
PREGNANT GILTS





GROWER PIGS





SAFETY TESTING

POST VACCINATION MONITORING (ALL GROUPS):

- **Rectal temperature**
- **Demeanour (Alertness)**
- **Injection site (Swelling)**
- **Weight (Flank-to-flank)**

Demeanour – “Seeking cordial”
Eagerness for raspberry cordial

Weight– Flank-to-flank

Group	Initial FF (cm) W0	Change in FF (cm) W6
Control	82.0	10.7
JEV-1ml	83.5	12.5
JEV-2ml	83.5	11.4

Injection site –
No erythema after injection, no significant injection abscesses.

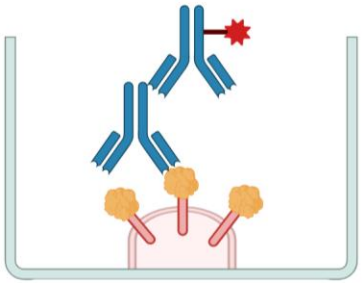
Pregnant Gilts – Normal farrowing success rates

Gestation Length (d)	Piglets Born	Mummies
115	12	1
115	13	0
112	10	2
115	14	1

IMMUNOLOGY DATA

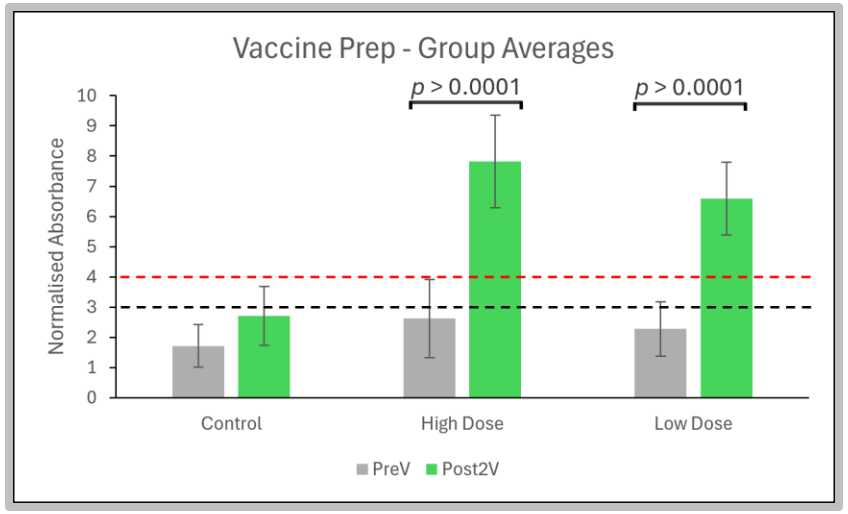
VACCINATED ANIMALS HAVE RESPONDED TO THE VACCINE PREPARATION

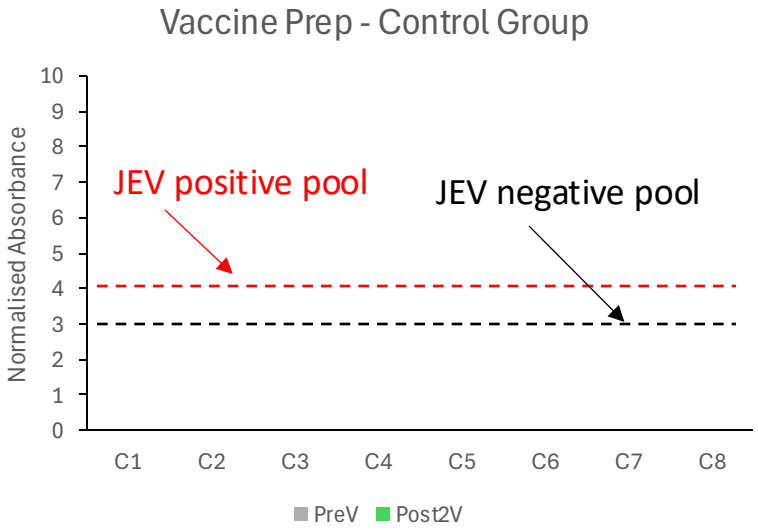
3 Anti-Porcine IgG

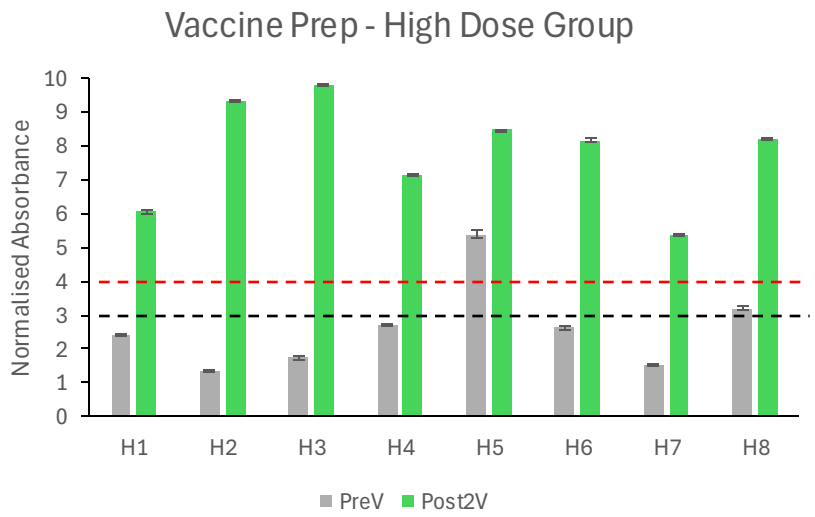


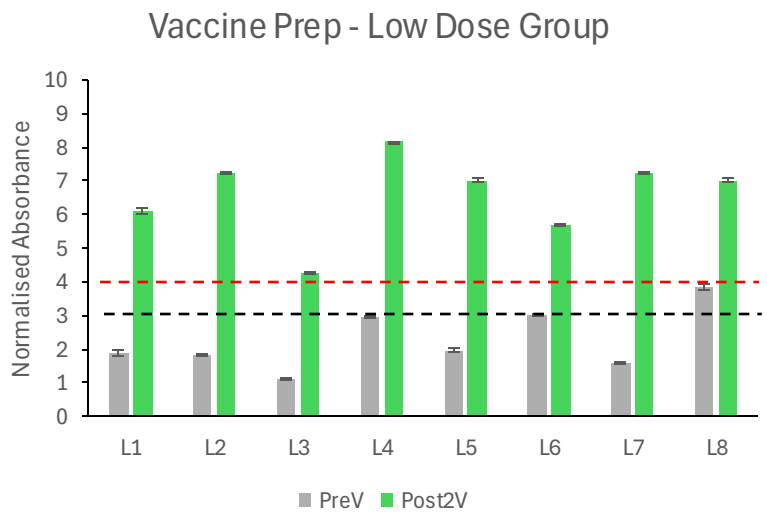
2 Pig Serum
Week 0 = PreV
Week 6 = Post2V

1 Vaccine Prep









WHAT'S NEXT

FURTHER ANALYSIS OF SPECIFIC ANTIBODY RESPONSE

- SAMPLES BEING PROCESSED AT AAHL, GEELONG ACDP
- NS1 SPECIFIC MONOCLONAL ELISA
- JEV PLAQUE REDUCTION NEUTRALIZATION TEST

ASSESSMENT OF PROTECTION AFTER NATURAL JEV INFECTION

- PERIODIC VACCINATION OF GILTS DURING POTENTIAL PEAK SEASON AND MONITOR BIRTHING SUCCESS

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