From Piggy Chews to Surveillance Clues

How oral fluids supported JEV Surveillance

Australian Pig Veterinarians Conference September 2024

AGRICULTURE VICTORIA


AWB

Images and videos courtesy of Sandy Adsett, Rivalea Veterinarian

Using Oral Fluids

- Currently used in North America for:
    - actinobacillus pleuro-pneumonia (APP)
    - Porcine Reproductive and Respiratory Syndrome (PRRS)
    - Porcine Endemic Diarrhoea (PED)
    - Porcine Circovirus Type 2 (PCV-2)
    - Influenza A
    - infection with *Mycoplasma hyopneumoniae*
- Experimental studies show nasal and oral shedding of JEV with transmission to naïve co-inhabitants
- Lyons (2018) demonstrated the detection of JEV in oral fluids collected via chew ropes for up to 2 weeks pi.

Oral Fluid Collection

Zip tie or twine

Unbleached cotton rope
(we used 3 stand 16mm thick rope)

Large plastic bag for sample extraction

Sample tube

Specimen bag for secure transport

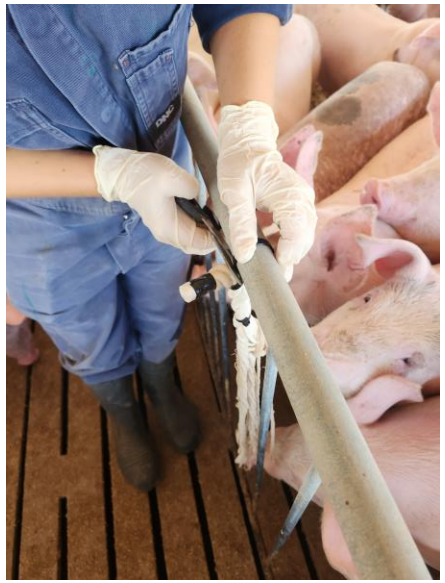
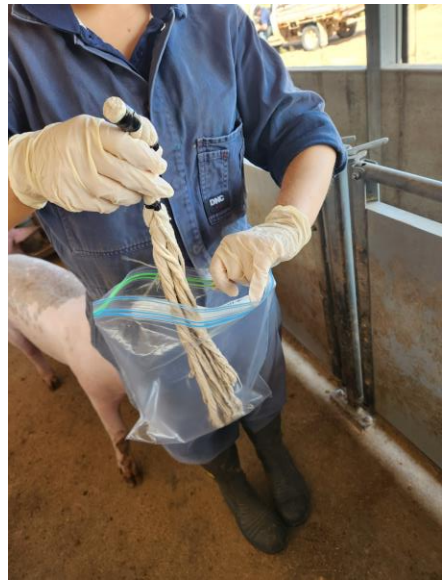
Gloves

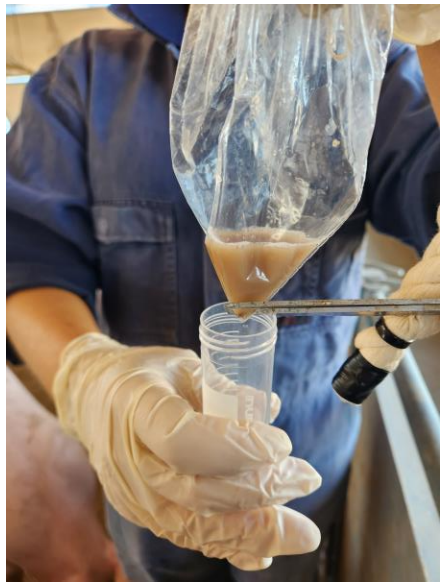




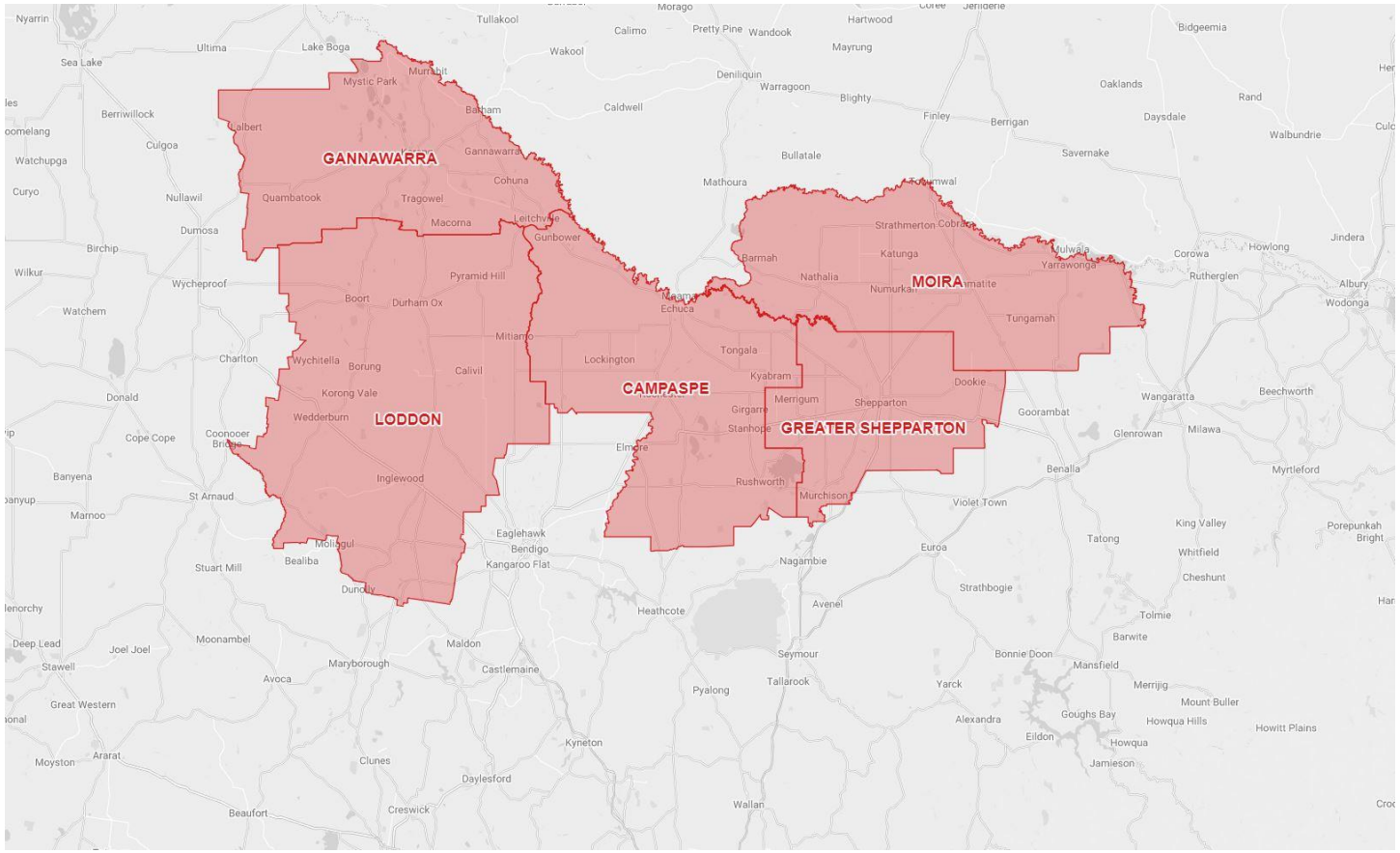
- Attach the cotton rope to a fence or post
- Ensure it is at shoulder height and the pigs can adequately reach it
- Leave it in the pen for 15 – 20 minutes
- Observe pigs to ensure the rope is not **completely destroyed**, dragged through the pen and potentially contaminated

- Ensure the rope is adequately saturated
- Carefully removed from the pen
- Avoid contact with any other surfaces
- Extract fluids by squeezing and wringing the rope in a plastic bag
- Allow fluid to pool at the base of the bag
- Cut the bag to expel fluids into sample tube


Piggery Identification and Engagement



Shires containing farms considered to be at high-risk of JEV infection, based on the 2021-22 outbreak.

- Identification of 5 high risk piggeries located in the Northern Victoria shires
    - Loddon (Farm 1)
    - Gannawarra (Farm 2)
    - Campaspe (Farm 3)
    - Greater Shepparton (Farm 4)
    - Moira (Farm 7)
- Visits to most farms to meet the piggery staff which would be assisting in the project

Oral Fluid Project Design

- 6 samples from separate pens containing 10 or more finisher pigs
    - Calculations reviewed by Ag Vic Epi employing a one-stage freedom analysis and sample size determination for demonstrating freedom using pooled testing
- Finisher pigs aged 18+ weeks old
    - Maternal antibody against JEV wanes at 16 weeks of age - Wang et al., 2012, Smith et al., 2020, Johnson et al., 2015

Mosquito Trapping

- 2-3 BG Sentinel traps with dry ice attractant set per farm per week
- Before dusk, the mosquito traps are set by farm staff
- CO2 released by the dry ice attracts mosquitoes to the trap
- Fan draws the mosquitoes into the trap
- Trap runs all night and so the mosquitoes cannot escape
- The following morning mosquito catch bags are collected
- Samples stored in freezer for a week before pick up the following Tuesday

Testing

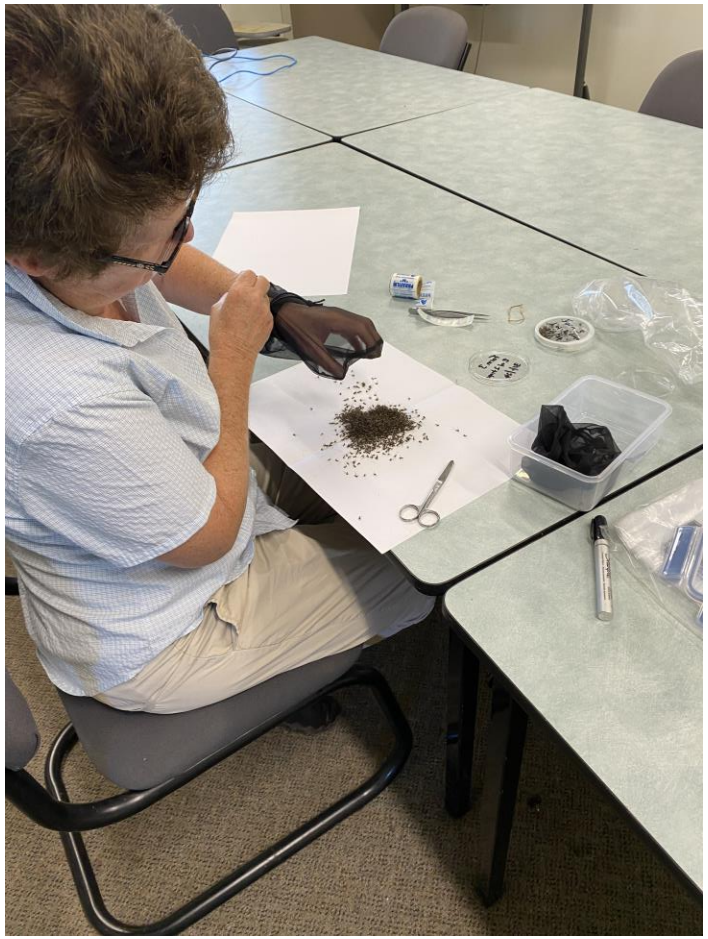
- All testing completed at the state laboratory - Agribio

Oral Fluids

- JEV PCR tests weekly – to detect JE Virus
- Flavivirus Antibody ELISA on sporadic occasions – to detect antibodies against

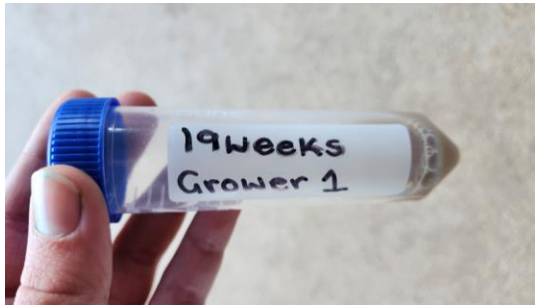
Mosquito Samples

- Morphological identification on every trap – to determine sex and species
- JEV PCR test on all female mosquitoes



Removal of the mosquitoes from the trap catch bags for packaging into petri dishes.

Tweezers were used to extract all mosquitoes captured.



Labels on the oral fluids were recorded for traceability.

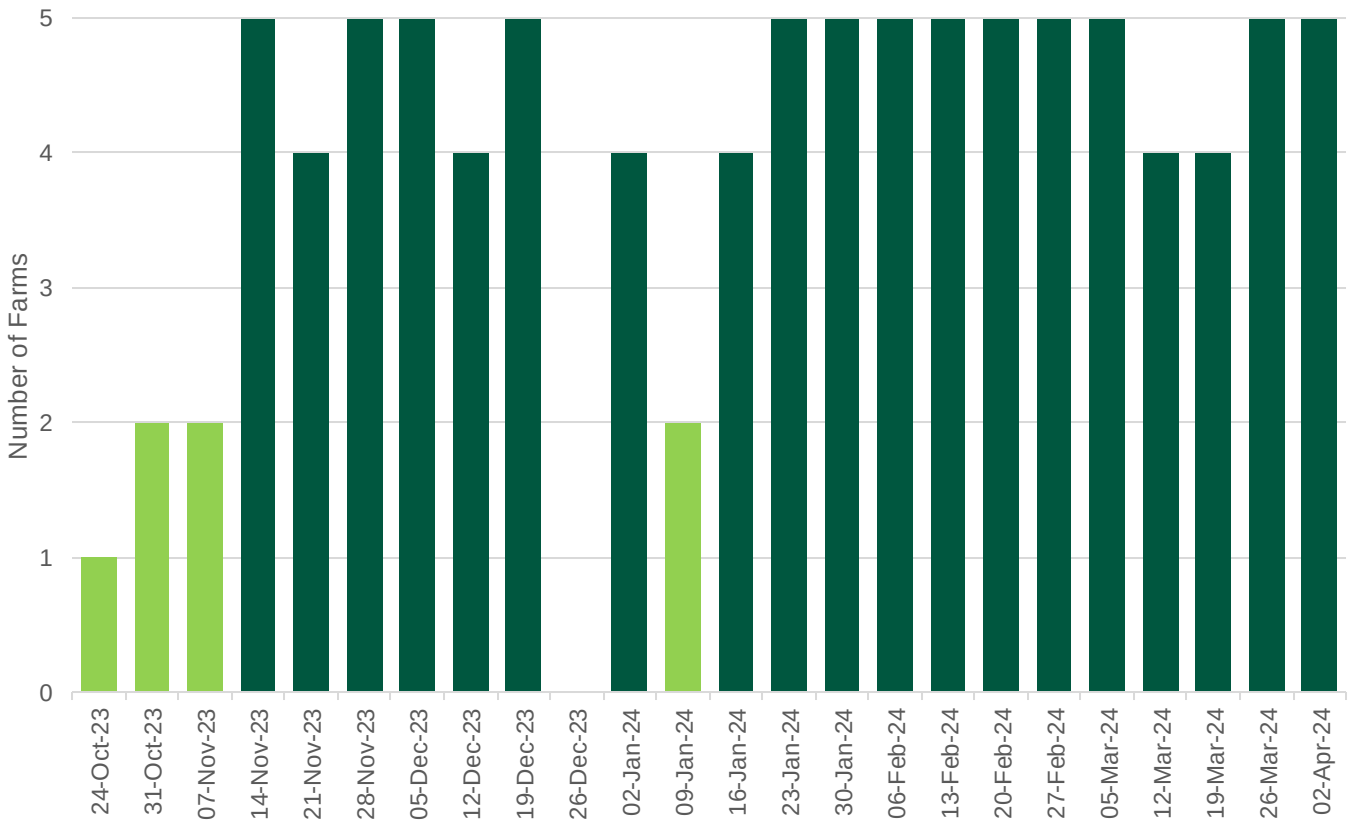
JEV Surveillance Activities Results

All

Negative.

Oral Fluid Surveillance Results

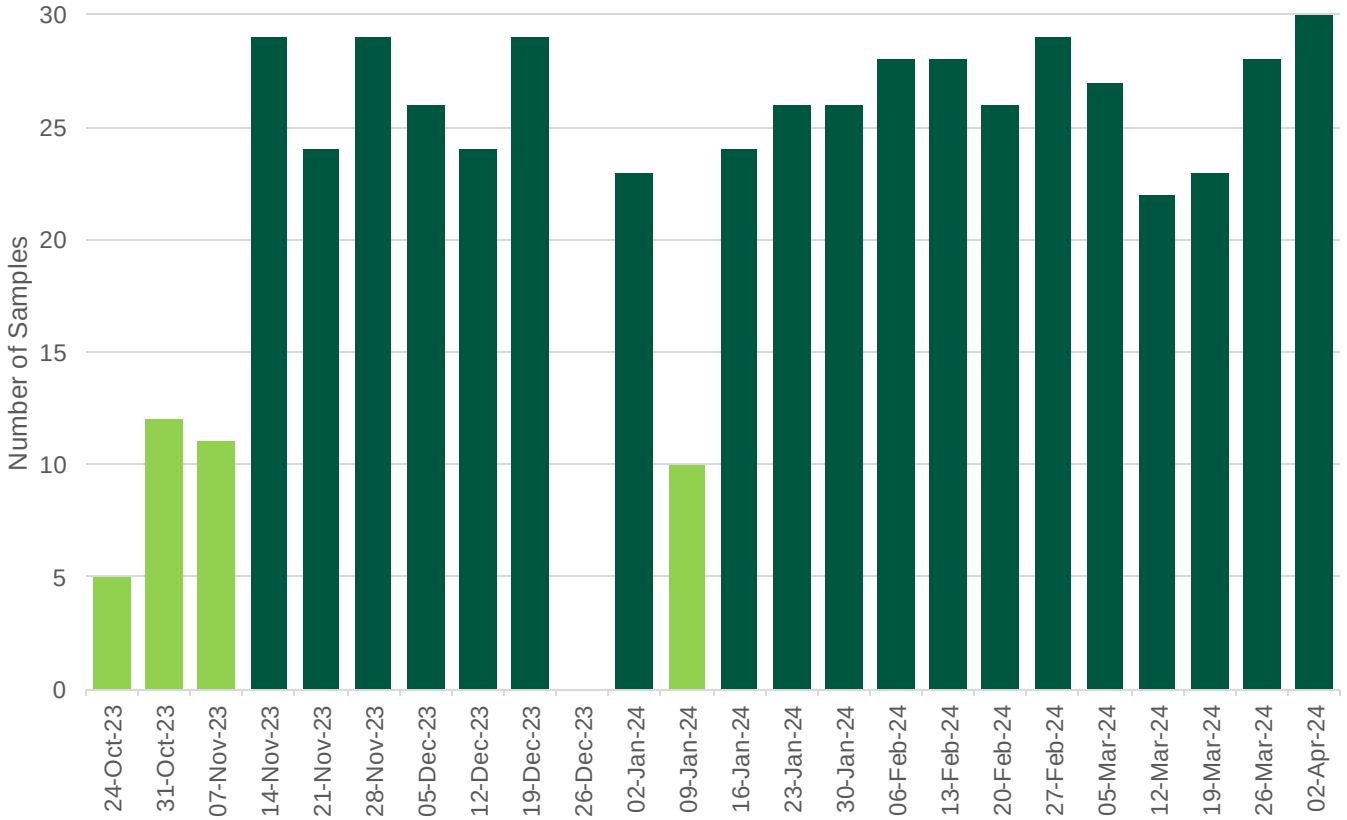
- 23 weeks of sample collection
    - First samples – October 24th 2023
    - Last samples – April 2nd 2024
- Total of 539 oral fluid samples were collected
    - All 539 samples tested via JEV PCR and returned negative results
    - 371 samples were tested via Flavivirus Antibody ELISA and returned negative results
- Average 26 samples per week (with outliers removed)



Farm participation throughout the surveillance project with the outliers in light green.

Oral Fluid Surveillance Results

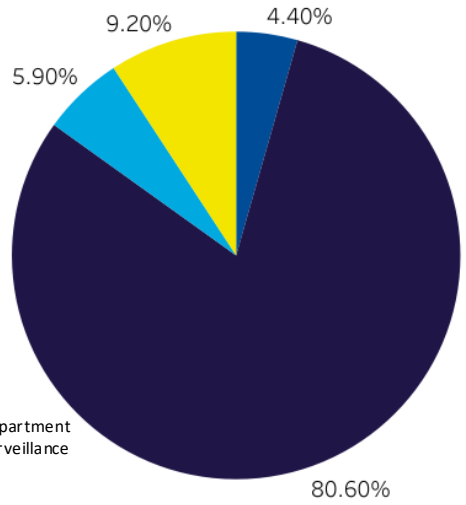
Farm Identification	Weeks participated	Total number of samples
Farm 1	19	88
Farm 2	20	120
Farm 3	18	102
Farm 4	20	118
Farm 7	19	111
	Total	539



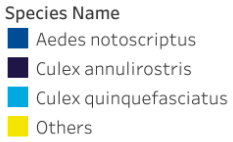
Samples obtained each week for the duration of surveillance project with the outliers in light green.

Mosquito Surveillance Overview

- 10 weeks of mosquito trapping and sample collection
- 60 Mosquito Traps Collected
- All JEV PCRs returned negative results
- *Culex annulitostris* was the most abundant species trapped on farm, aligning with the DoH mosquito surveillance

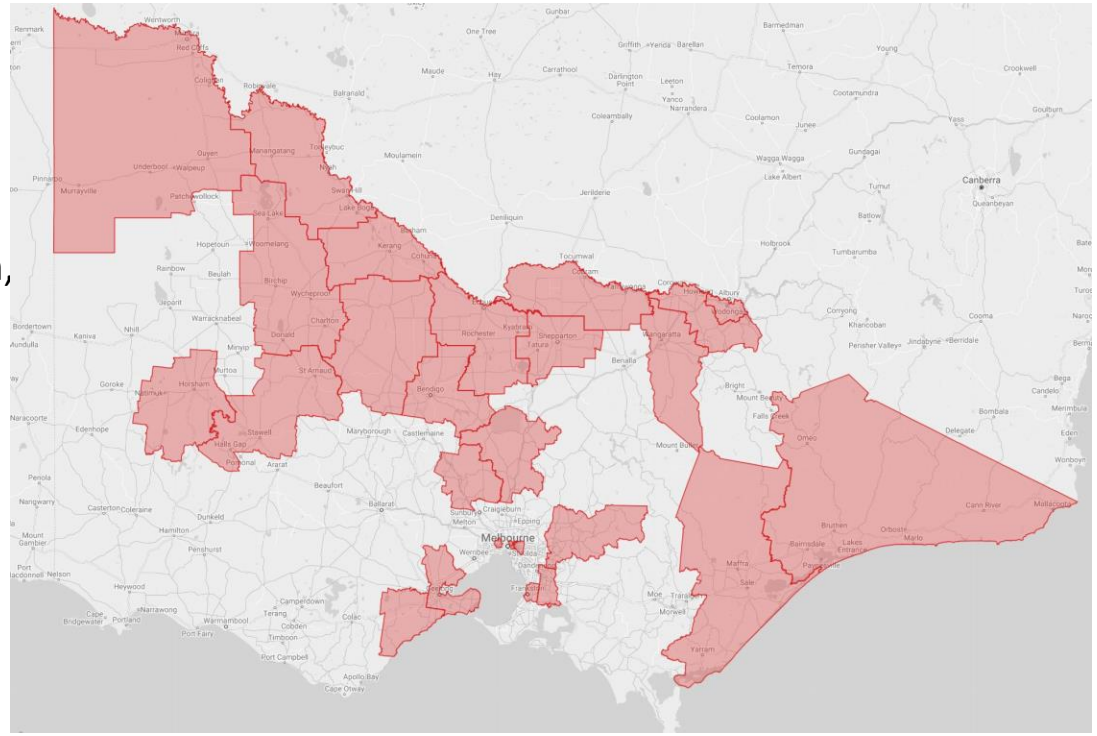


Retrieved form the Department of Health Mosquito Surveillance Report Page.



Proportion of trapped adult mosquitoes by species – North Central and East Victoria (Feb 2024)

(Campaspe, Greater Bendigo, Greater Shepparton, Macedon Ranges, Mitchell, Indigo, Moira, Wangaratta, Wodonga)



Local Governments (Shires) which participated in the Department of Health mosquito trapping and arbovirus surveillance program in the 2023-24 mosquito breeding season.

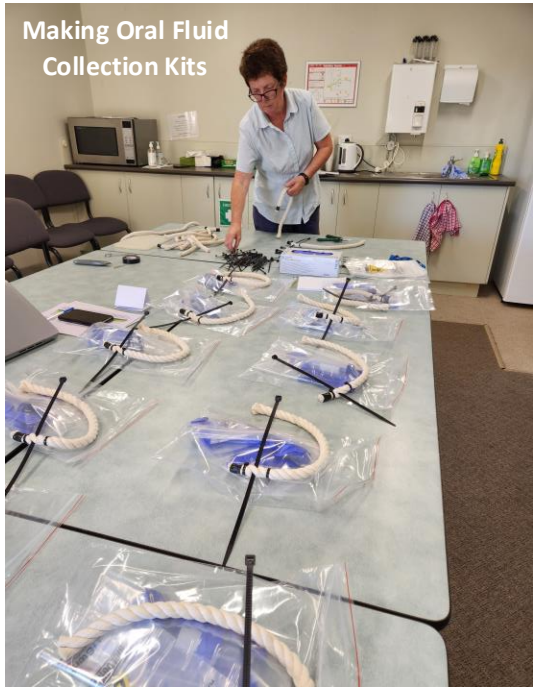
Conclusions

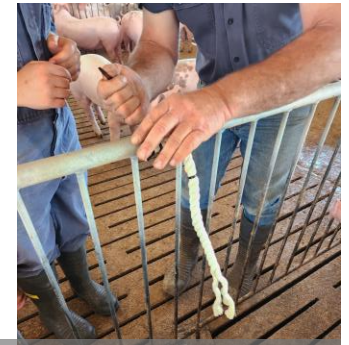
- Oral fluids collected via chew ropes:
    - Are already being regularly used in North America for routine surveillance
    - Offer a non-invasive sampling technique
    - Can effectively represent a population
    - Are easy to implement and do not require specialised staff
    - Are very well utilised by pigs!
    - Could play an important role in an EAD outbreak – potential exciting discussions ahead…

Thank you (and some happy snaps)

Thank you to all who contributed to this project and made it all possible:

- Regina Fogarty (Agriculture Victoria)
- Sally Salmon, Sarah Hall and Nicole McLaughlin from the Epi team (Agriculture Victoria)
- Chrissie Kracht (Rivalea)
- Hugo Dunlop (APIAM)
- Bernie Gleeson (SunPork Group)
- Ben Linn (Vet and Stock Consulting)
- Jono Van Dissel (APIAM)
- Peter Neville, Xin Fang, and other Department of Health Colleagues
- Peter Mee and the AgriBio team
- Kongolia Farms
- **All producers and farm staff for all the dedication to the project and collecting samples each week**





Kongolia Farms staff helping me with the SOP