Birds and Bugs and Bites (Oh My!)

Applied Epidemiology in One Health

A thesis submitted for the degree of Master of Philosophy in Applied Epidemiology of the Australian National University

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Originality statement

I hereby declare that this submission is my own work and to the best of my knowledge contains no materials previously published or written by another person, or substantial proportions of material which have been accepted for the award of any other degree or diploma at the Australian National University or any other educational institution, except where due acknowledgement is made in the text. The work presented in this these was completed between February 2023 and December 2024 for the degree of Master of Philosophy in Applied Epidemiology at the Australian National University. Any contribution made to the research by others, with whom I have worked is explicitly acknowledged in this thesis. I also declare that the intellectual content of this thesis is the product of my own work, except to the extent that assistance from others in the project's design and conception or in style, presentation, or linguistic expression is acknowledged.

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Abbreviations

ACDP	Australian Centre for Disease Preparedness
AHA	Animal Health Australia
AI	Avian influenza
AIV	Avian influenza virus
APM	Avian paramyxovirus
AUD	Australian dollars
BSL	Queensland Biosecurity Sciences Laboratory
BVL	Northern Territory Berrimah Veterinary Laboratory
CCEAD	Consultative Committee on Emergency Animal Disease
CEBO	Chief Environmental Biosecurity Officer
COVID-19	Coronavirus disease of 2019
CSIRO	Commonwealth Scientific and Industrial Research Organisation
CT	Cycle threshold
CVO	Chief Veterinary Officer
DAFF	Commonwealth Department of Agriculture, Fisheries and Forestry
DDLS	DPIRD Diagnostics and Laboratory Services
DFAT	Department of Foreign Affairs and Trade
DoHAC	Commonwealth Department of Health and Aged Care
DPIRD	Western Australia Department of Primary Industries and Regional Development
ESL	Epidemiology, Surveillance and Laboratory section
ELISA	Enzyme Linked Immunosorbent Assay
EMAI	New South Wales Elizabeth MacArthur Agricultural Institute
FAO	Food and Agriculture Organisation
GIS	Geographic Information System
HA	Haemagglutinin
HASEDS	Human Animal Spillover and Emerging Disease Scanning group
HPAI	High pathogenicity avian influenza

IBD	Infectious bursal disease
IBDV	Infectious bursal disease virus
IBH	Inclusion body hepatitis
ISVEE	International Symposium for Veterinary Epidemiology and Economics
JE	Japanese encephalitis
JEV	Japanese encephalitis virus
LFF	Lesson from the field
LMIC	Lower- middle income countries
LPAI	Low pathogenicity avian influenza
LSD	Lumpy skin disease
MAE	Master of Philosophy in Applied Epidemiology
MAL	Solomon Islands Ministry of Agriculture and Livestock
MOG	Machinery of Government
NA	Neuraminidase
NAIWB	National Avian Influenza Wild Bird surveillance program
NAQS	Northern Australia Quarantine Strategy
NATA	National Association of Testing Authorities
NDV	Newcastle disease virus
OCVO	Office of the Chief Veterinary Officer
PCR	Polymerase chain reaction
PEPAH	Pacific Engagement Program for Animal Health
PVP	Predictive value positive
RNA	Ribonucleic acid
SAFETYNET	South Asia Field Epidemiology and Technology Network
SDG	Sustainable Development Goal
SPC	The Pacific Community
TEPHINET	Training Programs in Epidemiology and Public Health Interventions Network
VESTA	Veterinary Epidemiology and Surveillance Throughout Australia network
vvIBD	Very virulent infectious bursal disease

vvIBDV	Very virulent infectious bursal disease virus
WHA	Wildlife Health Australia
US CDC	United States Center for Disease Control and Preparedness
UV	Ultraviolet radiation

Abstract

I completed my Master of Philosophy in Applied Epidemiology (MAE) at the Commonwealth Department of Agriculture, Fisheries and Forestry (DAFF) in Canberra, ACT. The Department of Agriculture, Fisheries and Forestry focuses on a range of issues of importance to Australian primary producers and the public, including biosecurity, agricultural production, and trade. Along with State and Territory Governments, DAFF has strong connections with industry partners, ranging from industry representatives such as the Meat and Livestock Association to commercial producers such as SunPork, one of the largest pork suppliers in Australia. The Department of Agriculture, Fisheries and Forestry also has strong relationships with advisory bodies such as Wildlife Health Australia (WHA) and Animal Health Australia (AHA), and with university research partners such as the University of Melbourne.

My projects focused on animal diseases and diseases of One Health significance. I described the effects of Japanese encephalitis virus on Australian piggeries, I evaluated the Northern Australian Quarantine Strategy component of the National Avian Influenza Wild Birds surveillance program, and I investigated an outbreak of very virulent infectious bursal disease in Solomon Islands chickens. This thesis also describes other experiences I had during the MAE, including two field trips I went on to support two of my projects, my teaching experiences, my involvement in working groups and my role coordinating the Veterinary Epidemiology and Surveillance Throughout Australia network.

Chapter One:

Introduction

Field placement overview

The Commonwealth Department of Agriculture, Fisheries and Forestry (DAFF) works to strengthen Australia's biosecurity (the measures put in place to prevent the introduction of harmful species into an area) and to support the growth of Australia's agricultural industries. DAFF has strong connections with state and territory governments as well as with industry partners, ranging from peak representative bodies such as the Australian Chicken Meat Foundation to commercial producers such as SunPork, one of the largest pork suppliers in Australia. DAFF also has strong relationships with other organisations such as Wildlife Health Australia (WHA) and Animal Health Australia (AHA), and with research partners such as the University of Melbourne. I led or assisted with a range of activities while completing the Master of Philosophy in Applied Epidemiology (MAE) at DAFF, many of which were made possible through these relationships.

Throughout my placement at DAFF, I worked across the One Health team in the Office of the Chief Veterinary Officer (OCVO) and the Epidemiology, Surveillance and Laboratory (ESL) team within the Animal Health Policy branch. Both teams are part of the Biosecurity, Operations and Compliance Group at DAFF. The One Health team in particular works with representatives from other sectors including public health, wildlife health and environment agencies. The team has a role in coordinating responses to zoonotic diseases and national leadership of animal health sector efforts to mitigate antimicrobial resistance. Within ESL I worked specifically in the Epidemiology and Surveillance team. This team is a small, skilled group of veterinary and non-veterinary epidemiologists whose role includes assisting with animal disease responses at the national level. In 2023 the team contributed to the response to a lumpy skin disease (LSD) trade disruption and in 2024 assisted with state responses to detections of high pathogenicity avian influenza (HPAI) in poultry farms.

I was able to work with other teams across the department to fulfil my MAE competencies. I worked with the Northern Australian Quarantine Strategy team to complete my surveillance evaluation (Chapter Three) and the Pacific Engagement Program for Animal Health (PEPAH) team to fulfil my outbreak investigation competency (Chapter Four).

MAE experience

I came into the MAE with a strong public health background and no veterinary experience, and so I was interested in a placement at DAFF to learn about One Health from an animal health perspective. My placement at DAFF allowed me to work with animal data for the first time. This was a significant learning curve for me as animal data is often collected for different purposes compared to human health data. This may include monitoring production or supporting the

animal health status claims that are required for trade. There is also a greater level of uncertainty accepted in animal health compared to public health.

I went on two field trips during the MAE as part of work towards two of my major competencies; In August 2023, I visited one of the piggeries in New South Wales (NSW) impacted by Japanese encephalitis (JE) in the 2022 outbreak, and in June 2024, I travelled to the Solomon Islands to visit poultry farms in Honiara and surrounds. Both trips were invaluable learning experiences and have informed the content and write-up of my thesis chapters.

I am the first scholar to complete the MAE at DAFF, and it was challenging for me to apply the requirements for the MAE to projects focused on animal health. Additionally, prior to my placement at DAFF, my only understanding of One Health was zoonotic infections in humans, and I had very little knowledge of infectious diseases in animals and the effects of human and animal diseases on the environment. Completing the MAE at DAFF has helped me to develop strong skills in working with animal data, complementing my skills in working with public health data and improving my overall understanding of One Health.

I lived in regional NSW for the duration of the MAE. I split my time between working in the DAFF Canberra office and working from my home in the Southern Tablelands.

Summary of chapters and competencies

Analysis of a public health dataset and design and conduct an epidemiological study

I investigated the effects of JE on Australian domestic pig farms in 2022, including clinical signs, seropositivity and long-term reproductive outcomes. This project was a good starting point for me to learn about animal data, and I used data on sow mating and litter outcomes as a proxy for herd-level impacts of JE infection. Many production metrics are monitored by farmers and can be used to identify changes that can indicate a disease problem. My visit to a piggery gave me greater insight into the data I was exploring and helped me to appreciate the importance of fieldwork in epidemiology, even when just visiting a site. This project fulfilled my ‘analysis of a public health dataset’ and ‘design and conduct an epidemiological study’ competencies and is presented in Chapter Two. Reflections on my visit to a piggery are also presented in Chapter Six.

This chapter also includes a literature review in Appendix 2C, and I have prepared a late-stage draft of a manuscript for submission to a peer-reviewed journal based on this chapter.

Evaluation of a surveillance system

I evaluated the Northern Australia Quarantine Strategy (NAQS) active component of the National Avian Influenza Wild Birds (NAIWB) surveillance program for avian influenza viruses (AIVs). Since 2020, high pathogenicity avian influenza H5 clade 2.3.4.4b has been detected in every continent other than Australia, and so understanding Australia's northern-most surveillance system for AIVs was of interest to stakeholders. This project is presented in Chapter Three, and I will be presenting findings from this project to the International Symposium for Veterinary Epidemiology and Economics, which will be held in Sydney in November 2024.

Investigation of an outbreak or acute public health risk

I was involved in an overseas animal outbreak investigation after detections of very virulent infectious bursal disease were made in chickens in Solomon Islands. Following the investigation, I travelled to Honiara to visit poultry farms and assisted in conducting a follow-up livestock survey. This investigation is described in Chapter Four. Reflections on my visit to Solomon Islands are also presented in Chapter Six.

Teaching activities

To fulfil the MAE teaching requirements, I prepared a Lesson from the field (LFF) for my peers and a teaching session for first year MAE's. My LFF was based on my outbreak investigation (Chapter Four). My teaching session for first year's was titled 'One Health response: An Introduction', and included a scenario based on JE in Australia to demonstrate a One Health response. These experiences are described in Chapter Five.

Other experiences

My placement at DAFF included field trips, participation in scenario exercises, and management of a national network of veterinary and animal health professionals. I also prepared an article for the Three Chiefs Newsletter describing my experience completing the MAE at DAFF to fulfil the lay summary competency of the MAE. These and other activities are detailed in Chapter Six.

An overview of all chapters and competencies is provided in Table 1.1.

Table 1.1. Overview of chapters and competencies completed for the Master of Philosophy in Applied Epidemiology

	Chapter 2	Chapter 3	Chapter 4	Chapter 5	Chapter 6
Analysis of a Public Health Dataset	X				
Design and conduct an Epidemiological study	X				
Evaluation of a surveillance system		X			
Investigation of an outbreak or acute public health problem			X		
Literature review	X				
Oral conference presentation		X			
Peer reviewed publication	X				
Summary for lay audience					X
Teaching activities				X	

Chapter Two:
The effects of Japanese encephalitis virus infection on Australian domestic pig farms in Queensland, New South Wales and South Australia, January 2018 to August 2023

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Glossary

Abortion	Premature failure of pregnancy
Ardeid birds	Wading birds including herons, egrets and bitterns
Aspermia	Complete absence of semen resulting in infertility
Boar	An uncastrated male pig
Cloven-hoofed animals	Animals with two-toes including pigs, cows, and goats
Cohort	A group of pigs based on production stage
Effluent	Liquid sewerage/waste
Found open/ Not in pig	A breeding female that has been serviced and is thought to be pregnant but is found not pregnant
Gilt	A female selected for the breeding herd that has not yet farrowed any litters (Parity 0)
Infected premises	A farm that showed clinical evidence of infection with JEV and met the national case definition of JEV between February 2022 – July 2022.
Mummified foetus	Piglets that have died during pregnancy after bone development has started and that are born dead at the time of farrowing; sometimes colloquially referred to as “mummies” in the pork industry
Oestrus	The time a breeding female is fertile or “in heat”
Parity	The number of times a sow or gilt has farrowed a litter of piglets
Progeny pigs	Pigs that are not part of the breeding herd and are being grown to market weight for sale.
Selection	The process whereby gilts and boars are selected from progeny pigs to join the breeding herd
Service	The act of inseminating a sow or gilt in the breeding herd (can also refer to a natural mating, although this is not used very often for pig production).
Sow	A breeding female who has farrowed at least once (parity 1 or more)

Stillborn	Piglets that are fully developed but die before or during farrowing
Wean	Separation of a sow from her piglets, usually when piglets are approximately 3-4 weeks of age

Prologue

Background

In early 2022, New South Wales (NSW), Victoria (Vic), Queensland (Qld) and South Australia (SA) reported detections of Japanese encephalitis (JE) in domestic pig herds for the first time (1). Japanese encephalitis is caused by infection with the Japanese encephalitis virus (JEV), a flavivirus that can cause reproductive abnormalities in pregnant sows and their litters, resulting in significant losses for the pork industry. In response to the confirmation of JEV detections in pigs, the Consultative Committee on Emergency Animal Disease (CCEAD) was stood up. Japanese encephalitis was also designated a Communicable Disease Incident of National Significance by the Australian Government Department of Health and Aged care (2). Under the CCEAD, the JEV Animal Health Surveillance Working Group was formed to coordinate surveillance activities in animals. This working group included representatives from the Australian pork industry, state animal health agencies and the Australian Government Department of Agriculture, Fisheries and Forestry (DAFF). The working group developed a national surveillance plan for animals, and implemented some of the activities, included coordinating the collection of blood samples from a subset of known infected properties to investigate the seroprevalence of JEV in pigs at affected farms. The pork industry had several key questions they hoped to address through this seroprevalence study, all with a focus on the significance of the relationship between seroprevalence and on-farm clinical signs, to improve understanding of the epidemiology of the virus (Appendix 2A). This chapter describes the JEV seroprevalence at selected farms as well as clinical signs of JEV in pigs. This chapter also includes a survival analysis and other analyses exploring the effect of JEV infection on long term reproductive outcomes in sows.

This chapter fulfils two competencies for the MAE:

1. Analysis of a public health[1] dataset
2. Design and conduct an epidemiological study.

My role

In March 2023, my supervisor Rachel Iglesias approached me about completing this project for my data analysis competency. After attending my peer review session in course block two (September 2023), I decided to combine my ‘analysis of a public health dataset’ competency and my ‘design and conduct an epidemiological study’ competency into one chapter, and include some more complex analyses. My roles for this project included coordinating meetings with pork industry veterinarians, collating and cleaning the data, and data analysis. I also

[1] Animal production dataset used due to nature of placement at DAFF.

conducted a literature review focused on the risk factors for JEV seropositivity in pigs to meet the literature review minor competency for the Master of Philosophy in Applied Epidemiology (MAE), available in Appendix 2B. This project was designed in 2022, and I was not involved in any of the planning, including sample size calculations, selection of farms, or selection of pigs. More information about the planning for the project is available in Appendix 2E.

I sought advice from the Human Research Ethics and Animal Research Ethics Committees at ANU about the appropriate ethics required for this project. I began an ethics application but was advised that ethics approval was not required and was given an exemption (Appendix 2C). I later sought an amended ethics exemption after I changed the title of the project.

Animal health and public health impact

Japanese encephalitis virus is endemic in many countries in south-east Asia and the Pacific, but the 2022 incursion of JEV into sub-tropical temperate areas Australia was an unprecedented event. Despite the prevalence of JEV in other countries, the risk factors for JEV infection in pigs are not well understood, including how prevalence of clinical signs observed in pigs relate to seroprevalence. This project is the first to describe the seroprevalence of JEV at affected farms in Australia, and to describe and compare the varying clinical signs observed at multiple farms across naïve pig populations. This study also captures potential long term effects JEV can have on sow productivity not previously investigated. There are limited opportunities to investigate the impact of JEV transmission in fully susceptible (immunologically naïve) pig populations as pigs in endemic countries are likely to be exposed within the first year of life. The findings from this study may therefore assist with JEV preparedness activities in the future, including for future outbreaks in Australia and overseas in countries currently free from JEV (3, 4) .

Japanese encephalitis is considered a disease of One Health significance as the virus can also cause illness in humans, and JE is the most common cause of encephalitis in humans in many Asian countries (5). In endemic countries, proximity to pigs is a known risk factor for JEV infection in people and so understanding the disease in pigs is helpful for understanding the disease in humans as well. Disease investigations with One Health significance are commonly anthropocentric, and this chapter provides an animal-focused approach which can be used in conjunction with human studies to improve the overall understanding of JEV in Australia and overseas. This chapter also describes the uncertainty associated with JEV infection in swine.

Reflections and lessons learned

I was involved in the public health response to JEV in 2022, and so had knowledge of JEV, flaviviruses, and serosurveys prior to working on this project. However, this project was my first introduction to working in an animal health space and working with production data, and I spent

a significant amount of time learning the differences between animal data and public health data. This included looking at herds rather than individuals and working with data collected for production purposes rather than for clinical investigations. I also learned that veterinarians are required to work with a greater level of uncertainty compared to public health counterparts. This is highlighted by the limited published material available on the effects of JEV on animals including swine. There were also many veterinary and pork-industry terms (such as sow, farrow and service) and processes (such as selection) I had to familiarise myself with during this project.

Working on this project helped me learn the importance of understanding how and why data is collected before starting any analysis. It was difficult for me to decide what areas to focus on in this project as I was not using the data for its intended purpose, and there was a large amount of information available in the datasets. One industry veterinarian commented that I would be lucky to still have all my hair at the conclusion of the project, and I am grateful to the pork industry representatives I worked with who took the time to answer all my questions and talk me through pork production and production data. I was fortunate to visit to a pig farm in August 2023, to improve my understanding of the data I was analysing. After this visit I significantly changed my data analysis plan. More information about this visit is available in Chapter 6 (Other experiences).

This project helped me to develop my skills in analysis of animal data and animal disease investigations, use of R and R studio, data analysis, and scientific communication. It was also my first time conducting a survival analysis. I kept pig veterinarians engaged throughout the project, though this could have been better managed by holding more frequent meetings during the first year of my MAE, and by having a more robust timeline for this project.

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Abstract

Background: Japanese encephalitis virus (JEV) was detected for the first time in Australian piggeries in early 2022. JEV can cause reproductive issues in swine, resulting in significant economic losses for the pork industry. There is limited published evidence on the effects of JEV on pigs and the pork industry, with most studies focusing on JEV in humans and how seroprevalence in pigs relates to human outbreaks. This chapter describes the incursion of JEV at 8 farms in sub-tropical and temperate parts of Australia, including clinical signs, seroprevalence and the effects of JEV on long term reproductive outcomes for sows.

Methods: Serology results from pigs in different cohorts at 8 different farms were used to calculate seroprevalence across farms and cohorts. Production information used to monitor sow reproductive performance was provided by industry veterinarians and used to calculate the proportion of mummified foetuses and stillborn piglets born per litter per week at each farm. Rolled averages were also calculated and compared across all farms. A survival analysis was performed to investigate the effect of JEV infection on sow longevity in the herd.

Results: The seroprevalence of JEV varied across the farms in the study and ranged from 0% to 70.5%. Older pigs had a higher seroprevalence than younger pigs, and farms estimated to have been first infected between November 2021 and December 2021 had longer outbreaks with more pronounced effects compared to farms infected from January 2022. In general, seropositive sows were removed from the breeding herd earlier than seronegative sows, particularly if they had blood collected at parity 0 or parity 3. There was no relationship between seroprevalence and shed type or distance from permanent water bodies, and no clear relationship between seroprevalence and magnitude of clinical signs at the herd level.

Conclusion: The incursion of JEV into southeastern Australia was an unprecedented event. This study demonstrated that seroprevalence varied across farms, some sows evaded infection with the virus, and infection in gilts may be associated with prolonged reproductive abnormalities. Future studies should include a larger sample size of farms and pigs and would ideally commence sample collection earlier in the transmission season to improve the understanding of JEV in pigs.

Introduction

Japanese encephalitis virus (JEV) is a mosquito-borne flavivirus that can cause disease in humans and animals (6). Ardeid birds[2] and pigs are amplifying hosts of the virus, and presence of the virus in these species is often seen as a precursor for disease in humans (7, 8). Japanese encephalitis virus can cause reproductive issues in gilts and sows, including reduced litter sizes, difficultly conceiving, abortions, prolonged gestation, shaky piglets, anencephaly and limb deformities in piglets, stillbirths and mummified foetuses (1, 6, 9-13). These signs are believed to depend on the stage of gestation at the time of maternal infection (14) and infection is often not evident until gestating piglets are born some weeks later. In addition, infected boars can also experience infertility (11, 13). These reproductive issues are associated with significant economic losses for farmers and the pork industry as they lead to reduction in numbers of pigs available to be sold. Reproductive issues may also result in premature removal of the sow from the breeding herd and boars from reproductive centres. While JEV is transmitted to pigs by mosquitoes, there is some evidence that pigs also shed virus through nasal and vaginal secretions and that this may result in pig-to-pig transmission of JEV (3, 16, 17). The importance of this mode of transmission is not clear but it was not considered to be epidemiologically significant during the Australian outbreak.

Japanese encephalitis virus is endemic to parts of Asia and the Western Pacific region (9, 18). Historically, sporadic cases of Japanese encephalitis (JE) have occurred in humans in the Torres Strait and Cape York peninsula in Australia, and three locally-acquired human cases were diagnosed in these areas in 1995, 1998 and 2021 (18, 19). Past surveillance programs have also demonstrated evidence of infection with JEV in feral pigs in northern areas of Australia (18). These infections were thought to be seasonal introductions from neighbouring countries and not evidence that the virus was circulating within Australia (19). Prior to the detections of JEV in southern areas of Australia in 2022 the risk of JEV spreading south of the Cape York Peninsula was considered low (18, 20).

Between February and June 2022, JEV was identified at over 80 pig farms across New South Wales (NSW), Victoria, Queensland (Qld) and South Australia (SA) (1, 18, 21). All states reported infection in one or more farms within the same week (13). During this time, the pork industry experienced significant losses through increases in unviable litters as well as interruptions to normal stock movements, including movement of reproductive material from affected farms. It is estimated that 60% of Australia's sow herd were affected[3], with the overall

[2] Studies have focused on ardeid birds however other bird species could also be amplifying hosts
[3] 60% of sows in Australia are on farms that showed evidence of infection with JEV

production loss estimated at 3%-6% (between $320,000 and 380,000 AUD per 1000 sows) (13, 22). Cases in humans were confirmed in the same period as pigs (2, 4, 8).

The Australian pork industry contributes an estimated $6 billion to the Australian economy and there are over 4,500 pork production sites in Australia housing approximately 2.5 million pigs (23). Farming set-ups are varied across Australia, and farms have different herd sizes, production stages, and farming systems (23). Some farms manage pigs at all stages of pig production at a single site and others manage pigs across multiple sites (24). For the latter, sites may be specialised based on pork production stage, such as sows on one site and weaned progeny pigs on another site, and movement of pigs between sites is carefully monitored (24). From a farm management perspective, an important distinction between farms is whether or not there are pigs that are part of a breeding herd, or if all pigs are progeny pigs. Female pigs reach sexual maturity at approximately 24-30 weeks of age, and boars are used for breeding from approximately 30 weeks old (24). An overview of pork production stages is available in Figure 2.1 and Appendix 2D.

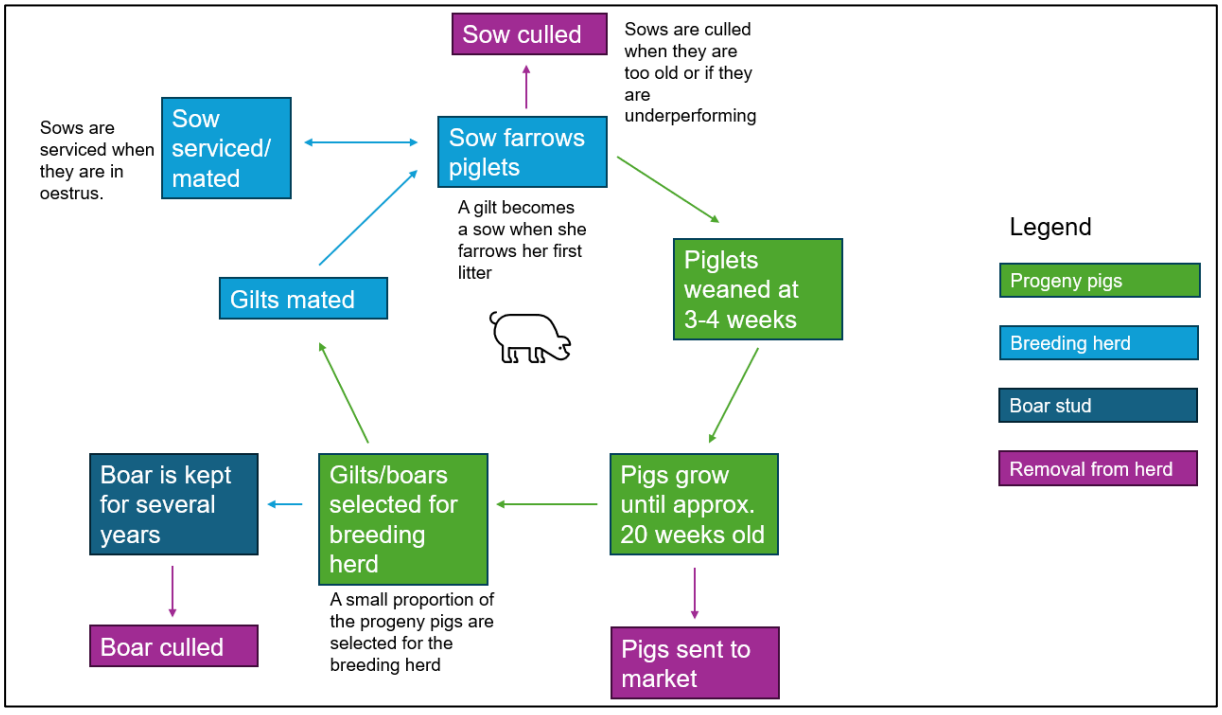

Figure 2.1. Overview of pork production stages in Australia *(figure drawn by Michaela Gilbert, 2024)*

High standards of biosecurity are maintained on pig farms, including prohibition of pork products being brought into sites and quarantine requirements for incoming pigs and staff that have had contact with pigs and other cloven-hoofed animals outside of the workplace (25). All staff are required to change clothes when arriving and leaving the site, with some sites also requiring staff to shower when arriving and leaving (25). These practices are vastly different to

pork production in many countries where JEV is endemic. For example, small-scale traditional farming continues to make up the majority of pork production operations in Vietnam (6, 26) and, similarly, smallholder and back-yard farming dominates the pork industry in India (27). In Japan, pig farming has become more commercialised in recent decades, however JEV was first identified in Japan in 1871 and became endemic in Japan prior to these production changes (5, 28).

Research into the effects of JEV on pigs is limited (6), including research investigating the risk factors and outcomes for JEV infection in Australian piggeries (29). It is well-established that conditions favourable to mosquitoes are associated with increased risk of JEV transmission (7, 30-33) though most of these studies focus on the relationship between seropositivity in pigs and the number of human cases in a region (7, 31-33). While previous studies in Asia have investigated the seroprevalence and risk factors for JEV seropositivity among pigs in endemic areas (7, 30, 32, 34) these studies have not quantified the clinical outcomes observed in swine infected with JEV.

We describe the clinical signs and investigate the seroprevalence of JEV at 8 pig farms across SA, NSW and Qld. For one farm, we report the seroprevalence and effects of JEV in boars. We describe and compare the varying clinical signs observed at multiple farms across a naïve pig population in southern parts of Australia, as well as analysing the seroprevalence and effect of JEV of sow performance in a herd. The findings from this study may assist with JEV preparedness activities in the future, including for future herds in Australia and herds overseas (3, 4).

Methods

Study design

This project has two parts:

1. Descriptive analysis of clinical signs, farm environments and serology
2. Survival analysis and long-term reproductive outcomes for sows.

Two types of data were used in this study by both components of the project. The first was routinely collected production data for the participating farms. Production data is used to monitor herd productivity and reproductive performance of individual animals in the herd and helps managers to make decisions about herd management. Ancillary information about participating farms, including location, was also used. The second type of data was prospectively collected serology data from a subset of the infected premises identified during the 2022 outbreak in south-eastern Australia. This data was collected with the intention that it could be used to answer the questions of interest to the industry veterinarians.

Study hypothesis

The primary hypotheses of the project are:

1. Farms with higher seroprevalence of JEV have more severe farm level impacts of JE outbreaks
2. Severity of farm level impacts is influenced by environmental factors
3. Sows infected with JEV experience ongoing reproductive problems after recovery and are removed from the breeding herd prematurely

The severity of impact was determined by the increase of reproductive abnormalities experienced on each farm, and the length of time these abnormalities lasted.

Pork industry representatives prepared hypothesis early in the 2022 outbreak based on the limited literature available at the time. Some of these included:

1. The duration of the farm epidemic as indicated by clinical signs lasts for only 50 days, until all the infected sows farrow or the mosquito season is over.
2. The prevalence of infection varies with each infected farm.
3. Some sows escape infection during pregnancy
4. The prevalence of infection in finishing pigs varies with region
5. Growing pigs carry maternal antibodies until at least 8 weeks of age
6. Growing pigs are seronegative by 16 weeks of age

More information about these hypotheses is available in Appendix 2A.

For details on farm selection and data collection, please see Appendix 2E.

Data analysis

Pig veterinarians responsible for the health of the study farms provided the following information for 8 farms:

- Production data for 2018-2023 (earlier years' data for comparative purposes)
- Farm coordinates
- Farm maps
- Shed information
- Outbreak dates
- Clinical overview
- Benchmarks
- Serology results and laboratory reports.

For the purposes of this analysis, each farm was randomly assigned an identification letter to maintain confidentiality (Farm A – Farm H).

Descriptive analysis of farm environments, clinical signs and serology

Farm environments

Coordinates provided by farm veterinarians were searched on GoogleEarth to identify lakes, rivers and creeks near farms and to measure the distance between these designated features and pig farms in the study.

Clinical signs

The severity of JEV impact on each farm was measured by the length of time a farm experienced reproductive outcomes and the intensity of the abnormal reproductive outcomes observed during the outbreak period, especially the increase in mummified piglets born per litter per week. Pig veterinarians provided production information used to monitor sow performance including important activity dates (date of sow arrival in herd, date serviced, date farrowed, date removed from herd), parity at time of service, sow ID, removal type, number of live born piglets in litter, number of mummified foetuses in litter and number of stillborn piglets in litter. Production data for each farm was imported and analysed in RStudio 2023.06.1-524 (34). Data for service events prior to the first of January 2018 and for farrowing events after the 17th of August 2023 were removed for consistency of data across all farms. The average proportion of mummified foetuses and stillborn piglets born per litter per week was calculated and plotted in a histogram for each farm. Weekly rolling averages for each farm were also calculated individually and plotted together.

Data on key dates and clinical signs of JEV observed at each farm were provided verbally and by email and summarised for all farms. Clinical signs described were:

1. mummified foetuses
2. stillborn piglets
3. shaky piglets
4. congenital abnormalities in piglets (anencephaly, limb deformities)
5. aspermia in boars.

Dates clinical signs were observed and dates sows farrowed were used to estimate the period of transmission for all farms. This was done by calculating the difference between the date abnormal litters were observed and the date a sow/gilt would have been in her second trimester. This was done for the entire period clinical signs were reported.

Serology

Veterinarians classified pigs bled for this study by cohort. Cohort names were standardised[4] across all farms for analysis (Table 2.1).

Table 2.1. Pig cohort identifiers and descriptions

Pig cohort	Progeny or breeding herd?	Description
4wk	Progeny	Pigs aged 4 weeks old. These are pigs that are newly weaned.
8wk	Progeny	Pigs aged 8 weeks old
12wk	Progeny	Pigs aged 12 weeks old.
16-18wk	Progeny	Pigs aged between 16-18 weeks old
20+wk	Progeny	Pigs aged 20 weeks or older. These are pigs approaching sale.
Unmated gilts	Breeding herd	Female pigs aged 16-18 weeks old that are selected for the breeding herd, but have not been serviced/mated yet.
First trimester	Breeding herd	Pigs that are 2 – 5 weeks pregnant
Second trimester	Breeding herd	Pigs that are 6 – 10 weeks pregnant
Third trimester	Breeding herd	Pigs that are 11 – 15 weeks pregnant
Boars	Breeding herd	Male pigs that are housed in boar studs and provide semen for artificial insemination of gilts and sows

Each pig that was bled was allocated a final serological result (seropositive, seronegative or indeterminate) based on the serological test results and discussions with laboratory scientists and pig veterinarians about the accuracy and interpretation of these tests. An overview of these tests is provided in Appendix 2E. Crude seroprevalence was calculated for each farm and each cohort. Standard error was also calculated for the crude seroprevalence of each farm and cohort.

[4] For some farms, information on shed locations or previous litters was included in raw cohort names

Retrospective survival analysis and long-term reproductive outcomes for sows

Seropositive sows and gilts were selected for the survival analysis from the population of pigs that had blood tested for antibodies to JEV in the serology investigation. Criteria for inclusion included:

- A serology result was available with a positive or negative result negative (not indeterminate)
- The serology result could be matched to production data for the animal, using a unique identification number
- The date of bleed for the serology was earlier than the recorded removal date for the sow/ gilt.

Seronegative sows and gilts were selected to match seropositive sows and gilts by the following criteria:

1. Parity at the time of JEV serology collection
2. Period of gestation the gilt/sow was at the time of JEV serology collection
3. Farm location at the time of JEV serology collection.

Selected seropositive sows that could not be matched with a seronegative sow on all three parameters were excluded.

A consistent dataset was assembled for each sow included in the study including the following variables:

- Date sow joined the herd
- Date sow was removed from the herd (the 17th of August 2023 was added as the end date for sows that were still in the breeding herd at the time of data extraction)
- Date sows had JEV serology collected
- Parity (provided for each pregnancy)
- Removal type
- Survival analysis matched ID
- Serology result

A “Bleed” variable was also created. This variable included:

1. A “bleed” flag to indicate the pregnancy that the sow/gilt was bled at
2. A “before” flag to indicate the pregnancy prior to the JEV bleed
3. An “after” flag to indicate the pregnancy following the JEV bleed.

Other pregnancies included in the extract were given a “Bleed” value of “N/A” for all sows and gilts.

Longevity of sow in the breeding herd

Length of time in the breeding herd (the number of days from arrival in herd to removal from herd or end of study) was calculated for all selected sows. Median lengths of time in the breeding herd were calculated for seropositive sows and seronegative sows. Length of time in the herd was also stratified by parity at time of JEV bleed.

The outcome measure for this analysis was the date a sow/gilt was culled or destroyed. Sows/gilts were censored if they died, were transferred, or were still present in the breeding herd when the study ended. Enrolment date was the date a sow joined the breeding herd as a gilt. Cox regression was used to analyse the difference between seropositive sows and gilts and matched seronegative sows and gilts. Log rank tests were used to test the difference between survival curves.

Effects on pregnancy

The following lengths were calculated for seropositive and seronegative sows included in the study:

1. Wean to next farrow: Length of time (in days) between when piglets were weaned and when a sow farrowed her next litter. This is a cumulation of the time taken for the sow to be in oestrus again, the time taken for a sow to become pregnant (which may require multiple services) and the length of gestation
2. Gestation length: Length of time (in days) between date of first service and date sow farrowed. This is a cumulation of the time taken for a sow to become pregnant (which may require multiple services) and the length of gestation (gestation length).

For both lengths, medians were calculated for seropositive sows and seronegative sows and significance determined using Wilcoxon rank tests. This was performed for the pregnancy that pigs had serology collected, and the pregnancies that preceded and followed this pregnancy. Lengths were stratified by parity, and the following exclusions were made:

1. JEV bleed: 6 gilts that were parity 0 at the time of JEV bleed were excluded for both gestation length and time between wean to next farrow as these pigs had not weaned a litter prior to JEV bleed
2. Before JEV bleed: 16 gilts that were parity 0 in the pregnancy before JEV bleed and so had not weaned a litter before this were excluded for time between wean to next farrow only

3. Before JEV bleed: 6 gilts that were parity 0 at the time of JEV bleed were excluded for both gestation length and time between wean to next farrow as these pigs had not joined the breeding herd prior to JEV bleed
4. After JEV bleed: 5 sows were excluded for both gestation length and time between wean to next farrow as these pigs did not have a pregnancy following JEV bleed. This includes sows that were transferred.

Ethics

Ethics was not required for this project as per the Australian National University Human Research Ethics Committee and the Australian National University Animal Research Ethics Committee (Appendix 2C).

Results

Descriptive analysis of farm environments, clinical signs, and serology

Overview of farms and farm environments

Japanese encephalitis virus was first confirmed in pig farms in Australia in February 2022, with some farms included in this study experiencing signs from as early as January 2022, and some experiencing signs until the end of June 2022 (Figure 2.2). Farms included in this study had varied herd size, shed type, local environment, transmission period and length of outbreak (Appendix 2F).

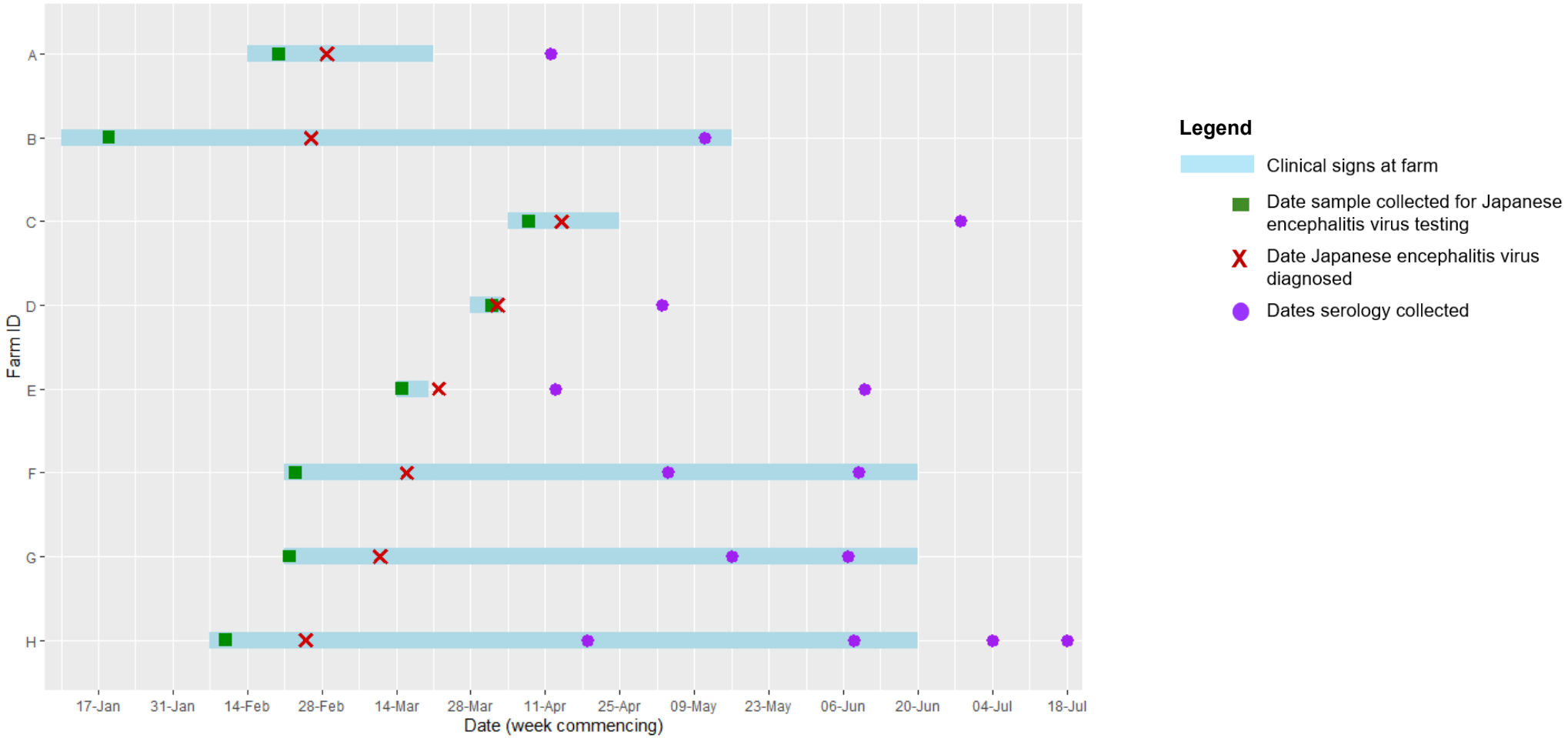

Figure 2.2. Timeline of Japanese encephalitis clinical signs and significant dates[5] at study farms, Australia 2022

[5] "Date sample collected for JEV testing" refers to the collection date of the sample/s that identified JEV was present on each farm. "Dates serology collected" refer to dates that serology was collected as part of this seroprevalence investigation.

Clinical outcomes

A total of 150,974 sows and 705,142 farrowing events across 8 farms were included in this study. Between January and June 2022, the proportion of mummified foetuses born per litter per week increased substantially for Farm A, Farm B, Farm F, Farm G and Farm H. For Farm C, Farm D and Farm E, the proportion of mummified foetuses did not markedly increase between January and June 2022. The proportion of mummified foetuses per week was lower than previous years for Farm C (Figure 2.3, Appendix 2G).

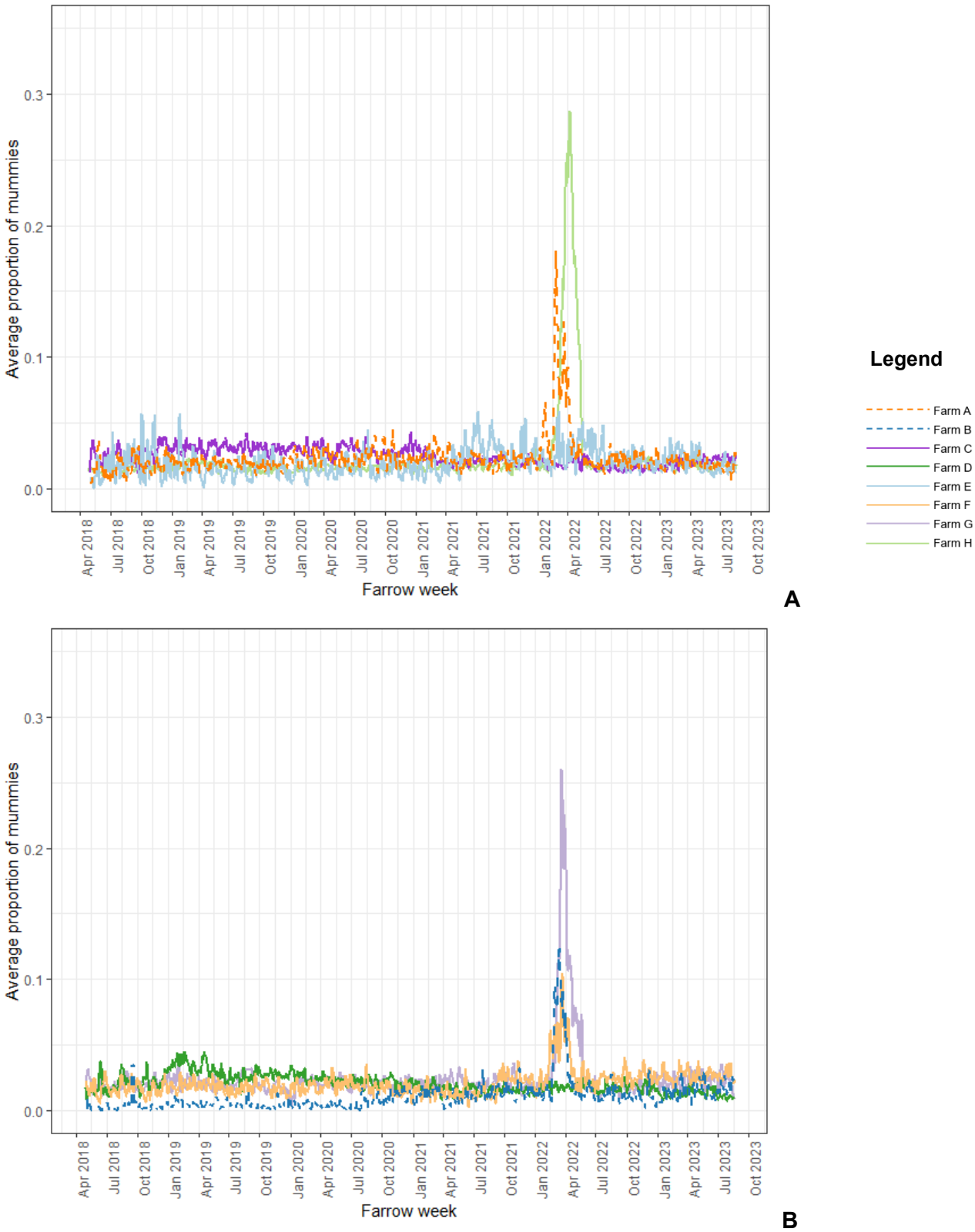

Figure 2.3. Average weekly proportion of mummified foetuses born per litter at all farms[6] by farrow week, 2018-2023

[6] Farms split into two groups of 4 for presentation purposes only. Pane A shows Farm A, Farm C, Farm E and Farm H. Pane B shows Farm B, Farm D, Farm F and Farm G.

Between October 2021 and June 2022, the proportion of stillborn piglets born per week increased slightly for Farm B, Farm G and Farm H. The proportion of stillborn piglets per week did not increase for Farm A, Farm C, Farm D, Farm E and Farm F during this time (Appendix 2G).

Serology

The number of samples collected at each farm and for each cohort varied (Table 2.2). A total of 1,637 samples were collected for this project, but 223 results were excluded from analysis due to missing data.

Table 2.2. Number of samples[a] collected for Japanese encephalitis virus testing for each pig cohort at each farm, 2022

		Pig cohort										Total sampled	Herd size (total pigs on-site)	Proportion of herd sampled
Farm ID		4wk	8wk	12wk	16-18wk	20+wk	Gilts – not mated	1st T* sows	2nd T* sows	3rd T* sows	Boars			
	A	.	.	.	.	.	.	21	23	85	.	129	10,000	0.129
	B	20	20	.	20	20	.	19	23	20	.	142	25,000	0.006
	C	20	20	22	20	20	.	21	20	21	.	164	20,000	0.008
	D	19	20	.	20	20	.	20	20	20	.	139	16,000	0.087
	E[b]	40	40	21	40	40	.	.	.	.	.	181	36,000	0.005
	F	20	20	20	20	20	.	19	20	19	.	158	45,000	0.004
	G	20	20	20	20	20	.	.	80	40	.	220	21,000	0.010
	H	.	.	.	57	87	90	.	.	.	47	281	103,000	0.003
Total		139	140	83	197	227	170	100	186	205	47	1414		

*T denotes trimester

a. Excludes 223 samples with unknown cohort or unknown farm ID

b. Farm E is two properties. Cohorts with 40 pigs had 20 pigs of that cohort bled at each of the different properties

Of the 1414 samples collected, 440 (31%) tested positive to JEV. Crude seroprevalence at each farm ranged from 0% (Farm C) to 70% (Farm G).

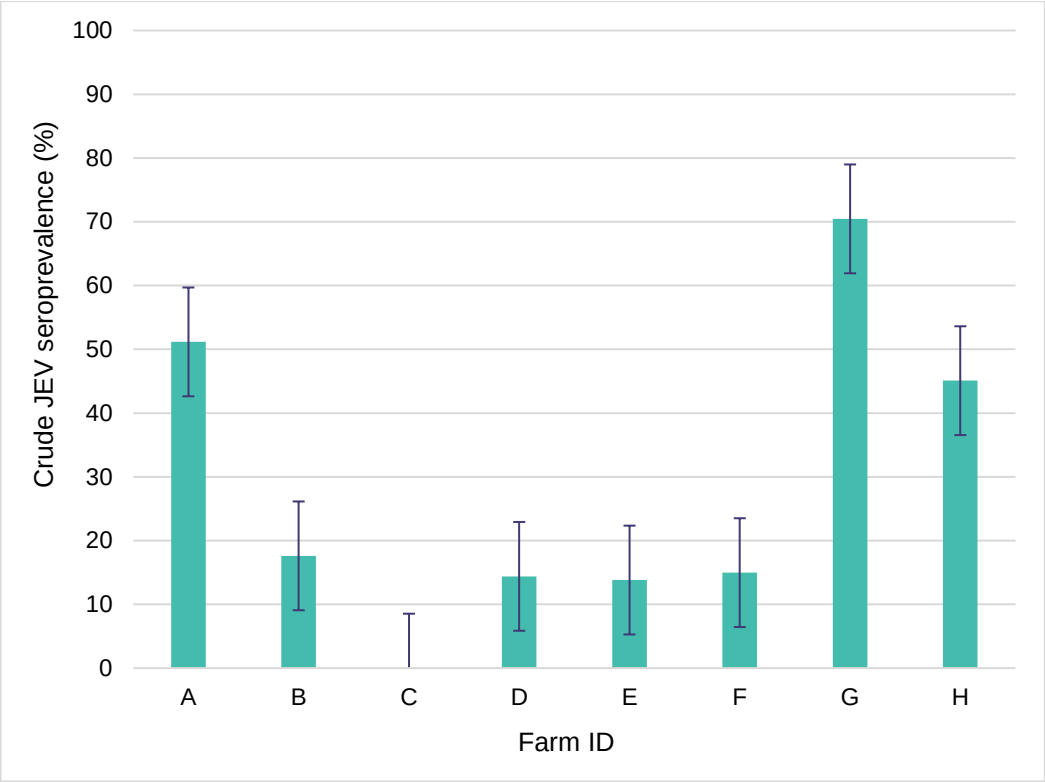

Figure 2.4. Crude Japanese encephalitis virus seroprevalence with standard error bars, by farm, Australia, 2022

Proportions of seropositive results varied by cohort. When pooled across all farms, third trimester sows had the highest seropositivity rate of all cohorts (50%) and 12-week-old pigs had the lowest (4%) (Figure 2.5, Appendix 2H). These cohorts accounted for 23% and less than 1% of all positive samples respectively.

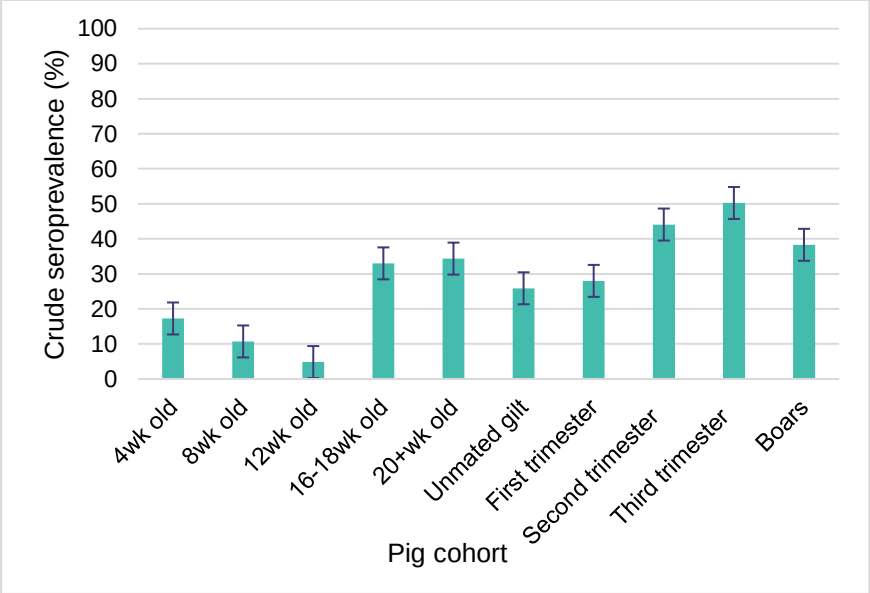

Figure 2.5. Proportion of results seropositive for Japanese encephalitis virus for each cohort across all farms with standard error bars, Australia, 2022

Retrospective survival analyses and long-term reproductive outcomes for sows

Demographics of selected pigs

Sixty-four sows, 32 seropositive for JEV and 32 seronegative for JEV were included in the survival analysis in the comparisons of sow reproductive outcomes (Table 2.3).

Table 2.3. Cumulative frequency of all sows included in analysis by parity at time of Japanese encephalitis virus testing

Parity at JEV bleed	Frequency (%)	Cumulative percentage
0	6 (9.4)	9.4
1	16 (25.0)	34.4
2	26 (40.1)	74.5
3	8 (12.5)	87
4	2 (3.1)	90.1
5	6 (9.4)	99.5
Total	**64 (100)**	**100**

Length in herd

Length of time in herd and end type varied for seropositive sows and seronegative sows. For 66% of pairs, the pig that tested negative to JEV survived longer in the herd than the pig that tested positive to JEV (Figure 2.6).

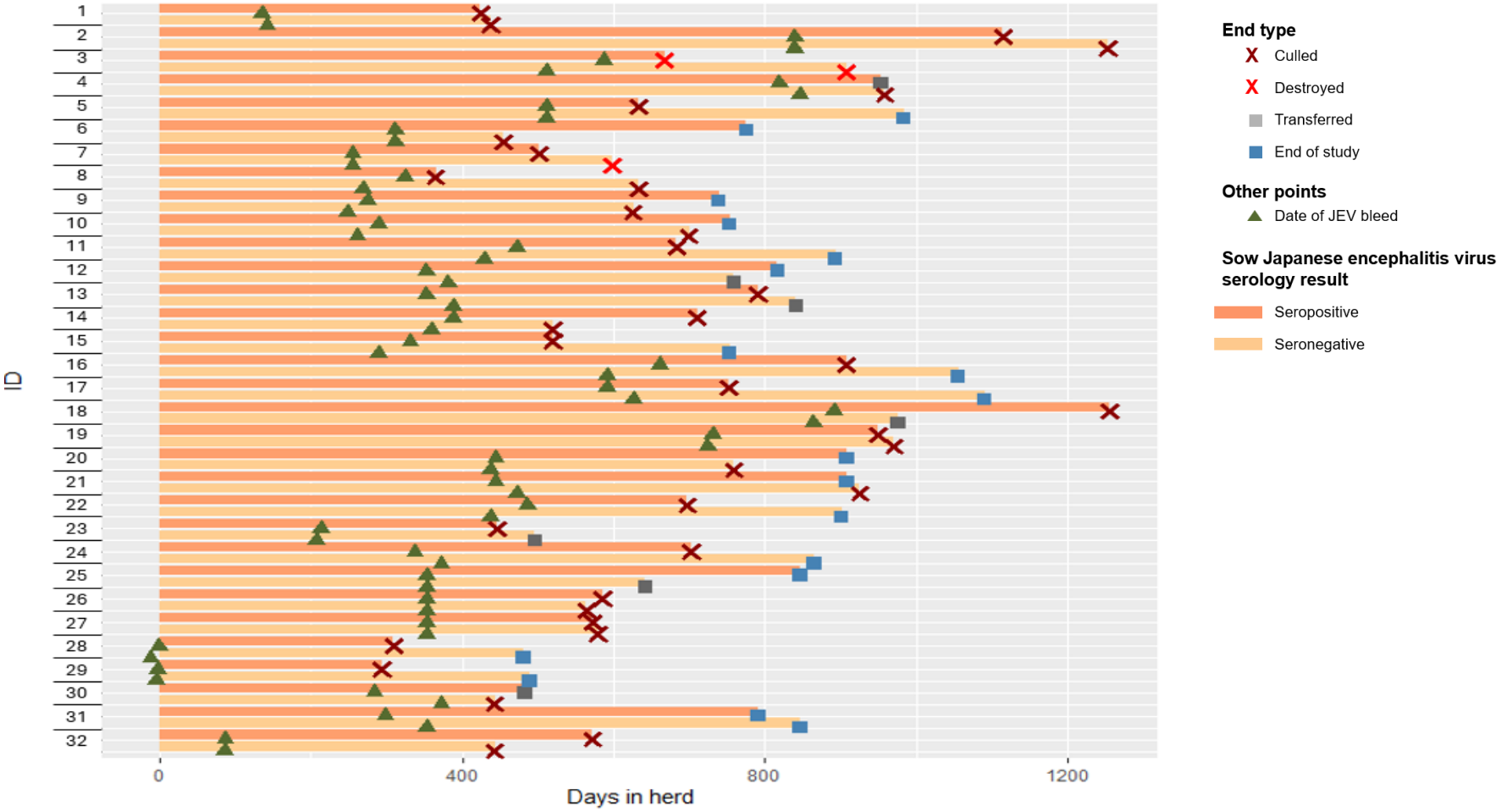

Figure 2.6. Length of time in breeding herd, end type, and date of collection for Japanese encephalitis virus testing for pigs included in survival analysis, by sow Japanese encephalitis virus serology result[7] and pair ID, Australia, 2022

[7] Seropositive sows are those first from the top, followed immediately by corresponding seronegative sow below

Overall, the median length of time in the herd was longer for seronegative sows and gilts compared to seropositive sows and gilts (756 days (range: 438-1253) and 706 days (range: 294-1255) respectively, $p = 0.46$). Stratifying by parity, sows that had higher parity at the time of bleed lasted longer in the breeding herd than sows that had lower parity at the time of JEV bleed. The greatest variation in length in breeding herd occurred for sows that were at parity 0 and parity 3 at the time of JEV bleed, with parity 0 seropositive gilts having a median length in the herd of 309 days (range: 294 – 572) and seronegative gilts having a median length of 481 days (range: 443 – 489, $p= 0.7$). For parity 3 sows, seropositive sows had a median length of time in the herd of 709 days (range: 673 – 907) and seronegative sows had a median length of 1019 days (range: 907 – 1090, $p <0.05$) (Figure 2.7).

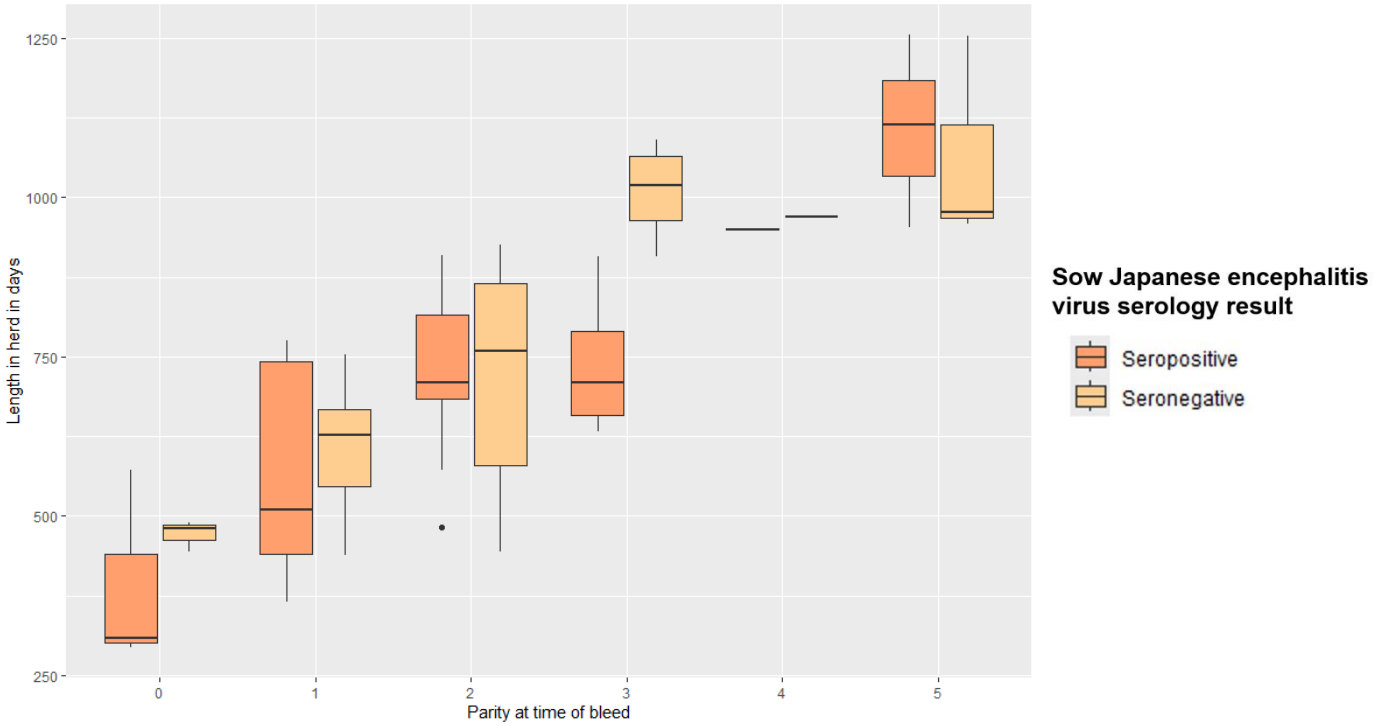

Figure 2.7. Length of time in breeding herd by parity at time of collection of bloods for Japanese encephalitis virus testing and serology result, Australia, 2022

At the end of the study, the survival probability was 0% for both seropositive and seronegative pigs. Fifty percent of the seropositive pigs were removed from the herd in less than two years, and 50% of the seronegative pigs were removed from the herd by two and a half years (Figure 2.8). Overall, the difference between survival curves was calculated at $X^2 = 1.03$ on 1 df, $p = 0.3$.

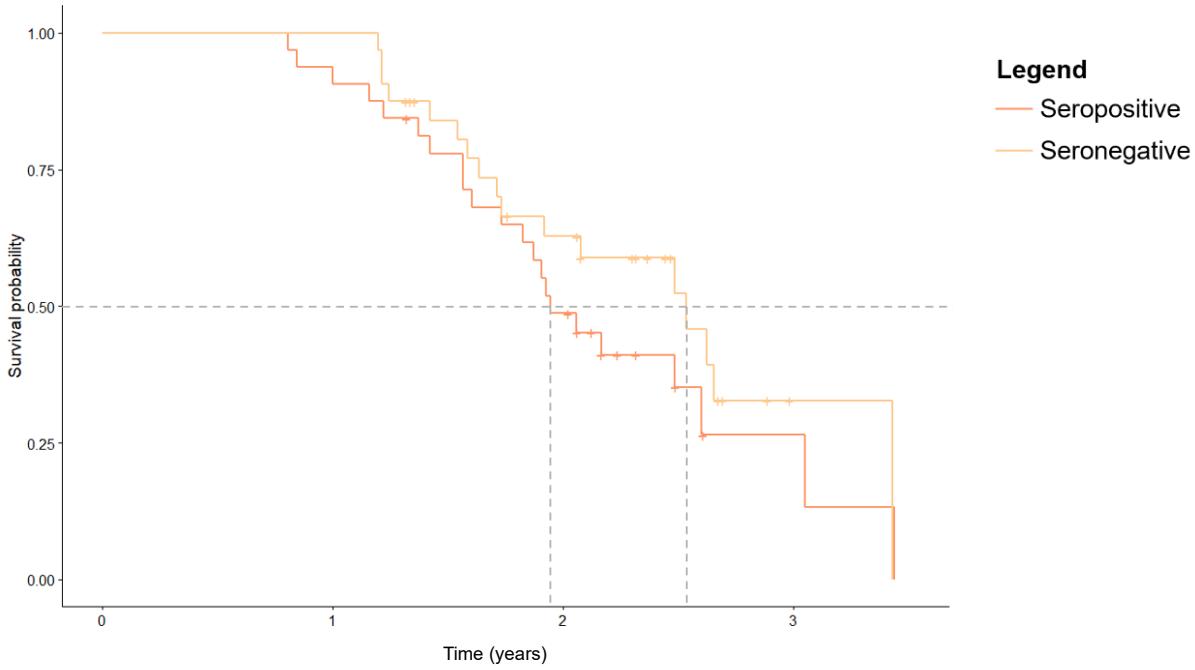

Figure 2.8. Survival curve of time in breeding herd for pigs by Japanese encephalitis virus result[8], Australia, 2022

Adjusting for parity, there was a significant difference in length in time in the herd between seropositive and seronegative pigs (X^2 = 33.39 on 6 df, p <0.05) (Figure 2.9). At the end of the study, seronegative pigs that were parity 0 at the time of JEV bleed had a 67% survival probability while their seropositive counterparts had 0% survival probability. Seronegative pigs that were parity 3 at the time of JEV bleed had a survival probability of 75%, compared to their seropositive counterparts that had 0% survival probability. Seropositive pigs that were parity 1 at the time of JEV bleed had a survival probability of 38%, compared to a survival probability of 15% in seronegative counterparts.

[8] Excludes 2 seropositive pigs parity 0 at the time of JEV bleed and 1 seropositive pig parity 1 at the time of JEV bleed

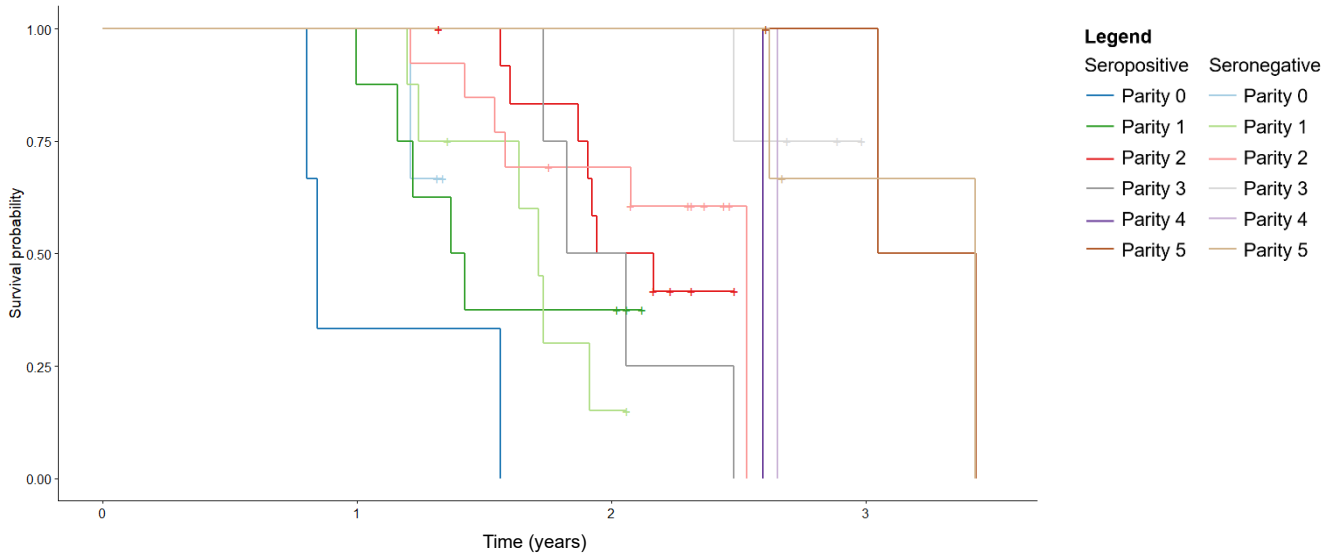

Figure 2.9. Survival curve of time in breeding herd for pigs by parity and Japanese encephalitis result[9], Australia, 2022

Pregnancy outcomes

Wean to next farrow

Overall, both seropositive sows and seronegative sows had a similar median length of time between last wean and next farrow date for the pregnancy that JEV testing occurred (120.5 and 120 days respectively, p = 0.95). The range of time between last wean and next farrow date was greater for seropositive sows (117-168 days) compared to seronegative sows (118-144 days). Median times were consistent between the pregnancy of JEV bleed and the pregnancy following JEV bleed for both groups. Stratifying by parity, seropositive pigs that were parity 1 at the time of JEV bleed had a higher median length of time between the previous wean date (weaning that occurred during the JEV outbreak) and next farrow date (first farrowing subsequent to JEV outbreak) (139 days, range: 121 – 168) compared to parity 1 seronegative pigs (122 days, range: 119 – 130, p= 0.12) (Figure 2.10).

[9] Excludes 2 seropositive pigs parity 0 at the time of JEV bleed and 1 seropositive pig parity 1 at the time of JEV bleed

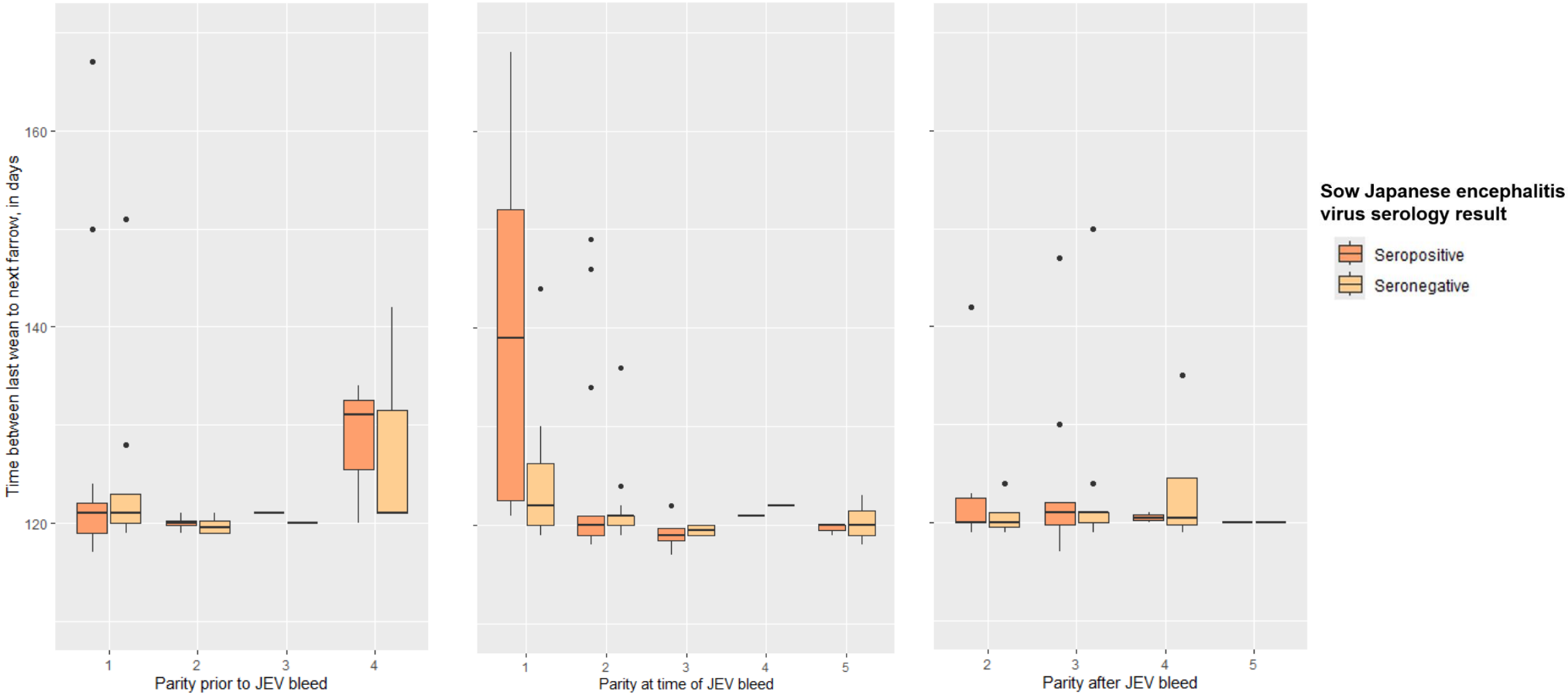

Figure 2.10. Time between last wean and next farrow date for pregnancy with Japanese encephalitis virus testing[10] and pregnancies before[11] and after[12] Japanese encephalitis virus testing, by result and parity, Australia 2022.

[10] Excludes 6 parity 0 pigs
[11] Excludes 16 pigs that were parity 0 at pregnancy before JEV bleed and 6 pigs not part of breeding herd prior to JEV bleed (gilts at time of JEV bleed)
[12] Excludes 5 pigs that did not have a pregnancy following JEV bleed

Gestation length

Overall, both seropositive sows and seronegative sows had a median time of 116 days between first service and farrow date for the pregnancy that JEV testing occurred. The range of gestation times was greater for seropositive pigs (113-164 days) compared to seronegative pigs (114-139 days). Median times were consistent between the pregnancy prior to JEV bleed and the pregnancy following JEV bleed for both groups. Stratifying by parity, seropositive pigs that were parity 1 at the time of JEV bleed had a higher median gestation length and larger range (119 days, range: 115 – 164) compared to their seronegative counterparts (116 days, range: 115 – 121, $p = 0.41$) (Figure 2.11).

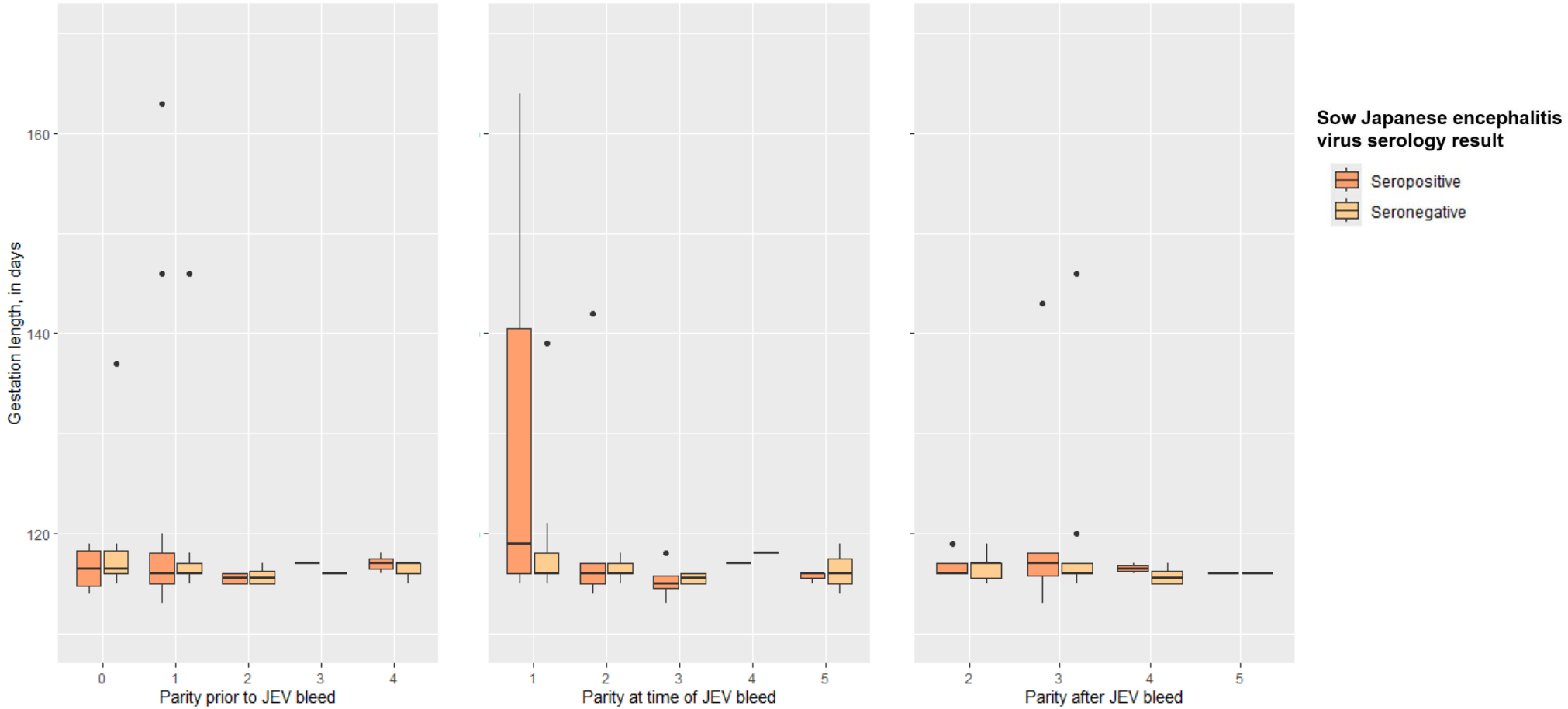

Figure 2.11. Gestation length for pregnancy with Japanese encephalitis virus testing[13] and pregnancies before[14] and after[15] Japanese encephalitis virus testing, by result and parity, Australia, 2022

[13] Excludes 6 parity 0 pigs
[14] Excludes 6 pigs not part of breeding herd prior to JEV bleed (gilts at time of JEV bleed)
[15] Excludes 5 pigs that did not have a pregnancy following JEV bleed

Discussion

The incursion of JEV into southern parts of Australia was an unprecedented event that presented unique challenges for pork producers, as well as for the animal health sector and the public health sector. There was a delay in identifying JEV as there is a time delay of several weeks to months between infection of the sow during gestation and birth of affected litters, and infected sows do not show evidence of infection prior to farrowing. The risk of incursions of JEV in sub-tropical and temperate regions of Australia was considered low, so the index of suspicion to prompt investigation for this disease was also low (18, 20). This was also observed in human investigations. The syndrome for JE can also be caused by other porcine diseases (35).

JEV manifested differently at each of the eight pig farms included in this study. Reported duration of clinical signs of JEV were observed varied from 1 to 20 weeks at farms (median 11.5 weeks), with no farms in the study having an outbreak lasting 50 days (7 weeks) as hypothesised by pig veterinarians. The four farms that experienced clinical signs for between 18-20 weeks (Farm B, Farm F, Farm G and Farm H) were all located in similar agricultural regions with the same jurisdictional surveillance reporting guidelines. These farms also experienced significant flooding prior to December 2021, however all farms in this study reported flooding around this time. There was no association between seroprevalence and length of time clinical signs were observed, size of farm, or distance from closest river, creek or lake, indicating that these factors are not useful predictors of the extent of transmission within a herd. There was not enough information to determine if shed type or shed location on farm was a risk factor for JEV seropositivity. However, Farm C, with a crude seroprevalence of 0%, experienced comparatively fewer clinical signs compared to other farms. Farm C was identified as an infected property in April 2022, and this was the only farm to have all sheds enclosed, which may have reduced the exposure and transmission period of JEV.

Transmission periods and likely time of first infection varied at each farm, and can be estimated by the developmental stage of piglets at the time of farrowing. The seropositivity of JEV in older progeny pigs can also be used to infer when transmission was occurring on the farm. It is important to note that the seropositivity calculated at these farms are point-in-time based on the time of sample collection, and time of sample collection varied for farms in this study. Farms that experienced initial transmission and infection between November and December 2021 (Farms A, B, F, G and H) had longer periods of clinically obvious disease and potentially longer periods of ongoing JEV transmission (via mosquitoes) than farms infected between mid-January and March 2022 (Farms C, D and E). Farms A, B, F, G and H also experienced a higher proportion of mummified foetuses within the herd for a longer period. Farm C, D and E

did not experience these large increases. The relationship between environmental factors and JEV outbreaks in Australian pig farms has recently been explored (29) and suggests that increased temporary surface water may increase risks for JEV outbreaks. It is possible that Farms A, B, F, G and H had sustained suitability for mosquito habitats such as temporary surface water or there was sufficient prevalence of viremia among pigs for continued transmission via mosquitoes that farms C, D and E did not experience. Further investigation is needed to determine the significance of temporary surface water on the increased risk of infection. Preventing mosquitos from biting pigs, including removing mosquito habitats, reducing accessibility of mosquitoes to pigs and applying integrated mosquito controls could help to reduce effects of JEV on herds, and this was recommended at the time of infection at farms (36). Surveillance programs for JEV at pig farms may also help to identify the presence of JEV early, prior to observations of clinical signs. These programs could target progeny pigs aged over 16 weeks of age, including unmated gilts and boars selected for breeding. Surveillance of environmental water sources, ardeid and other birds and mosquitoes could also assist with early detection of JEV transmission risks in an area and allow pig farms and the community to implement mitigation strategies to avoid infection of humans and pigs, including enhanced surveillance breeding herds prior to clinical signs in pigs.

Industry veterinarians hypothesised that the seroprevalence of JEV varied at infected farms, and this study provided evidence in support of this hypothesis. Previous studies indicate that maternal antibodies wane as a pig ages, lasting until about 12-16 weeks of age (30). Industry veterinarians wanted to determine if this held true in the Australian outbreak, and hypothesised that maternal antibodies to JEV are carried until at least 8 weeks of age. Seropositivity in 4-week-old and 8-week-old pigs could indicate detection of maternal antibodies or primary infection. For older cohorts, seroconversion following infection is more likely. As the samples were collected several months after JEV was first confirmed on farms, the significance of the results in cohorts must be interpreted with caution. More specific antibody testing on samples collected 4 weeks apart or serial bleeds over the transmission season would be necessary to demonstrate an increase or decrease in antibody titres, to allow a more accurate estimation of when pigs were infected and if seropositivity was a result of maternal antibodies or natural infection.

In endemic countries with year-round transmission, pigs are usually infected with JEV within one year of birth as by this age they have had sufficient time to be exposed to mosquitoes carrying JEV (36). Older pigs are therefore more likely to be seropositive for JEV. In this study, progeny pigs aged 20 weeks and older as well as sows in their second or third trimester accounted for 60% of positive results across all farms. This could be a result of sampling bias, as 44% of all collected samples were from one of these cohorts.

The JEV serology result of a sow cannot be used to definitively determine what her future reproductive performance may be. Survival analysis demonstrated that overall, seronegative sows had a higher probability of remaining in the herd for longer compared to seropositive sows. The overall difference in survival curves was not significant between these groups. Matching and validating production data and serological results for individual sows was a challenging and largely manual process that limited the number of sows that could be included in the analysis, resulting in a small sample size and probably limited statistical power. This meant that while some pigs in the survival analysis survived beyond the study period, the probability of survival was calculated as 0% overall. Prior to the last event recorded before the end of the study period, seropositive pigs had a survival rate of 13% while seronegative pigs had a survival rate of 33%. This suggests sows that were infected with JEV were culled from the breeding herd earlier than they otherwise might have been. This could indicate that infection with JEV had a negative impact on reproductive performance that contributed to their removal. This is an important economic impact of outbreaks on farms, although less obvious that the impacts of piglet losses.

When stratified by parity, the difference in survival curves was significant between seropositive and seronegative sows, especially those at parity 0, 1, and 3 at the time of JEV testing. It is not clear why these parities showed significant differences, and it must be noted that both parity 0 and parity 3 pigs had small sample sizes (6 and 8 respectively). Parity 1 pigs had one of the largest sample sizes in the analyses (16 pigs). Generally, pigs at parity 0 have lower reproductive performance (such as number of pigs born alive, number of weaned piglets and number of returns to service) compared to sows at parity 2, 3, and 4 (37). It is possible that infection with JEV could have further decreased the reproductive performance in pigs infected before parity 1, resulting in their removal from the herd. This does not explain why seronegative parity 3 pigs had a higher probability of survival in the herd compared to their seropositive counterparts, or why seropositive parity 1 pigs had a higher probability of survival in the herd compared to their seronegative counterparts. All pigs at parity 5 were removed from the herd before the end of the study, though this is not surprising as these pigs were older than other pigs in the study, and age is a factor in decisions for removal of pigs from the breeding herd (37). It is also important to note that sows are routinely turned over at a rate of 50-60% annually. The overall decreased longevity in the herd for seropositive sows compared to seronegative sows indicates JEV could result in premature removal from the herd for this cohort, but a larger sample size, especially for pigs bled at parity 0 and parity 3, is needed to confirm this.

Seropositive pigs that were Parity 1 at the time of JEV bleed had longer gestation lengths and time between wean to next farrow compared to their seronegative matched pairs, though these results were not statistically significant. Longer gestation times and times between wean to

next farrow may indicate a pig required repeated servicing to become pregnant, or the pig experienced early pregnancy loss. Prolonged time between last wean and next farrow could also indicate that oestrus was delayed, or that or that sows were not serviced at the first oestrus following weaning. These seropositive parity 1 pigs may have been infected with JEV as gilts, and the reproductive performance of gilts is influenced by many factors. However, the comparatively normal gestation lengths and times between weaning and farrowing the next litter in the seronegative parity 1 pigs suggests that infection with JEV in gilts may cause prolonged reproductive issues not seen in pigs that are parity 1 or higher at the time of infection with JEV. This phenomenon did not continue into the following pregnancy for this cohort and due to the small sample size requires further investigation at all parities. Pigs that were bled at other parities did not show a significant difference in mean gestation length, time between last wean and next farrow, or longevity in the herd regardless of seropositivity. However, pigs that tested positive to JEV had higher incidence of positive outliers in all these categories which could have significant economic impacts for pork producers. These outliers are also more likely to be culled for poor reproductive performance, reducing the survival of seropositive animals in the herd.

This project was limited to the use of production data which is collected primarily for monitoring pig performance rather than for specific monitoring of health conditions. The relevant clinical signs of JEV recorded in this data were limited to numbers of mummified foetuses and stillbirths, and information such as number of clinically affected (e.g. shaky or deformed) piglets of those born alive was not available. The data provided differed between producers which complicated analysis, however all data indicated if a sow had been serviced more than once and if her most recent service had failed. Not all data provided further information on services, or if a sow experienced abortions or was found open or not in pig[16] so these could not be explored. These outcomes could therefore not be analysed or compared. Additionally, while this data included if a sow was culled, the reason she was culled was not included. Sows can be culled for several reasons in addition to reproductive performance, such as illness, injury or age. As this information was not available it could not be determined why sows were removed at any given time. Another limitation of this study was that selection of farms and pigs, which occurred prior to my involvement, was not random. The project was also limited by the use of different testing methods at different laboratories and the tendency of flaviviruses to cross-react. These factors may have affected the findings from this study and results should be interpreted with caution.

[16] While not provided in the specific extract used in this project, this information is collected and recorded by all farms. Other extracts were deemed more suitable for this project.

In humans, flaviviruses such as JEV can have a wide range of effects including encephalitis, long-term sequalae and death, however 99% of people infected with the virus show no clinical signs (29). There are limited studies examining the varied clinical effects of JEV on pigs and this could be an area of future research to assist with predicting the effects of JEV on a herd.

Conclusion

Over 80 Australian pig farms were confirmed to be infected with JEV in early 2022. This project descriptively assessed a small subset of these farms and demonstrated that the way JEV manifests in a herd is variable, seroprevalence varied across farms, some sows evaded infection with the virus, and infection in gilts may be associated with ongoing impacts on fertility. Effects of infection with JEV are predominately observed in litters, but this project showed that seropositive sows were more likely to be removed from the breeding herd earlier than seronegative sows at the same parity. Japanese encephalitis virus can have significant economic effects on the pork industry, and due to the unpredictability of clinical signs, the delay between infection in sows and observation of clinical signs in piglets, mosquito surveillance and control remains a key strategy to mitigate transmission and the effects of JEV in pigs. Future studies should include a larger sample size of farms to further improve the understanding of JEV.

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Appendix 2A: Surveillance for Japanese encephalitis virus on infected premises – Questions proposed by pork industry veterinarians (March 2022)

Table 2A.1 Hypothesis and methods proposed by pork industry representatives for Japanese encephalitis virus surveillance activities, March 2022

	Hypothesis	Method
1	The duration of the farm epidemic as indicated by clinical signs lasts for only 50 days, until all the infected sows farrow or the mosquito season is over.	On farm data analysis x 10 IPs
2	The prevalence of infection varies with each infected farm.	Sero-sampling sows in different pregnancy trimesters on 10 IPs
3	Some sows escape infection during pregnancy	Sero-sampling sows in different pregnancy trimesters on 10 IPs
4	The prevalence of infection in finishing pigs varies with region.	Sero-sampling of pigs at slaughter from different farms at different latitudes or geographic locations in affected states
5	Growing pigs carry maternal antibodies until at least 8 weeks of age	Sero-sample pigs at 4 and 8 weeks of age
6	Growing pigs are seronegative by 16 weeks of age	Sero-sample pigs at 16 weeks of age

Pork industry veterinarians prepared 15 questions for consideration. 12 of these related to “Phase 1” of the surveillance activities. Of these, 6 are addressed by this chapter.

Note: Discussions for this project began in 2022, including the involvement of a MAE scholar from the 2022-2023 cohort.

Appendix 2B Literature review search terms and results

A literature review was conducted on Scopus, Web of Science and PubMed to 31 July 2024 to identify previous studies investigating risk factors associated with seroprevalence of JEV in pigs.

Search terms

Table 2B.1. Logic grid of search terms for literature review to identify previous studies investigating risk factors associated with seroprevalence of Japanese encephalitis virus in pigs

Disease	Animal	Outcome	Risk
“Japanese Encephalitis”	"Pig" OR "Pork" OR "Sow" OR "swine" OR "Sus domesticus" OR "piglet" OR "gilt"	"Serology" OR "Prevalence " OR "Seroprevalence"	“Risk”

“Risk” was included as a broad search term due to the limited number of published studies available. Articles were limited to those published in English with full-text available. A total of 101 articles were identified. After excluding duplicates, 63 articles were screened by title and abstract, and 17 full-text articles were reviewed (Figure 2C.1). Twelve articles met the criteria for inclusion as per the below criteria.

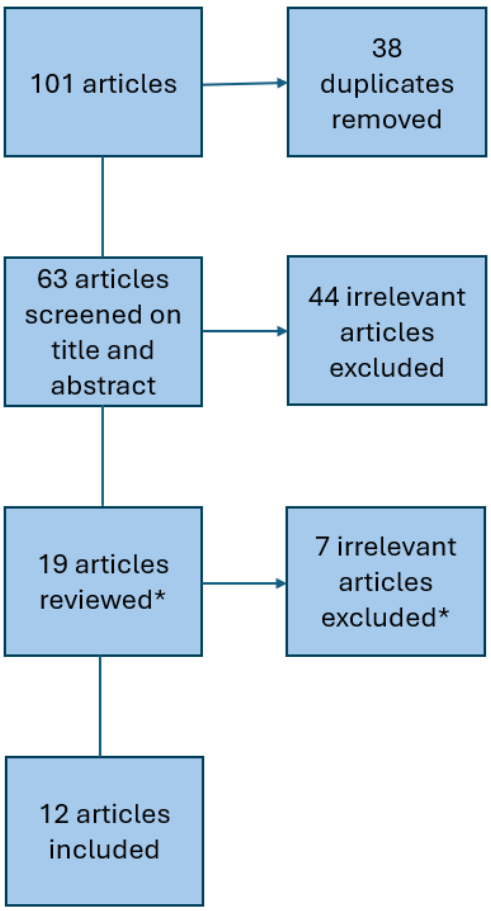
Inclusion criteria:

- Japanese Encephalitis
- Pigs
- Serological testing
- Risk factors associated with seropositivity

Exclusion criteria:

- Human studies
- Studies in other animals
- Studies about other diseases
- Studies describing seroprevalence only (no mention of risk factors)
- Vaccine development

- Diagnostic test development



*Full text was not available for 2 articles

Figure 2B.1. Logic tree for literature review to identify previous studies investigating risk factors associated with seroprevalence of Japanese encephalitis virus in pigs

Results

Most of the 12 studies included in the literature review were set in Asia, including 3 (25%) in India. Articles were published between 2012 and 2023, with 4 (33%) published in 2022. Results were varied among the different studies but age group (n = 6), different provinces/districts (n = 5), stagnant water (including wells or open water tanks) or water-logged areas (n = 3), season (n = 2), farm size (n = 2) and pig breed (n = 2) were found to be significantly associated with seropositivity. One study found that decreased elevation was associated with increased seroprevalence in pigs, and one study found 100% seroprevalence across sampled pigs and so risk factors could not be analysed. Proximity to mosquito breeding habitats such as rice paddies was found to be associated with seropositivity in one study, however this study provided only descriptive results. There were discrepancies regarding which age group had

the highest risk of seropositivity, but most studies identified older pigs as having the greatest risk.

Studies used different methods to test seropositivity of swine. This included use of tests that were not verified against gold standard tests, and cross reactivity of JEV with other flaviviruses was also mentioned.

Articles

Reference No.	Reference
1.	Adeleke, R., et al. (2023). "Serological Investigation of Japanese Encephalitis Virus Infection in Commercially Reared Pigs, Southwestern Nigeria." Vet Ital 59(4).
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8.	Kumar S.S. et al. (2023). "Sero-molecular epidemiology of Japanese encephalitis virus in swine population of western Uttar Pradesh, India: Unraveling the geographical expansion of the virus." Journal of Vector Borne Diseases 60(3): 7.

9.	Pham-Thanh, L., et al. (2022). "Zoonotic Flavivirus Exposure in Peri-Urban and Suburban Pig-Keeping in Hanoi, Vietnam, and the Knowledge and Preventive Practices of Pig Farmers." Trop Med Infect Dis 7(5).
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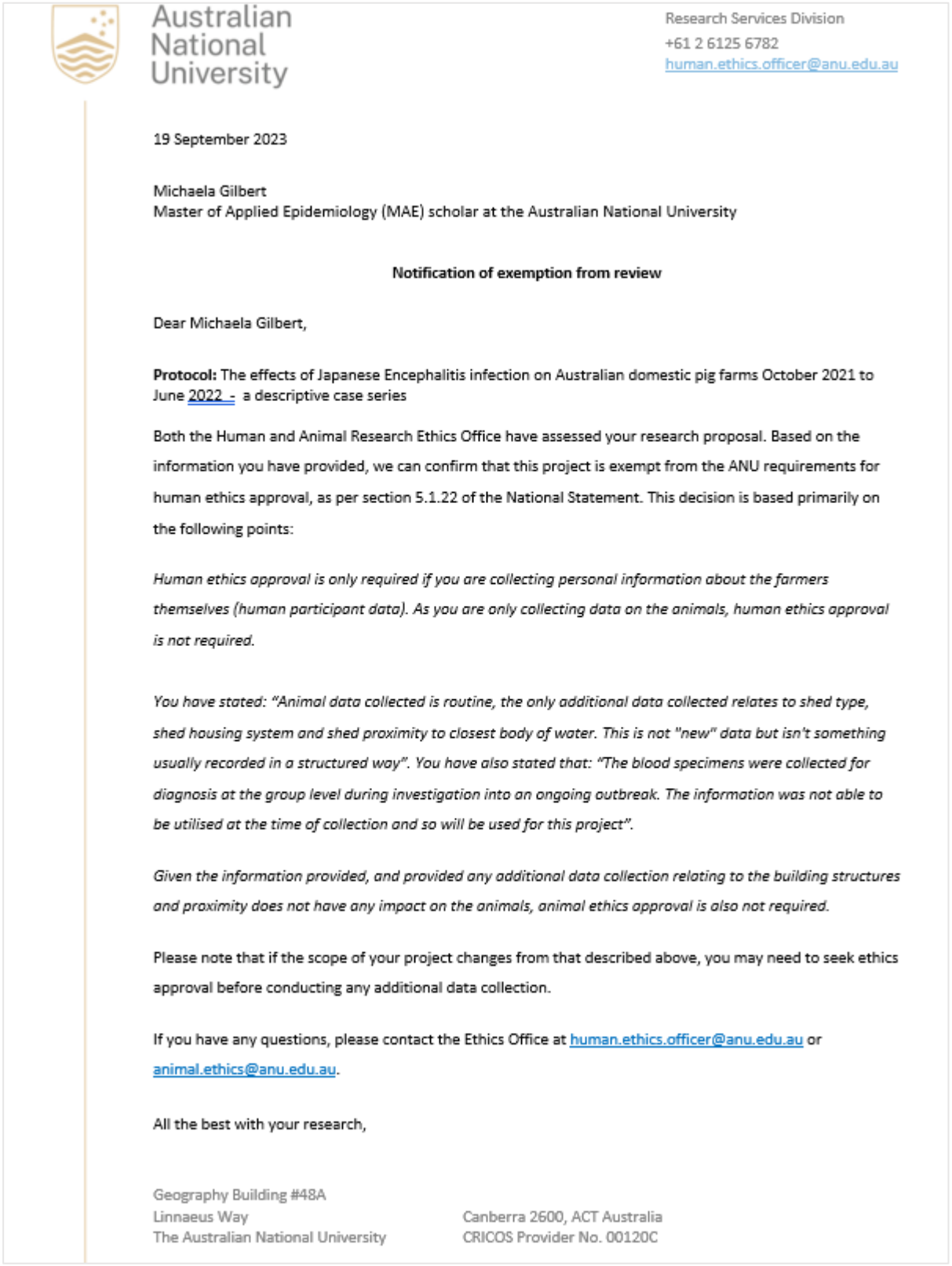
Appendix 2C: Ethics exemptions



Australian National University

Research Services Division
+61 2 6125 6782
human.ethics.officer@anu.edu.au

19 September 2023

Michaela Gilbert
Master of Applied Epidemiology (MAE) scholar at the Australian National University

Notification of exemption from review

Dear Michaela Gilbert,

Protocol: The effects of Japanese Encephalitis infection on Australian domestic pig farms October 2021 to June 2022 - a descriptive case series

Both the Human and Animal Research Ethics Office have assessed your research proposal. Based on the information you have provided, we can confirm that this project is exempt from the ANU requirements for human ethics approval, as per section 5.1.22 of the National Statement. This decision is based primarily on the following points:

Human ethics approval is only required if you are collecting personal information about the farmers themselves (human participant data). As you are only collecting data on the animals, human ethics approval is not required.

You have stated: "Animal data collected is routine, the only additional data collected relates to shed type, shed housing system and shed proximity to closest body of water. This is not "new" data but isn't something usually recorded in a structured way". You have also stated that: "The blood specimens were collected for diagnosis at the group level during investigation into an ongoing outbreak. The information was not able to be utilised at the time of collection and so will be used for this project".

Given the information provided, and provided any additional data collection relating to the building structures and proximity does not have any impact on the animals, animal ethics approval is also not required.

Please note that if the scope of your project changes from that described above, you may need to seek ethics approval before conducting any additional data collection.

If you have any questions, please contact the Ethics Office at human.ethics.officer@anu.edu.au or animal.ethics@anu.edu.au.

All the best with your research,

Geography Building #48A
Linnaeus Way
The Australian National University

Canberra 2600, ACT Australia
CRICOS Provider No. 00120C

Figure 2C.1 Original letter of exemption dated 13 September 2023

Human Ethics Office - Approval Letter
30/Oct/2024

Ms Michaela Gilbert
Md Rezanur Rahaman (Supervisor) Team

I am writing to advise you that your application for Exemption from Human Research Ethics Review has been approved and you may now commence your project as outlined in your application.

Project #	H/2024/1188
Project Title	The effects of Japanese encephalitis virus infection on Australian domestic pig farms in Queensland, New South Wales and South Australia, January 2018 to August 2023
Application Version	V1.0
Approval Date	30/Oct/2024
Expiry Date	N/A
Responsible Person / Principal Investigator	Michaela Gilbert
Approving committee	Human Ethics Office
Link	https://rems.anu.edu.au/projects/form/viewform?categoryID=10024&projectFormID=8124&projectID=5170

Dear Researcher,

I am pleased to advise that the HREC Chair has approved your Human Exemption application.

We wish you every success in your project.

Kind regards

Human Ethics Office

Yours sincerely,

Human Ethics Officer
Research and Innovation Services
+61 2 6125 6782
human.ethics.officer@anu.edu.au

Figure 2C.2. Amended letter of exemption (after title change) dated 30 October 2024

Appendix 2D: Overview of pork production

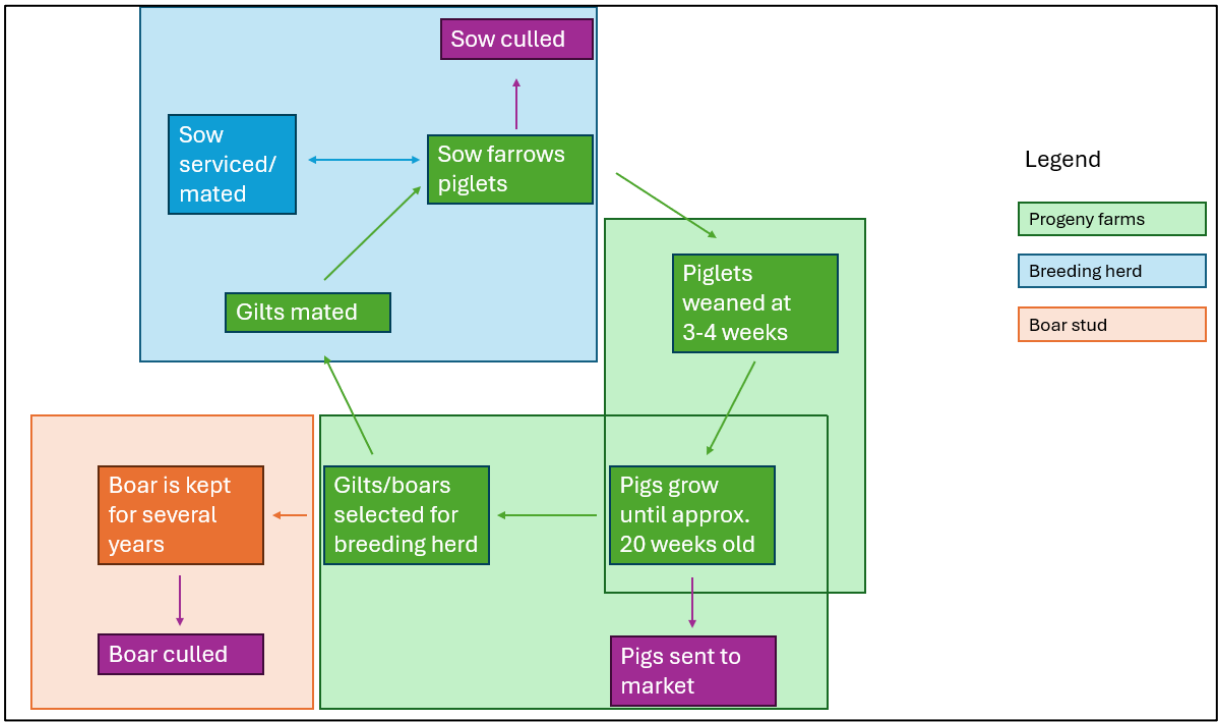


Figure 2D.1. Overview of potential pig movements through breeding herds and progeny pigs *Prepared by Michaela Gilbert 2024*

Farms may include a breeding herd only, progeny pigs only, or both a breeding herd and progeny pigs. Some farms also include a boar stud, although the majority of boar studs are located discretely in different locations to breeding farms. For farms that include only a breeding herd or progeny pigs, there are established networks of pig movements to other sites where pigs progress through different production stages. For example, a defined farm will select gilts from progeny pigs to send to a breeding farm, or piglets from a breeding farm will be moved to a specific progeny site for growing out.

Appendix 2E: Farm selection and data collection methods

Planning for this project began in 2022 and included collection of serology from 10 infected properties across NSW, Vic, SA and Qld. Only 8 properties were included in this analysis and none of these were from Victoria. Efforts were made to contact the additional farms, but this was unsuccessful and so these farms were excluded from the project. Pork industry representatives purposively selected a range of infected premises giving consideration to farm location and the severity of impacts observed, as well as logistics of sampling (such as staff availability). Selection aimed to capture a range of locations and observed levels of impact from infection with JEV. Level of impact was determined by the increase of reproductive abnormalities experienced on each farm, and the length of time these abnormalities lasted.

Sample sizes were calculated using Epitools. Between April and June 2022, whole blood was collected from pigs by veterinarians and allowed to clot at room temperature for 30 minutes and refrigerated. Blood samples were submitted to animal health laboratories based on farm location (Table 2E.1).

Table 2E.1. Summary of laboratories used by each state for Japanese encephalitis virus testing in commercial pigs, Australia, 2022

State	Number of farms	Laboratory samples tested at
NSW	4	Elizabeth McArthur Agricultural Institute (EMAI)
Qld	3	Biosecurity Services Laboratory (BSL) and Australian Centre for Disease Preparedness (ACDP)
SA	1	ACDP

Veterinarians aimed to collect blood samples from 20 pigs per cohort at each farm. Veterinarians practised random selection of pigs for bleeding where possible.

All samples were tested for total antibodies to JEV. Tests varied at each of the three laboratories (Table 2E.2) Laboratory representatives advised results were comparable across all 3 laboratories.

Table 2.E2. Laboratory tests performed at each laboratory for Japanese encephalitis virus testing in commercial pigs, Australia, 2022

Laboratory	Tests
EMAI	EMAI JE Enzyme-Linked Immunosorbent Assay (ELISA) with resolution
BSL	Cross-reactive Flavivirus ELISA; positives were forwarded to ACDP for further testing
ACDP	JEV competitive ELISA (c-ELISA) Kunjin virus blocking ELISA Murray Valley Encephalitis Virus (MVEV) ELISA

Appendix 2F. Overview of farms

Table 2F.1. Overview of farms and the Japanese encephalitis virus outbreak at each farm, Australia, 2022

Farm ID	Date JEV confirmed	First clinical signs[a]	Last clinical signs[a]	Estimated transmission period[b]	Length of clinical signs	Overview of clinical signs as reported by her veterinarian	Number of pigs on farm[c]	Shed types	Farm context	Distance to closest permanent water[d] (km)
A	1 March 2022	14 February 2022	21 March 2022	Earliest transmission: From November and December 2021[e] Latest transmission: until at least January 2022	5 weeks	Increase in mummified foetuses ("mummy storm")	10,000	• Enclosed tunnel vent • Open-sided	Farm experienced flooding week commencing 29 November 2021. Breeding herd only – replacement gilts sourced from Farm E.	2.8
B	26 February 2022	10 January 2022	16 May 2022	Earliest transmission: From November and December 2021 Latest transmission: until at least March 2022	20 weeks	Shaky piglets and mummified foetuses	25,000	• Enclosed tunnel vent • Open-sided	Farm situated in cropping area. Has pigs of all production stages. Includes boar stud.	0.81
C	14 April 2022	4 April 2022	25 April 2022	Earliest transmission: Late February/ early March Latest transmission: Not continued[f]	3 weeks	Very few affected litters	20,000	• Enclosed tunnel vent	Has pigs of all production stages that move across 3 different areas	2.65

D	2 April 2022	28 March 2022	28 March 2022	Earliest transmission: Late January 2022 Latest transmission: Not continued[f]	1 week	Piglet deformities in very few litters	16,000	• Open-sided	Farm situated in cropping area. Has pigs of all production stages	4.12
E	22 March 2022	14 March 2022	14 March 2022	Earliest Transmission: Early January 2022 Latest transmission: Not continued[f]	1 week	Very few affected litters, record gilt piglets born live in March 2022	36,000	• Enclosed tunnel vent • Open-sided	Farm is two separate properties, both experienced flooding week commencing 29 November 2021. Farm situated in area with irrigation and earth works. One property has progeny pigs and gilts only, the other has progeny pigs only.	1.98
F	16 March 2022	21 February 2022	20 June 2022	Earliest transmission: December 2021 to January 2022 Latest transmission: until at least April 2022	18 weeks	Increase in stillborn piglets and deformed litters February to mid-March. Increase in mummified foetuses mid-March to mid-June	45,000	• Open-sided	Has pigs of all production stages. Gilts located on separate property to other pigs on farm.	2.13
G	11 March 2022	21 February 2022	20 June 2022	Earliest transmission: December 2021 to January 2022 Latest transmission: until at least April 2022	18 weeks	Shaky piglets in February. Increase in stillborn piglets and deformed litters February to mid-March. Increase in	21,000	• Open-sided	Has pigs of all production stages.	0.73

						mummified foetuses mid-March to mid-June				
H	21 February 2022	07 February 2022	20 June 2022	Earliest transmission: December 2021 to January 2022 Latest transmission: until at least April 2022	20 weeks	Shaky piglets and 16% aspermia in boars in February. 50% aspermia in boars and increase in stillborn piglets and deformed litters February to mid-March. Increase in mummified foetuses mid-March to mid-June and deformed litters until mid-March	103,000	• Open-sided	Several co-located areas with frequent movement of pigs between each area. Has pigs of all production stages. Includes boar stud.	3.77

a. Week commencing

b. Transmission period estimate based on date clinical signs first observed/reported and date clinical signs last observed/reported (Figure 2.2).

c. Approximate number of all pigs on farm, including estimate of piglets prior to weaning

d. Excludes effluent and freshwater dams and temporary water sources. Includes seasonal creeks.

e. Note farm reported onset of clinical signs in February 2022 however farm experienced an increase in the proportion of mummies born per week per litter in January 2022.

f. Latest transmission “Not continued” indicates farm likely did not have significant ongoing transmission beyond initial infection of farm.

Appendix 2G: Proportion of stillborn piglets born per week

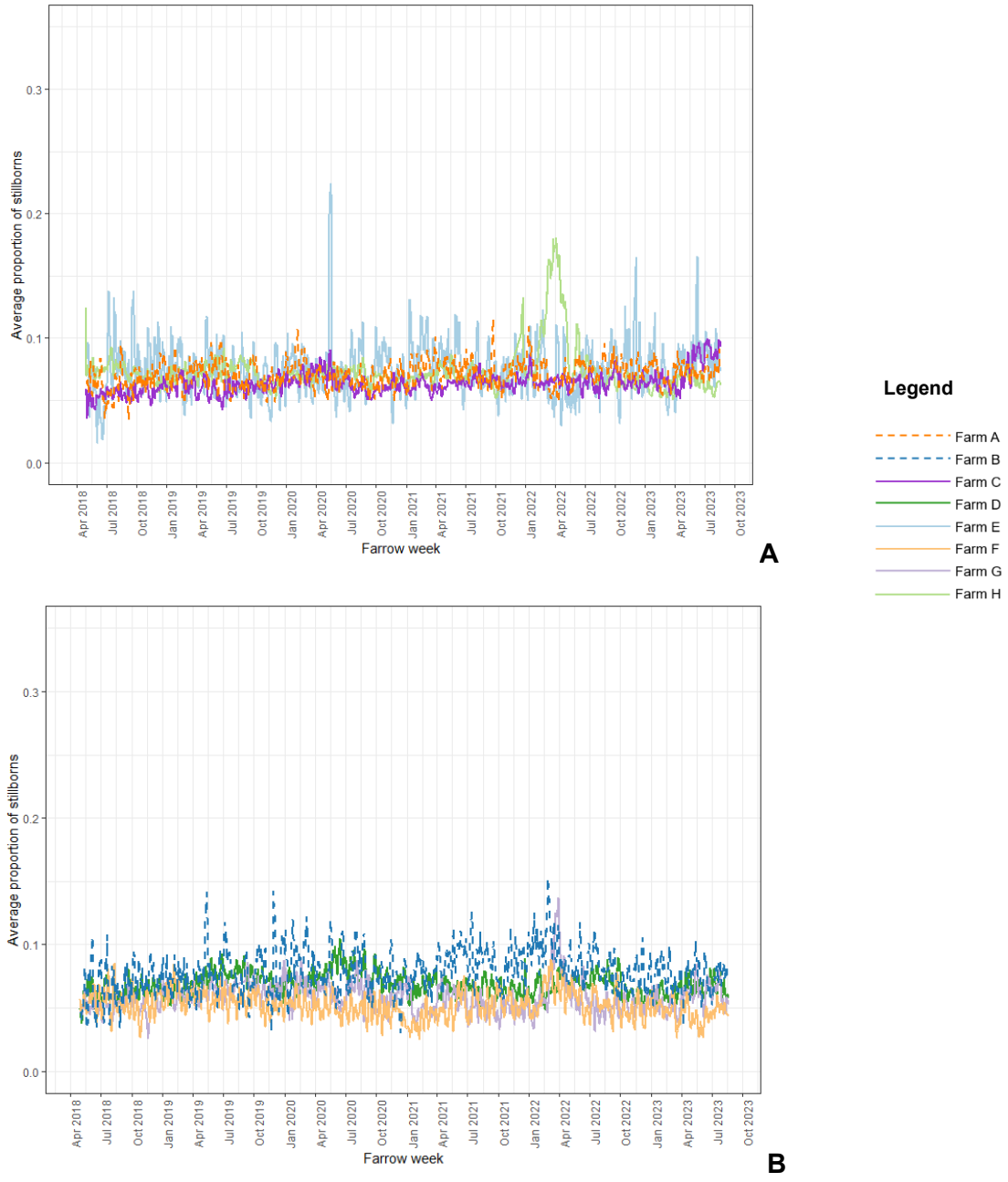


Figure 2G.1. Average weekly proportion of stillborn piglets born per litter at all farms by farrow week, 2018-2023[17]

[17] Farms split into two groups of 4 for presentation purposes only. Pane A shows Farm A, Farm C, Farm E and Farm H. Pane B shows Farm B, Farm D, Farm F and Farm G.

Appendix 2H: Supplementary figures – seroprevalence

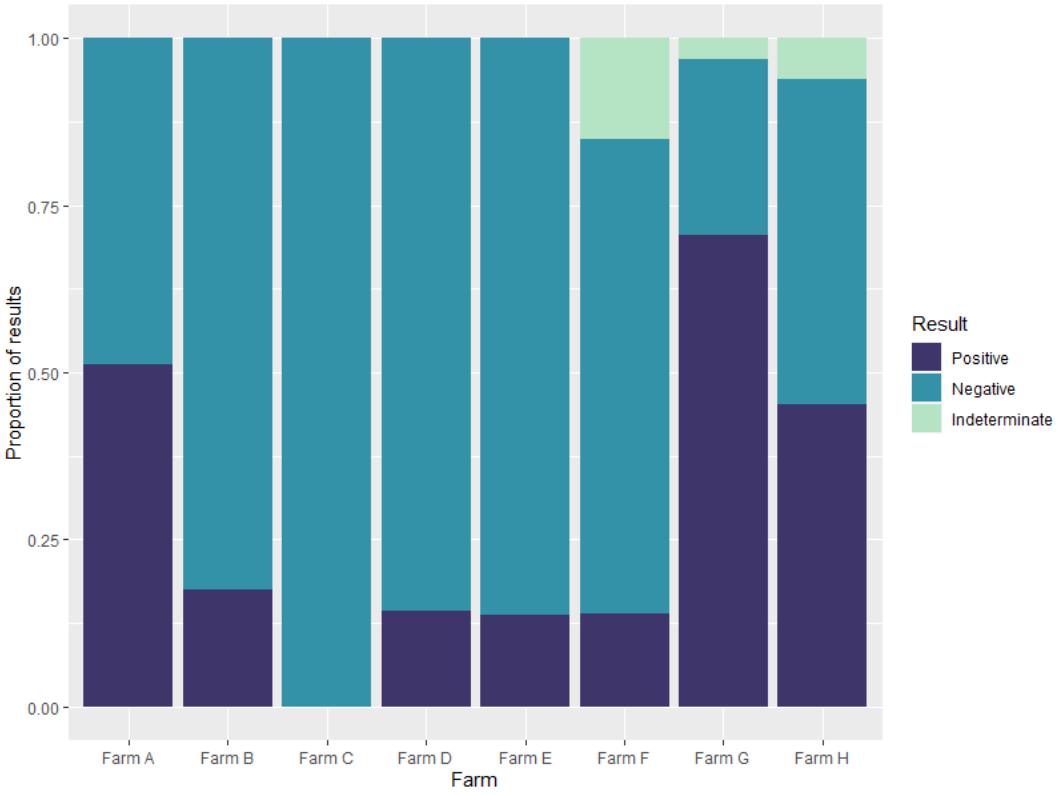


Figure 2H.1. Proportion of Japanese encephalitis virus serology results at each affected farm, Australia, 2022

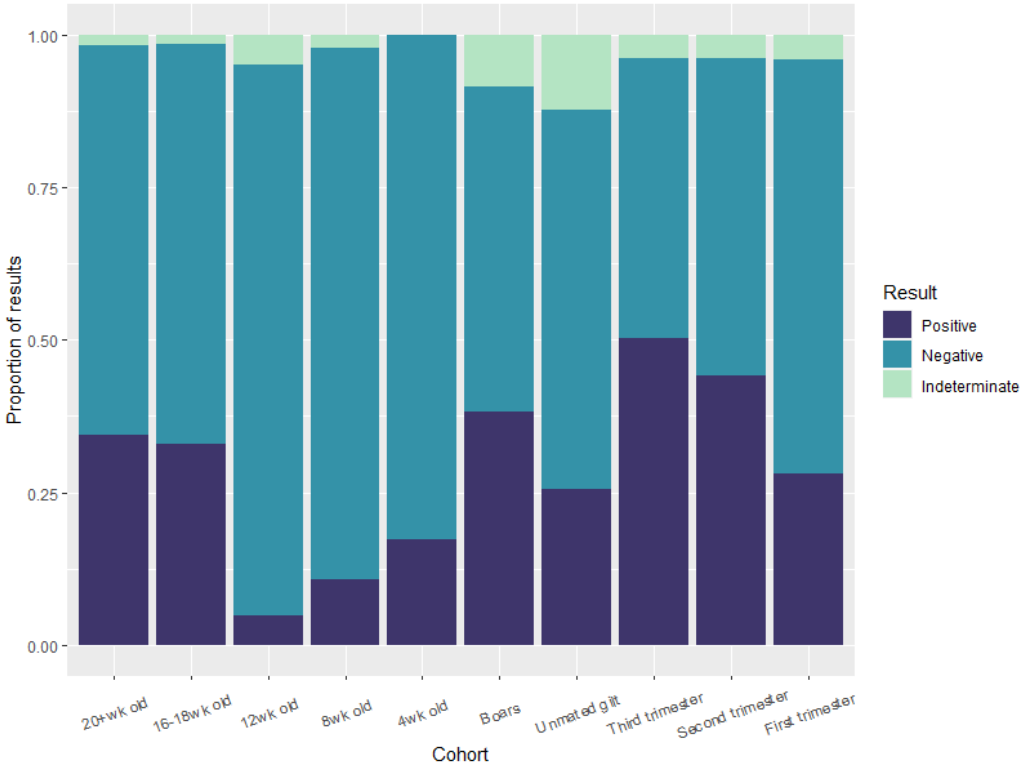


Figure 2H.2. Proportion of Japanese encephalitis virus serology results pooled across farms within each cohort, Australia, 2022

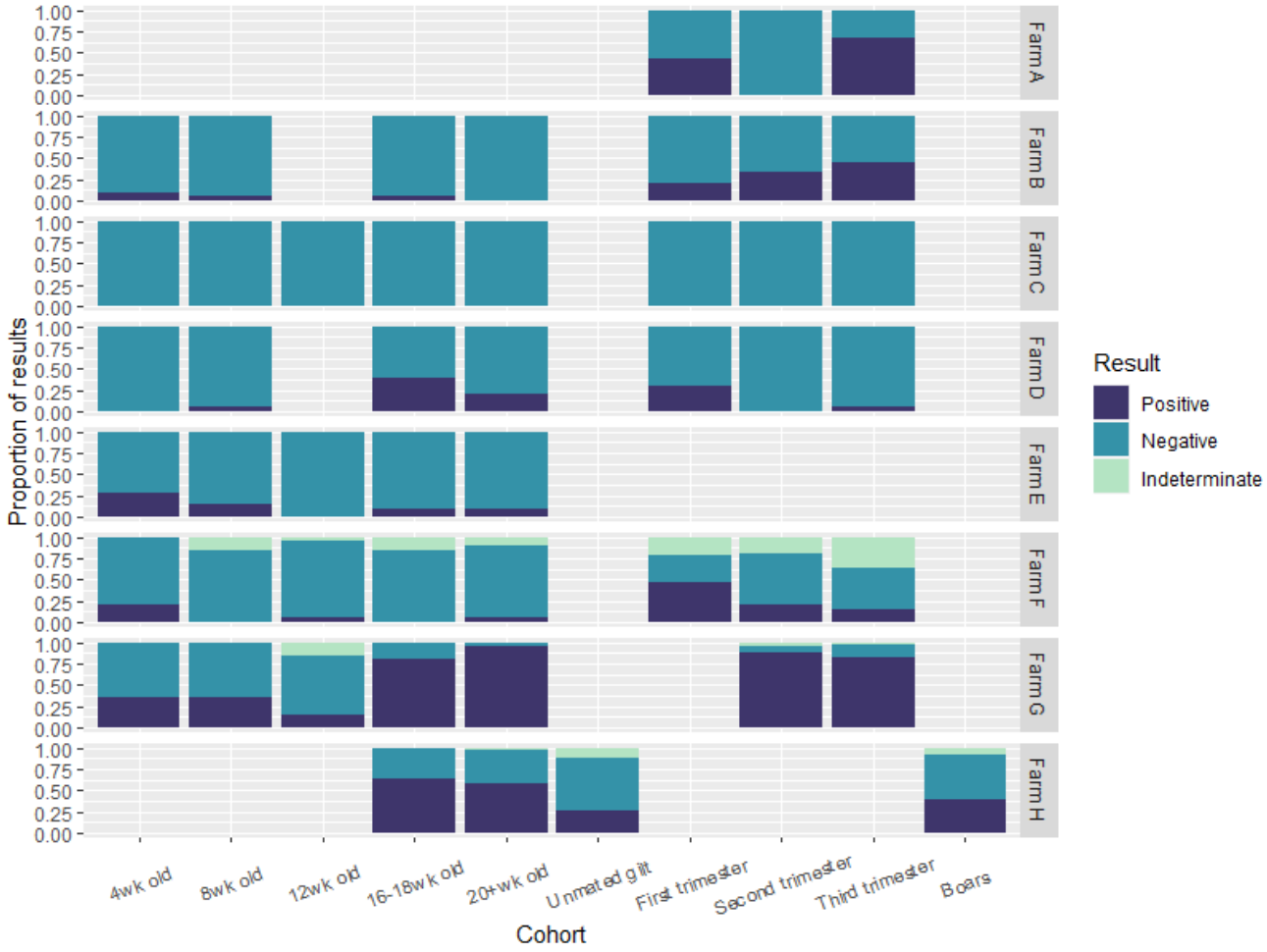


Figure 2H.3. Japanese encephalitis virus serology results at each farm by cohort, Australia, 2022

Chapter Three:

Evaluation of the Northern Australia Quarantine Strategy active component of the National Avian Influenza Wild Bird surveillance program 2024

Table of Contents

Glossary

Clade	A grouping of organisms based on a single common ancestor
Cleavage site	The specific region of a protein that is cut to facilitate the function of an enzyme or organism, such as entry of a virus into a cell
Cold chain	Appropriate storage of biological products (such as samples or vaccines) throughout transport, generally maintaining a temperature of between 2°C - 8°C
Cycle Threshold (CT)	The minimum number of cycles a thermocycler performs to amplify nucleic acid to reach a detection threshold based on fluorescence. The greater the cycle threshold, the less nucleic acid was present in the original sample. Cycle threshold values may be used as diagnostic cut points.
Proof of freedom	Appropriate evidence that a specific pest or pathogen is absent from a region
eNAT®	A virus transport media that inactivates the virus and preserves it for molecular diagnostics, allowing environmental samples for nucleic acid testing to be transported without the need for cold chain
General surveillance	Used in animal health contexts as an equivalent to passive surveillance[18]
Hemagglutinin	One of two influenza A virus surface proteins used to classify viruses into subtypes
Migratory	Regular seasonal movement of birds along migration flyways due to food availability, may coincide with breeding for some species
Neuraminidase	One of two influenza A virus surface proteins used to classify viruses into subtypes
Nomadic	Sporadic movement of birds across large distances, not on migratory pathways. Movement is due to water and food availability
Ornithology	The branch of zoology focused on the study of birds

[18] General surveillance has different definitions in different contexts. The listed definition is used throughout this chapter.

Poultry	Domesticated birds bred for eggs and meat, including chickens, ducks and turkeys
Qualtrics	Qualtrics (CoreXM) – a web-based research software program used to create surveys
Questionnaire	A series of questions used to collect information
REDCap	Research Electronic Data Capture – a secure, web-based software platform designed to support data capture for research studies
Seabirds	Birds that spend large amounts of time in marine environments such as cormorants and pelicans – the classification of "seabirds" is based on behaviour rather than taxonomy and different birds from different orders are considered part of this group. Another key marker for seabird classification is their diet that primarily consists of fish
Shorebirds	Wading birds such as plovers, curlews and sandpipers, part of the order Charadriiformes
Survey	Scheduled collection and testing of samples (specimens) for surveillance purposes, generally to understand "point in time" prevalence of target diseases.
Targeted surveillance	Used in animal health contexts as an equivalent to active surveillance[19]
Waterfowl	Birds such as ducks, swans and geese, part of the order Anseriformes

[19] Targeted surveillance has different definitions in different contexts. The listed definition is used throughout this chapter.

Prologue

Background

Surveillance of avian influenza viruses (AIVs) is an important preparedness activity for understanding the risks avian influenza (AI) presents for poultry industries and the health of other species globally. Wild birds, particularly waterfowl and shorebirds, are the natural reservoir for AIVs, and may carry the virus without clinical evidence of infection. Monitoring programs in many countries target migratory species of waterfowl and shorebirds that may carry the virus to new areas, and waterfowl in particular are known to easily shed the virus. In Australia, all jurisdictions have an existing program for active surveillance of AIs in wild birds that contributes to the National Avian Influenza Wild Birds (NAIWB) program, coordinated by Wildlife Health Australia (WHA). This program also includes an active surveillance component delivered by the Northern Australian Quarantine Strategy (NAQS) within the Department of Agriculture, Fisheries and Forestry (DAFF).

Australia's northern coastline poses unique risks for disease incursions including AIVs. These risks include environmental and climatic conditions, remoteness of areas, and close proximity to neighbouring countries (1). While both the overall NAQS program and NAIWB program have been reviewed and/or audited (Appendix 3B), a formal evaluation of the NAQS active surveillance component of the NAIWB program has not been previously undertaken. The NAQS AI active surveillance system is currently going through a period of significant transition, including increased numbers of surveillance sites and target bird species. This is in response to the H5 clade 2.3.4.4b AIV strain circulating globally, and also aligns with the Northern Australia Biosecurity Strategy 2030 (1). This chapter presents an evaluation of the NAQS AI active surveillance system against the attributes of simplicity, timeliness, stability, flexibility and data quality.

My role

I have an interest in influenza viruses, including AIVs. I initially approached WHA about evaluating the active surveillance components of the entire NAIWB program, but due to time and resource constraints, I decided to focus solely on the NAQS active surveillance component instead. Wildlife Health Australia supported this decision and invited me to quarterly NAIWB steering group meetings where I spoke regularly about the project. My roles for this project included describing the system, identifying the attributes to evaluate the system against, data collection, and analysis and write up of the findings of the evaluation. This project was also included as an activity under the High Pathogenicity Avian Influenza (HPAI) Preparedness Taskforce that was established in July 2024.

I used the following strategies to collect data and evaluate the system:

1. Review NAQS and NAIWB documents including grey literature.
2. Review methods and protocols used by the four laboratories involved in the system
3. Create a questionnaire for stakeholders using REDCap, and analyse the responses
4. Interview the Technical Manager of the NAQS Animal Health team
5. Analyse 12 years' worth of surveillance data produced by the program

An abstract based on this project was accepted for an oral presentation at the International Symposium on Veterinary Epidemiology and Economics (ISVEE). This presentation fulfils the conference attendance minor competency of the Master of Philosophy in Applied Epidemiology (MAE). The ISVEE conference will be held in Sydney in November 2024 (after the time of submission). A late draft of the slides prepared for this presentation is included in Appendix 3I.

Public health and animal health impacts

Avian influenza viruses are important pathogens of poultry, and HPAI strains can cause significant losses for poultry producers. Avian influenza viruses sometimes spread to poultry from wild birds and cause a spectrum of clinical disease in poultry depending on the strain. Once the virus is identified in a commercial poultry flock, the entire flock may be euthanised to prevent further spread and potential development of HPAI strains if H5 or H7 viruses are involved. The disease thus has implications for animal welfare, including for mammals as well as birds. Moreover, detection of HPAIs also has implications for Australia's trade arrangements, and surveillance of AIVs contributes evidence to support Australia's animal health status claims, including proof of freedom from high pathogenicity strains (2). Australia's poultry industry predominately operates south of the Tropic of Capricorn, and detections of novel viruses entering from the north along Australia's northern coastline can provide early warning for potential AI infections in southern regions. Additionally, since 2021 a strain of HPAI known as H5N1 clade 2.3.4.4b has been spreading globally and at the time of submission (October 2024) has been detected in all continents except Australia. As well as causing deaths in poultry, this strain of the virus has caused deaths in wild birds, and several spillover events of the virus have caused death and illness in mammals, including some human cases. Given this, HPAI is a disease of interest to One Health as it can affect wildlife and humans as well as poultry and livestock. While the NAQS surveillance activities are a discrete component of the NAIWB surveillance program, some findings from this surveillance evaluation can be applied to other areas of NAIWB, improving AI active surveillance across Australia, including with respect to the clade 2.3.4.4b viruses.

Surveillance system evaluations are not commonly practiced by animal health agencies in Australia, but this project has demonstrated the importance of evaluations to improve surveillance of animal diseases, and provides an example of how this can be done. Since I

commenced this project, DAFF has explored evaluating other surveillance systems, including for lumpy skin disease (LSD).

Reflections and lessons learned

This evaluation was a large project, consisting of multiple approaches to evaluate different attributes of the NAQS active surveillance component of the NAIWB surveillance program. In consultation with the NAQS Technical manager for Animal Health, I chose to evaluate the system against five attributes, which was difficult in the time frame I had for this project. Available guidelines for surveillance system evaluations have a strong public health focus and there were limited animal health surveillance evaluations for me to draw on when planning the project. However, I was able to use resources gathered for an evaluation of the NAQS feral animal surveillance system (prepared by Shellee Williams (MAE 2005-06)) that was under development when I started my placement with DAFF.

I intended to distribute my stakeholder questionnaire in November 2023.This was delayed as REDCap had a system outage and was unavailable for several weeks. Rather than send the questionnaire out close to the Christmas shutdown period and summer school holidays, I elected to instead send the questionnaire out in March 2024, to align with the first NAIWB steering group meeting of the year. This affected the timelines of all my projects, and preparing the questionnaire on Qualtrics at the end of 2023 instead of waiting for REDCap to be available would have reduced delays for this project. I had understood that the REDCap issues would be resolved within a few business days, although this proved not to be the case. The experience taught me to have a backup plan in case applications fail, and to be ready to use it when time is limited. The questionnaire was open until the end of June 2024, and while I sent email reminders and spoke about the questionnaire at NAIWB steering group meetings only 37 people started the questionnaire and of these only 18 people completed it. Some respondents also answered questions for the larger NAIWB surveillance program rather than the NAQS component, and being more selective with my respondents may have helped to limit this. The questionnaire could also have been improved to allow for more flexibility in answering questions. For example, one responder commented, in relation to a question with a yes/no answer, “Please note: I have provided NO as a response so I could add comments here…”, which is not ideal and could have been avoided by allowing comments for all questions and responses.

This project was an exercise in time management, and I learned that surveillance evaluations are large projects that take longer than expected to complete. It was particularly difficult for me to work on this project as the surveillance program was undergoing a period of change during my evaluation (e.g. new sites and changes in participating laboratories). I learned a lot about

AIVs, the NAQS program, and the network of animal health laboratories in Australia. I also developed skills in using REDCap, questionnaire development and data analysis.

Acknowledgements

The following people and groups were instrumental in this project being completed:

- Guy Weerasinghe and the Northern Australia Quarantine Strategy (NAQS) team, for helping me develop the evaluation and providing data and information about the program
- Andrew Breed, Rachel Iglesias and Troy Laidlow, for assisting with the development of the REDCap questionnaire used in the evaluation and providing guidance on avian influenza
- Tiggy Grillo, Paul Eden, Silvia Ban, Keren Cox-Witton and the team at Wildlife Health Australia, for supporting the evaluation and allowing me to speak about the evaluation at National Avian Influenza Wild Bird (NAIWB) steering group meetings
- The NAIWB steering group, for their interest in the evaluation
- Berrimah Veterinary Laboratory, Biosecurity Services Laboratory, DPIRD Diagnostics and Laboratory Services, and the Australian Centre for Disease Preparedness, for providing information on laboratory testing for NAQS AI samples
- The Northern Territory Department of Industry, Tourism and Trade, Western Australia Department of Primary Industries and Regional Development and Queensland Department of Agriculture and Fisheries, for their support of the NAQS surveillance in the three northern jurisdictions
- Sharon Roche and Zarzeez Anindya, for suggesting an evaluation of the NAQS component of NAIWB as an MAE project
- Shellee Williams, for providing advice on surveillance evaluations for NAQS programs
- Sam Colquhon, for facilitating my use of REDCap through ANU.

Abstract

Introduction: Avian influenza viruses (AIVs) are globally widespread in wild birds, with some subtypes having the potential to cause disease and production impacts in poultry. The high pathogenicity avian influenza (HPAI) strain H5 clade 2.3.4.4b currently circulating globally has caused severe impacts associated with disease in wild birds and mammals including humans, presenting a significant One Health issue. Australia's National Avian Influenza in Wild Birds (NAIWB) surveillance program monitors AIVs in wild birds and includes an active surveillance component delivered by the Northern Australia Quarantine Strategy (NAQS). The NAIWB program and NAQS activities have undergone reviews, but the NAQS component has not been formally evaluated. Regular structured evaluations of surveillance programs provide valuable information about the strengths and weaknesses of a program including how the system could be improved.

Methods: We evaluated the NAQS component of the NAIWB active surveillance program against the surveillance attributes simplicity, stability, timeliness, data quality and flexibility using the methods outlined by the United States Centers for Disease Control and Prevention. We examined 12 years of data from the program and reviewed the protocols used by the four laboratories involved in the system. A questionnaire was also sent to stakeholders from the public health, animal health and environmental health sectors, including representatives from government, laboratories, universities, and wildlife groups.

Results and Discussion: This evaluation demonstrated that the NAQS component of the NAIWB active surveillance program is a flexible system, successfully adapting to changing requirements over time to meet its objectives. We found that the simplicity of the system could be improved and recommend that the recording of data be simplified and findings from the system be reported more frequently.

Conclusion: The NAQS component of the NAIWB program is a useful component of Australia's AIV surveillance activities that reliably collects data on AIVs present in Australia's north. External users agreed that the system is simple and stable, however the collation and analytical work required by the system is more complex and should be streamlined to improve the simplicity and timeliness of the system. The findings from this study can inform other components of the NAIWB active surveillance system, such as the structure of the NAIWB database and appropriate sampling frameworks, to further improve the usefulness of the program nationally.

Introduction

Avian influenza viruses (AIVs) are a diverse group of influenza A viruses, with some subtypes having the potential to cause disease and production impacts in poultry (3). All detections of influenza A viruses in birds are notifiable in Australia (4) and, as with other influenza A viruses, Avian influenzas (AIs) are further classified into subtypes based on the haemagglutinin (HA) and neuraminidase (NA) proteins on the virus surface. To date, there are 16 recognised HA subtypes and 9 recognised NA subtypes that exist in birds in different configurations (5-7). These subtypes are further classified into clades.

The effect the virus has on chickens broadly classifies AIVs into two groups:

1. Low pathogenicity AI (LPAI) strains, for viruses that infect respiratory and gastrointestinal cells only and have less severe impacts on poultry health (3, 6).
2. High pathogenicity AI (HPAI) strains, for viruses that can infect multiple cell types and cause systemic infection, resulting significant illness and a significant number of deaths in poultry. Only viruses with HA subtype H5 or H7 are known to have become HPAIs (6-9).

At the molecular level, HPAI viruses are distinguished from LPAI viruses by the amino acid sequence of the HA cleavage site, which is involved in the attachment of the virus to host cells (6, 9). Under the right circumstances, LPAI viruses can mutate into HPAI viruses (6, 9). A single bird can harbour multiple different influenza viruses with different subtypes and clades at the same time. It is hypothesised that all influenza A viruses originated in birds, before spilling over into other host species and (in some instances) adapting to be sustained in the new host population (6, 9, 10). Therefore, AIVs pose a threat to human health, including having the potential to cause pandemics (10).

Wild birds are reservoirs for AIVs, and viruses can spread to different regions of the globe along migratory bird flight pathways (5-7, 11). AIV surveillance activities predominately target waterfowl and shorebirds, with waterfowl (specifically ducks) considered the primary reservoir of AIVs (12). Shorebirds migrate south along the East-Asian Australasian Flyway (Figure 3.1) and arrive in Australia between September and August (13), and this is a potential pathway of entry for AIVs into Australia's north. There are also other flyways that birds migrate along, including the West Pacific Flyway (14). Australia is not along migratory flight pathways for waterfowl, however nomadic waterfowl move between Australia, Papua New Guinea and Indonesia in response to weather events and resource availability (15). This movement pattern of waterfowl is unique to Australia, and therefore risks of AIVs entering Australia are different to risks experienced in the Northern hemisphere (7) and shorebirds present a greater risk of introduction of novel strains in Australia compared to other countries (12). Low pathogenicity

avian influenzas can spread to poultry from contact with wild birds, and poultry management practices can allow LPAI viruses to become HPAI viruses, resulting in significant illness and death for exposed birds (6, 9). The exact mechanism for how LPAI viruses evolve into HPAI viruses within poultry flocks is not well understood (6).

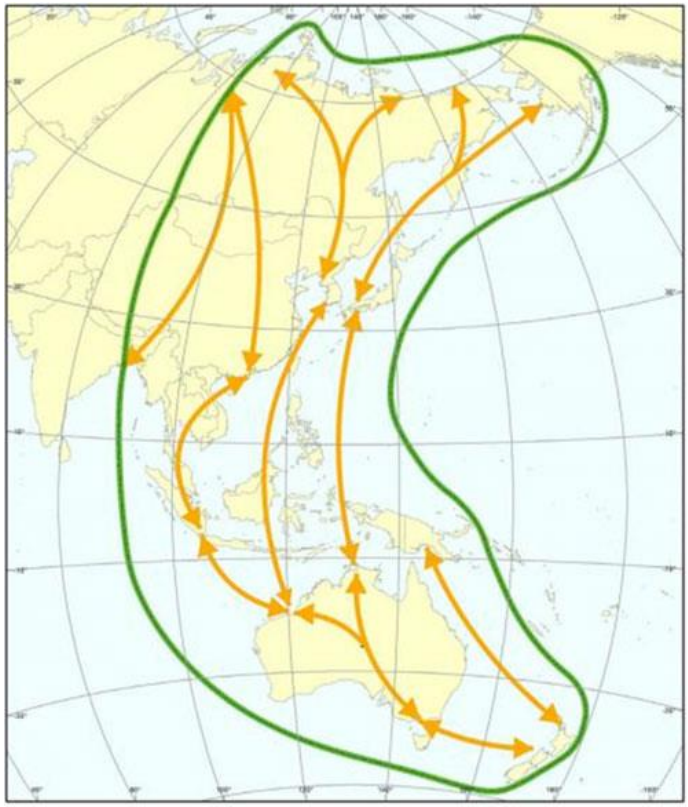


Figure 3.1. East Australian Flyway and bird migration pathways (13)

In recent years there has been increased focus on AIVs and the risk they pose to poultry, wild birds and other mammals including humans. Since 2021, a HPAI H5 clade 2.3.4.4b virus has been detected in Asia, Africa, Europe, the Middle East, the Americas and Antarctica (16, 17), but at the time of writing this chapter has not been detected in Australia. Infection with the HPAI H5 virus clade 2.3.4.4b is associated with mass deaths in wild birds and poultry, and spillover events have also caused deaths in mammals including sea lions, minks, foxes and racoons (18-20). Illness and death in these mammals have been preceded by mass deaths in wild birds and detection of the virus in these bird populations (18-20). Surveillance of AIVs in wild birds is thus important to inform preparedness activities and understand the viruses circulating within the region.

In Australia, surveillance for AIVs in wild birds is coordinated by Wildlife Health Australia (WHA), the national organisation for wildlife health in Australia. The National Avian Influenza Wild Birds (NAWIB) surveillance program includes active and passive surveillance activities, and all states and territories participate in active surveillance activities. The program was

established in 2006 in response to the emergence of a HPAI H5N1 virus in Asia, and the steering group was initially tasked with determining if the virus had spread to Australia (12). Members of the steering group include representatives from WHA, The Department of Agriculture, Fisheries and Forestry (DAFF), all state and territory animal/agriculture governments (including laboratory representatives), the Department of Health and Aged Care (DoHAC), the Department of Climate Change, Energy, the Environment and Water (DCCEEW), universities across Australia, the poultry industry, and other relevant groups. The NAIWB program is primarily funded by DAFF (21).

Across Australia's northern coastline, surveillance as part of the NAIWB program is undertaken by the Northern Australia Quarantine Strategy (NAQS), a biosecurity program within DAFF aimed at early detection (22). The NAQS program was established in 1989 and targets plant and animal pests and diseases that may cross into Australia from the north (22). NAQS also supports the Indigenous Ranger Biosecurity Surveillance program, and the Indigenous Ranger Network has a key role in biosecurity preparedness activities in Australia's north (22). The NAQS program commenced sampling of wild birds to test for diseases including AIVs in 1992 (22). Since 2004, NAQS has received specific funding for regular surveys of wild birds to test for HPAIs (22) and in 2006 became part of the newly established NAIWB surveillance program. The NAQS active surveillance involves regular collections of samples at locations across Northern Australia in Queensland (Qld), Western Australia (WA) and the Northern Territory (NT). These activities are delivered by scientific and operational staff based in Broome, Darwin, Nhulunbuy (Gove), Weipa, Bamaga, Cairns and throughout the Torres Strait Islands (23). The NAQS activities include assessing the risk of target pests and diseases (including HPAI) entering Australia, conducting surveys and monitoring activities, and providing surveillance data where required (24). A risk review of NAQS sampling sites is conducted every five to eight years for each targeted disease, the most recent of which was conducted in 2019 (25). An overview of the NAQS animal health surveillance system, including AI surveillance plans and surveillance objectives, is available in Appendix 3A.

While the NAQS program, including AI active surveillance activities, has been reviewed over time (Appendix 3B) many of these reviews focus on appropriateness of surveillance sites and results of surveillance activities, and a formal evaluation of the NAQS active surveillance component of the NAIWB surveillance program has not been undertaken. This evaluation is being performed to:

- Describe the purpose of the NAQS component of the NAIWB active surveillance system

- Assess the usefulness of the surveillance system against the attributes of simplicity, timeliness, flexibility, stability and data quality
- Make recommendations to NAQS on how the system could be improved
- Make general recommendations to WHA on how the NAIWB surveillance program could be improved.

This evaluation focuses on the active surveillance activities undertaken by NAQS only. Active surveillance activities performed by state governments and universities and all passive surveillance activities performed by NAIWB members (including NAQS) are out of scope for this evaluation.

Methods

System description

The description of the NAQS component of the NAIWB surveillance system was prepared using information gathered from discussions with the NAQS technical manager for animal health and other DAFF NAIWB members, and from reviews of NAQS documents and the WHA NAIWB operational plan. Laboratories also provided information that was used to describe the system.

Evaluation Framework and attributes

Evaluations of animal surveillance systems are not yet routinely practiced although the usefulness of these evaluations is recognised (26, 27). When they are undertaken, evaluation methods are often adapted from the Public Health sector (26, 27). For this project, the United States Centers for Disease Control and Prevention (US CDC) *Updated guidelines for Evaluating Public Health Surveillance Systems* (28) was used to describe and evaluate the NAQS component of the NAIWB active surveillance program against the attributes of simplicity, timeliness, flexibility, stability and data quality (Table 3.1).

The system was not evaluated for representativeness, sensitivity, predictive value positive (PVP) or acceptability. Please see Appendix 3C for more information.

Table 3.1 Summary of attributes used to evaluate the Northern Australian Quarantine Strategy component of the National Avian Influenza Wild Birds active surveillance system

Attributes (and their description)	**Questions to answer**	**Evaluation methods**
Simplicity *How easy the system is to use, and how simple the structure of the system is*	• How simple is it for stakeholders to engage with the surveillance system? • How much time is spent training users how to use the system? • How easily does the NAQS component of the system integrate with the rest of NAIWB?	• Review methods and protocols used by laboratories • Stakeholder questionnaire • Interview with Technical Manager • Analyse 12 years[a] of data • Review NAQS Documents
Timeliness *The speed or delay between steps in a surveillance system*	• Is the current sampling schedule appropriate for timely detection? • What are the protocols for testing samples? E.g. as soon as sample arrives, once a specific number of samples are in lab etc. • How long does it take for laboratories to report results once a sample has arrived at the laboratory? • How long does it take for results reported to NAQS to be reported to WHA?	• Review methods and protocols used by laboratories • Stakeholder questionnaire • Interview with Technical manager

Flexibility *How a system adapts to changing information or operating conditions e.g. requirements for* *additional cost in time, personnel, or allocated funding*	• Could the system easily be adapted to increase sampling? What additional resources would be required? • Could the system easily be adapted to decrease turnaround time for testing? What additional resources would be required? • Could the system be easily adapted to investigate another avian disease?	• Review methods and protocols used by laboratories • Stakeholder questionnaire • Interview with Technical Manager • Analyse 12 years[a] of data • Review NAQS documents
Stability *The reliability of the system to collect, manage and report data, and to be available for use when needed*	• Does the system meet its objectives? • Does the system meet objectives and guidelines stipulated by WHA? • Is the collection and reporting of data valid? • Is the system reliable?	• Review methods and protocols used by laboratories • Stakeholder questionnaire • Analyse 12 years[a] of data • Interview with Technical Manager
Data quality *The completeness and validity of the data recorded in the system*	• Is the data complete? • Is the data valid?	• Stakeholder questionnaire • Interview with Technical Manager • Analyse 12 years[a] of data

a. Data extract from September 2011 – December 2022

Data collection

Laboratory methods and protocols

Laboratories were contacted by email and asked to provide responses to a list of questions relevant to AIV testing for samples collected through NAQS active surveillance activities (Appendix 3D). A copy of the protocol used for AIV testing was also requested.

Responses and protocols were compared across the four laboratories to evaluate the simplicity, timeliness, stability and flexibility of the system.

Stakeholder questionnaire

A stakeholder questionnaire was developed with assistance from DAFF NAIWB representatives, including from NAQS, to evaluate the simplicity, timeliness, stability, flexibility and data quality of the system. Excluding participant information questions, a total of 85 possible questions were created, with 49 of these providing an option for additional comments. Questions included 39 Likert scale questions, 17 multiple choice questions, 9 dichotomous questions and one checkbox question. Questionnaire data were collected and managed using REDCap electronic data capture tools[20] hosted at The Australian National University National Centre for Epidemiology and Population Health (29, 30). The questionnaire was distributed via email link to members of the NAIWB steering group identified from the NAIWB steering group email distribution list and other key personnel at DAFF and state government departments. Steering group members were encouraged to forward the link to relevant people in their networks who may have an interest in responding to the questionnaire. Participants were kept anonymous and branching logic was embedded into the questionnaire so that participants were only asked questions that were relevant to their experience with the system.

Analysis of 12 years of data

Wildlife Health Australia provided an extract of 12 years of NAQS data from the NAIWB database for September 2011 – December 2022 via email. Completeness of fields, consistency of entries, validity of entries and changes over time were analysed to assess the flexibility, stability and data quality of the system. The information reported in individual fields was also used to assess the simplicity of the system.

Interview with Technical Manager

A structured interview was held with the technical manager of the NAQS animal surveillance program in two hour-long sessions over Microsoft Teams. Written notes were taken during both

[20] REDCap (Research Electronic Data Capture) is a secure, web-based software platform designed to support data capture for research studies, providing 1) an intuitive interface for validated data capture; 2) audit trails for tracking data manipulation and export procedures; 3) automated export procedures for seamless data downloads to common statistical packages; and 4) procedures for data integration and interoperability with external sources.

interview sessions, and a transcript was recorded in the second interview session. The information provided in this interview was used to describe the system based on the simplicity, stability, flexibility, and timeliness attributes. The interview also gave an overview of recent changes to the system, including the inclusion of external territories and additional organisations to conduct surveillance activities, and also included responses to questions asked in the stakeholder questionnaire.

Review NAQS and NAIWB documents

The NAQS documents stored on the DAFF network drive were used to examine NAQS protocols and changes over time including business plans, operational plans, and surveillance strategies. Past audits and risk reviews were also reviewed. These documents were used to evaluate the system against the attributes simplicity, stability and flexibility. This information was also used to describe the system.

Documents relating to the NAIWB program overall were also reviewed, with a focus on elements applicable to the NAQS active surveillance component.

Data analysis

Data was analysed using Microsoft® Excel® for Microsoft 365 MSO (Version 2403 Build 16.0.17425.20236) 64-bit and RStudio 2023.06.1-524 (31).

NAQS NAIWB extract

Numbers of samples collected and results for each sample were calculated by year, jurisdiction, collection date and collection site. Laboratories involved in testing and bird species recorded at the time of collection were reviewed and compared, and HA testing results were counted and reviewed over time.

Stakeholder questionnaire responses

Number of responses and proportion of each response was calculated for each question in the questionnaire. Responses were stratified by membership of the NAIWB steering group, organisation, jurisdiction or length of time with NAIWB where required. Comments were reviewed and REDCap was also used to supplement analysis of questionnaire data.

Ethics

This investigation received an ethics waiver from the ANU Human Research Ethics Committee as per protocol 2017/909, “Request for waiver of consent for use of data in research for Master’s in Applied Epidemiology students for ‘Outbreak Investigation’ and ‘Surveillance in Public Health’ projects”.

Results

System description

The NAQS component of the NAIWB active surveillance program operates across Qld, WA and NT (Figure 3.2) and NAQS works closely with governments of these jurisdictions to coordinate sampling and testing. NAQS "risk sites", including those for AI surveillance activities, are identified based on the likelihood of a particular pathogen entering and/or becoming established in particular area, and sites are frequently reviewed. WHA guidelines (12) recommend a minimum of 300 birds be sampled per sample site per calendar year for AI active surveillance activities, however faecal environmental swabs are also appropriate for testing. The frequency of sampling varies for each site (monthly, quarterly, or biannually) depending on variables such as weather, season, and staff availability. These factors are important for bird movement patterns, as well as the types of birds being sampled (24, 32). Safety is also an important factor for sampling, particularly with regards to crocodile habitats and wild buffalo (33, 34).

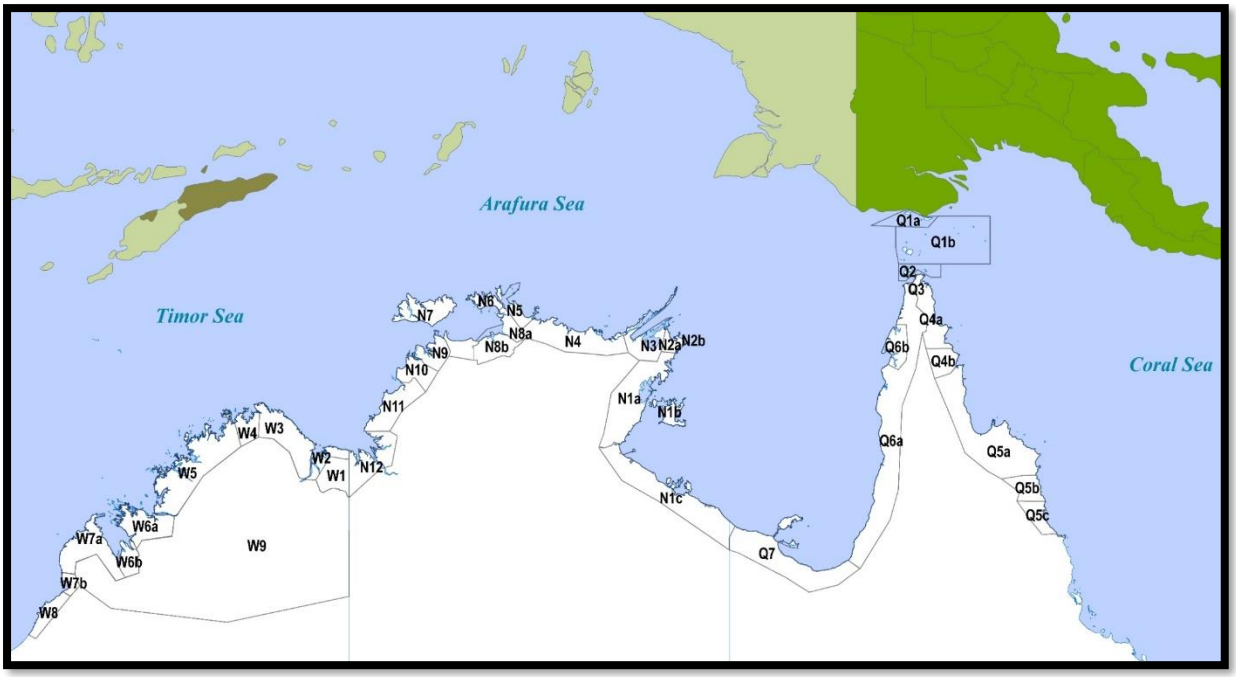


Figure 3.2. Risk sites for all Northern Australian Quarantine Strategy animal surveillance programs in Australia (33)

Sample collection and initial testing

The NAQS active surveillance activities for AIVs involves the NAQS team travelling to designated sites and collecting environmental faecal samples from apparently healthy birds (Table 3.2). In 2024, this has been expanded to include sampling in external territories, and the inclusion of collections by the Indigenous Ranger Network and Parks Australia. At the time of collection, data such as bird species present at the site is also noted. This information is

recorded manually or with the use of a field collection application on a device, and the NAQS field team email this information for review by the NAQS policy team. A new digital application is currently being trialled to improve the recording of data. The Indigenous Ranger network have their own methods for recording data and sending this information to NAQS.

Table 3.2. Overview of when and where to sample bird species for Northern Australia Quarantine Strategy avian influenza active surveillance activities (34)

Bird species	When to sample	Where to sample	Rationale
Waterfowl	Early hours of morning	Banks of wetlands and other bodies of water including water treatment plants	Waterfowl gather on land overnight and return to water in the early hours. Early morning sampling also limits ultraviolet radiation (UV) from degrading virus ribonucleic acid (RNA).
Shorebirds	Varies for different species. Early morning preferred	Tidal flats, preferably freshly wet sand	Early morning sampling limits UV from degrading virus RNA.
Seabirds	Early hours of morning where possible	Rocks and boulders close to the shoreline. Avoid nesting sites. Additional approaches being assessed include the use of reuseable tarpaulin placed at the base of trees the night before sampling.	Birds congregate on rocks and boulders close to shoreline. The new technique using tarpaulin enables easier collection in some areas with low wind and lack of rocks/boulders.

Testing

After collection, samples are transferred to the jurisdiction animal health laboratory (Berrimah Veterinary Laboratory (BVL) in NT, Department of Primary Industries and Regional Development Diagnostics and Laboratory Services (DDLS) in WA, and Biosecurity Sciences Laboratory (BSL) in Qld) for initial pan-Influenza A testing usually in pools of three. Pooled samples with a positive or indeterminate pan-Influenza A result are re-tested individually, and positive or indeterminate individual samples undergo H5 and H7 subtyping (however this is only NATA (National Association of Testing Authorities) accredited at the Australian Centre for Disease Preparedness (ACDP)). Samples collected in external territories are sent directly to ACDP for all testing and are not tested at subsequent laboratories. Results of testing at the

primary testing laboratory are reported to NAQS, and positive results are also reported to the jurisdiction chief veterinary officer (CVO).

Samples that test positive or indeterminate to the pan-Influenza A Polymerase Chain Reaction (PCR) at the jurisdictional testing laboratory are sent to ACDP for confirmatory Influenza A testing. Positive and intermediate samples are typed against all HA subtypes, and suitable samples may also undergo other testing including sequencing. Results of all testing are reported to the referral or primary testing laboratory. Positive results are reported to the jurisdictional CVO. If a HPAI strain is identified, the jurisdictional CVO will report this to the Australian CVO. If NAQS is alerted to any HPAI detections, NAQS will also notify the Australian CVO. There is no routine reporting strategy for ACDP to report findings to NAQS if samples were referred by a jurisdictional laboratory.

Collation and Analysis

The NAQS Technical manager for animal health manually collates all field data with laboratory data. This information is analysed by NAQS, and significant findings are shared within the NAQS team. The technical manager manually inputs information into an excel spreadsheet and submits this to WHA for inclusion in the NAIWB database on a monthly basis. Once submitted, WHA cleans NAQS data and data submitted from other areas of the system for analysis. WHA reports results at quarterly NAIWB meetings, and NAQS reports results annually in Animal Surveillance Quarterly. Information collected by the system is used to inform further actions for surveillance, such as additional surveys.

System overview

The NAQS active surveillance component of the NAIWB surveillance program requires significant coordination and the majority of actions are completed manually. After samples are collected, sample collection information is sent to the NAQS policy team for processing, while the physical sample is sent to the primary laboratory for testing. The result and sample information must then be merged manually. The reporting of results from the primary laboratory to NAQS is straightforward, but once the sample is tested at the secondary laboratory (ACDP), NAQS loses oversight of the results, and any additional testing done on positive samples. Reporting to jurisdictional CVOs is routinely actioned by laboratories, and NAQS also performs this role. As other components of the NAIWB surveillance program also report to WHA, WHA must be able to accommodate the varied laboratory testing and collection information into one database, and as the NAQS data differs to other components there are likely complexities with integrating this information. The recent inclusion of seabird surveillance and sampling by external organisations adds complexities in how data is recorded through the system. An overview of the system is available in Figure 3.3.

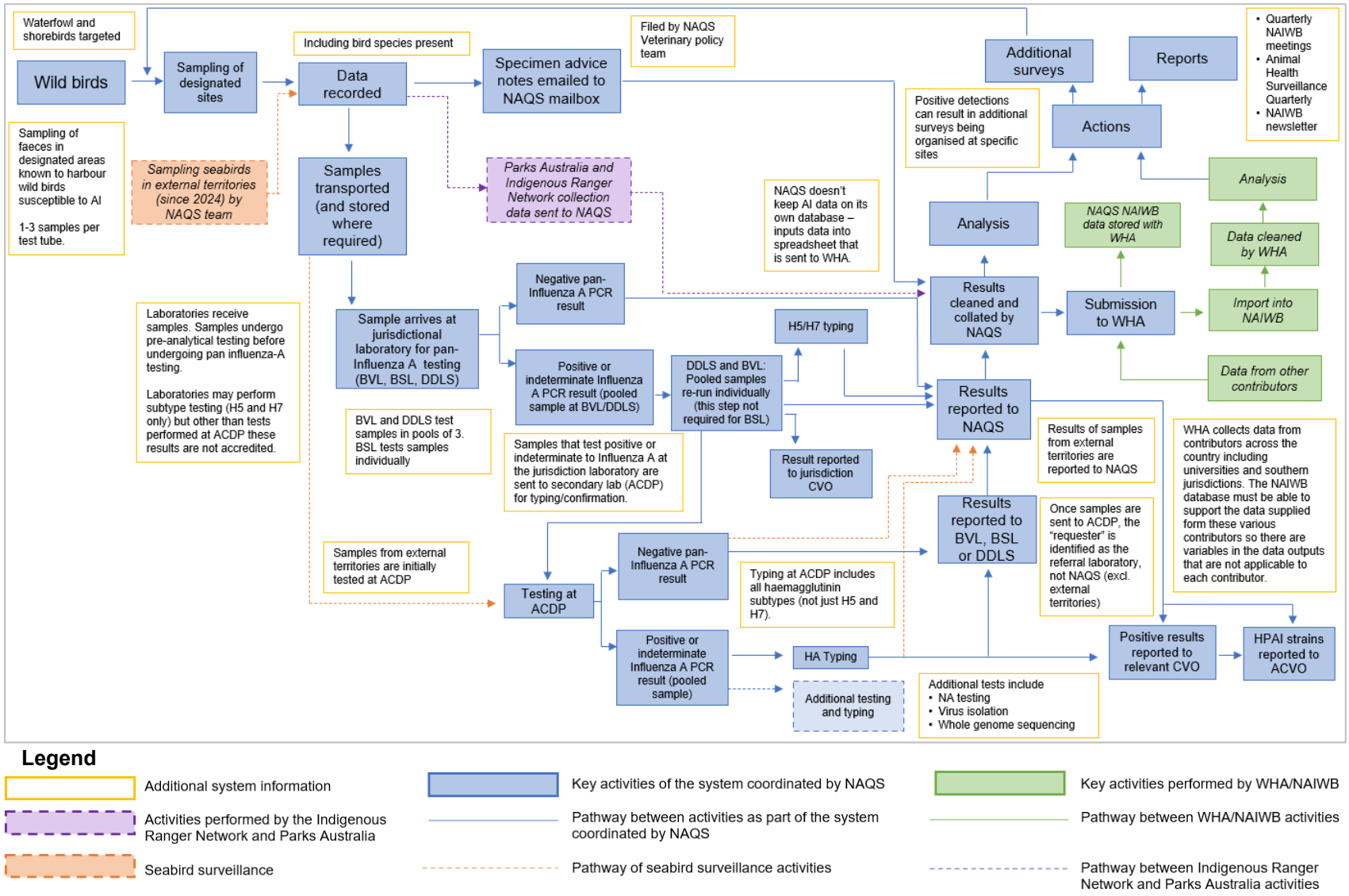


Figure 3.3 Overview of the Northern Australian Quarantine Strategy component of the National Avian Influenza Wild Birds Active Surveillance program

Analysis of surveillance system data

The surveillance data analysed in this project is an extract from the NAIWB system for NAQS data collected between September 2011 and December 2022. The NAIWB dataset includes 119 variables that may be completed for each sample, with 102 variables related to laboratory testing, laboratory results, or laboratory comments. The dataset also includes information on the sites where samples are collected and the bird species that are present at the time of collection. Between September 2011 and December 2022, 14,788 samples were collected as part of the NAQS component of the NAIWB active surveillance activities, with an average of 410 samples collected per jurisdiction per year (Table 3.3).

Table 3.3. Number of samples collected for the Northern Australia Quarantine Strategy component of the National Avian Influenza Wild Birds active surveillance program by year and jurisdiction, 2011 – 2022

Year	2011[a]	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022	Total
Qld	193	952	810	550	98	140	198	200	192	41	150	140	3664
WA	397	663	185	392	501	300	300	601	398	402	602	525	5266
NT	458	1200	302	378	300	300	596	607	293	598	462	364	5858
Total	1048	2815	1297	1320	899	740	1094	1408	883	1041	1214	1029	14788

a. 2011 includes 4 months of data only (September 2011 – December 2011)

A total of 257 samples tested positive for influenza A between September 2011 and December 2022. An additional 15 samples had a result of “indeterminate”. The number of positive samples has varied between 2011 and 2022, with the highest number of positive samples collected in 2012 (n = 50, 2% of all 2815 samples collected in 2012). The highest proportion of positive samples occurred in 2014 (n = 49, 4% of all 1320 samples collected in 2014). No samples collected in 2020 tested positive or indeterminate to the pan-Influenza A PCR (Figure 3.4).

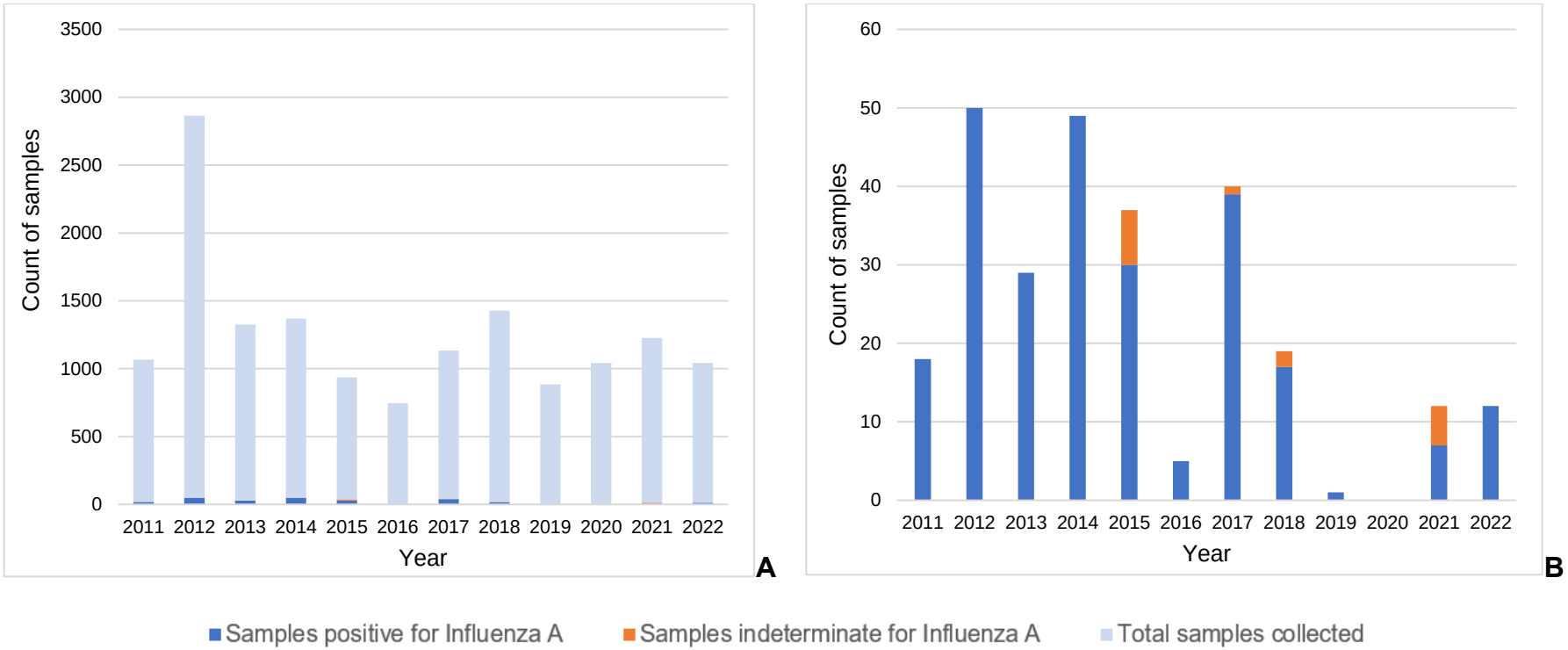


Figure 3.4. Number of samples tested for influenza A, all results (panel A) and positive and indeterminate[21] results only (panel B) as part of the Northern Australia Quarantine Strategy component of the National Avian Influenza Wild Birds active surveillance program, September 2011 – December 2022

[21] For samples tested at two laboratories with conflicting results, the result at the second laboratory is reported.

There was overall consistency in pan-influenza A results for samples tested at a primary laboratory and a secondary or reference laboratory. Between 2015 and 2019, all samples positive for influenza A at the primary testing laboratory subsequently tested positive for influenza A at the secondary or reference laboratory[22] (Appendix 3E).

Hemagglutinin subtype results for positive samples have varied between 2011 and 2022, with the hightest number of positive samples of any one HA subtype recorded for H1 in 2013 (29 samples) (Figure 3.5). The capacity for HA typing at different laboratories has changed over time. Some primary testing laboratories perform or have performed these assays, though this is currently limited to H5 and H7 typing at laboratories other than ACDP (Appendix 3F). Historically, samples positive for influenza A may not have HA results recorded from the primary testing laboratory, but be tested at the reference laboratory (ACDP) only. Some samples have HA typing done at both laboratories.

[22] For samples positive for influenza A at the primary laboratory that underwent testing at a secondary laboratory

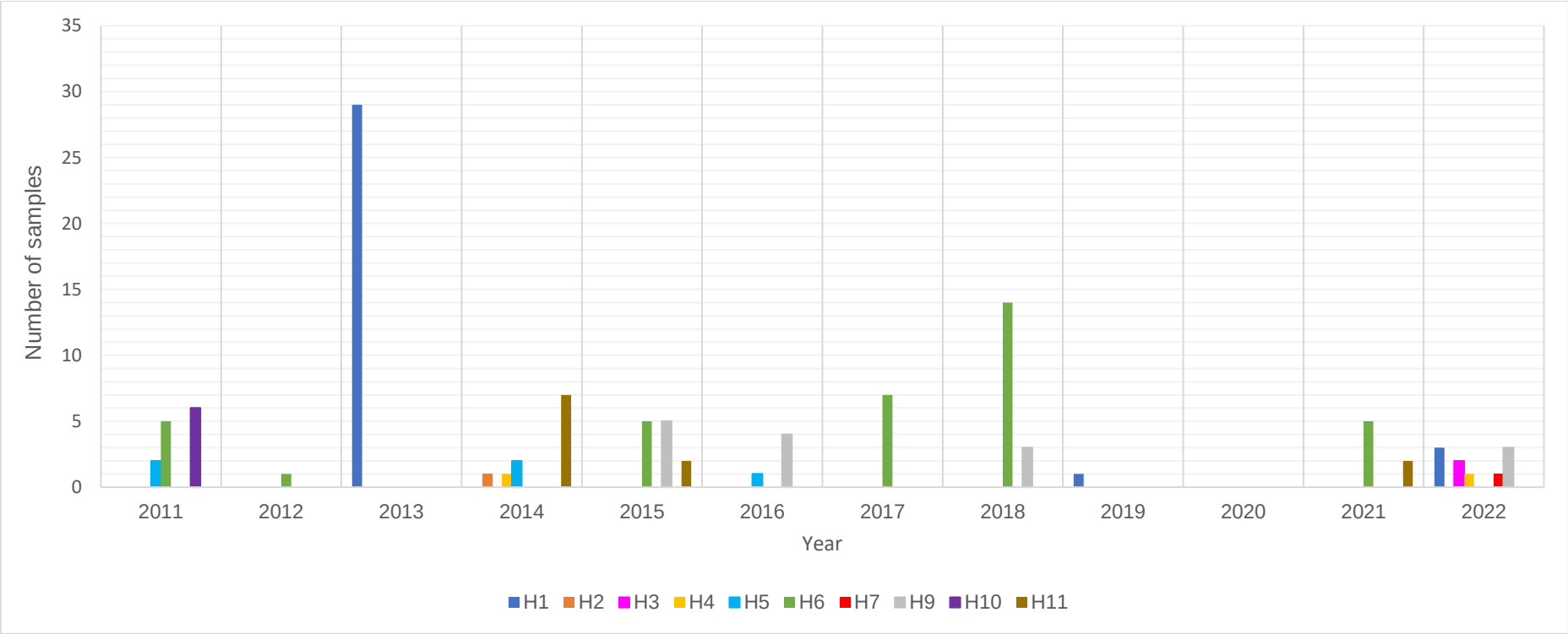


Figure 3.5. Hamaglutinin testing results[23,24,] on samples collected as part of the Northern Australia Quarantine Strategy component of the National Avian Influenza Wild Birds active surveillance program, all laboratories, Septmeber 2011 - December 2022, Australia

[23] For positive or indeterminate influenza A samples that were successfully typed
[24] HA subtype resulted from at least one typing test at testing laboratory

The most abundant bird species present at the time of sampling varied across all sites and years, with *Dendrocygna etyoni* (plumed whistling duck) the most commonly listed species (40% of entries listed). The listed taxonomic level of species present also varied. For some samples, more than one species was listed as the most abundant.

Laboratory protocols

The NAQS component of the NAIWB surveillance system relies on four different laboratories, each with their own processes and protocols. All four laboratories provided response to questions and copies of their influenza A testing protocols by email or Microsoft Teams.

As per the WHA NAIWB operational plan, laboratory testing for AIVs is at the discretion of each jurisdiction. The four laboratories involved in testing all complete pan-Influenza A testing on samples, but kits, cycle thresholds (CTs) and testing time vary (Table 3.4).

Table 3.4. Overview of testing at laboratories involved in the Northern Australian Quarantine Strategy component of the National Avina Influenza Wild Birds active surveillance program

Laboratory	PCR extraction kit	Positive CT cut-off	Sequencing capacity?	Haemagglutinin subtypes tested	Testing time (from time of specimen arrival to results reported)[25]	Pooled
BVL	AgPath-ID™ One-Step RT-PCR	40	No	H5 and H7	Within 10 business days	3
BSL	MagMAX CORE (KF DUO) or MagMAX Viral RNA Isolation Kit (KF Apex)	40	No	H5, H7 and H9	Within 5 business days	1
DDLS	AgPath-ID™ One-Step RT-PCR	37	Yes – not routinely performed	H5 and H7	Within 72 hours	3
ACDP	MagMAX-96 Viral RNA Isolation Kit (Ambion AMB 1836-5)	40	Yes	All subtypes[26]	Within 5 business days	3

[25] For pan-influenza A testing
[26] H5 and H7 PCR routinely performed. For other HA subtypes H1 to H16, test is not routine.

Laboratory staff reported that testing of samples is simple, however if results are positive or indeterminate further testing is managed on a case-by-case basis. One laboratory highlighted that AI testing is performed by multiple areas within the laboratory.

Samples collected by the NAQS active surveillance component of the NAIWB surveillance program are considered routine tests, and in some cases testing occurs only when a certain number of samples have been received by the laboratory. Laboratories operate under a triage system and, if required, measures are in place to ensure that the testing of NAQS active surveillance activities are prioritised for testing. Routinely collected samples are tested for influenza A in pools of three at most laboratories, but if a pooled sample tests positive then all three samples are re-tested separately before any further analysis or transport to ACDP (if initial testing is done at a different laboratory) occurs. The pan-influenza A PCR itself takes approximately five hours, and considering sample preparation, quality assurance checks and any H5 and H7 tests, testing generally takes about two business days including for urgent samples. As these tests are routine, the time from sample arrival to tests being completed varies from within 72 hours to within two weeks at each laboratory, and can take longer if more specific testing such as whole genome sequencing is performed (at the reference laboratory). Transport of samples to ACDP may be restricted for some laboratories, for example a courier service may only operate on specific weekdays. In these cases, the time it takes for samples to undergo testing and resolution of results can increase significantly.

Laboratories involved in the surveillance system carry excess stock of reagents needed for the testing of samples. Some laboratories also advised that they could draw on other laboratories for reagents if required, or order reagents in for arrival within 72 hours, to ensure testing occurs as required by the system. One laboratory highlighted that faecal environmental samples can cause inhibition of PCR, but that this has been mitigated through additional wash steps in sample preparation.

The ACDP advised that the AI H5 Australian lineage is updated as required, and that scientists are constantly assessing the need for any updates.

Stakeholder questionnaire

The stakeholder questionnaire was sent to 87 people including 68 NAIWB steering group members, but it is unknown how many additional people received the questionnaire link. A total of 37 people commenced the questionnaire, however only 18 (49%) complete responses were received. Seven (19%) partial responses were received, and 12 (32%) respondents only completed up to the introduction or participant information section of the questionnaire. The results presented in this section refer to responses from the 25 participants who completed or partially completed the survey.

Responses were received from all jurisdictions, including 6 from the NT (Figure 3.6).

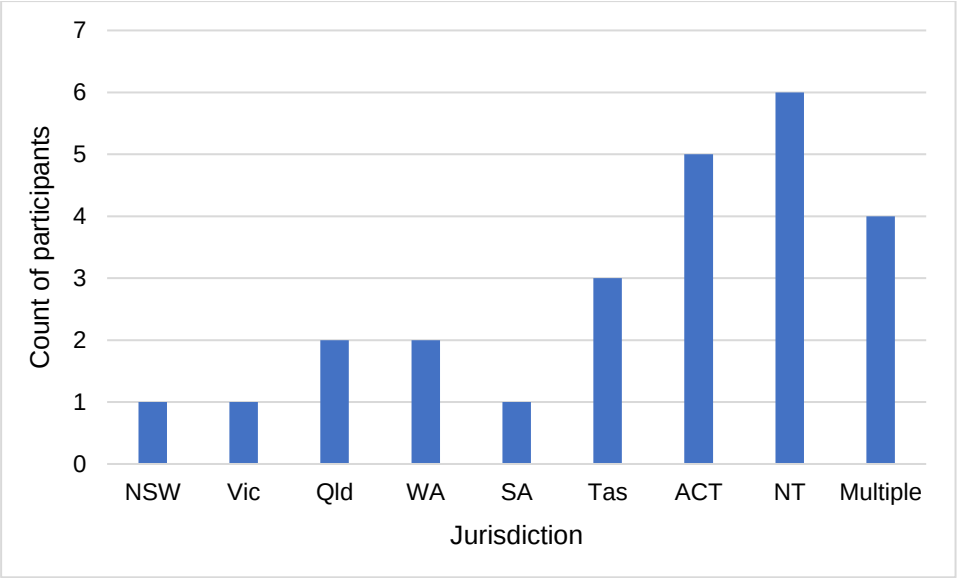


Figure 3.6. Number of responses to the evaluation of the Northern Australia Quarantine Strategy National Avian Influenza Wild Birds stakeholder questionnaire by jurisdiction[27] (n = 25)

Participants had varied affiliations, with 10 (40%) affiliated with a state government (Table 3.5). A total of 15 participants provided their sector, with 13 (52%) indicating they worked in Animal health and two (8%) in Human/Public health. The majority of participants (n = 15, 60%) were members of the NAIWB Steering Group.

[27] "Multiple" refers to between 2-8 jurisdictions listed by a respondent

Table 3.5. Number of responses to the evaluation of the Northern Australia Quarantine Strategy National Avian Influenza Wild Birds stakeholder questionnaire by affiliation (n = 25)

Affiliation	Count	Frequency %
State Government	10	40
Federal Government	5	20
Northern Australia Quarantine Strategy (NAQS)	3	12
Wildlife Health Australia	3	12
Industry	2	8
Diagnostic laboratory	1	4
University/research	1	4
Other	0	0
Total	25	100

Overall, participants reported that sample collection, sample transport, testing and interpretation and communication of reported information are simple to complete and meet the data entry needs of the system. Training new staff in these areas is also regarded as simple. The majority of participants (60%) also agreed that communicating the information collected by NAQS is simple. Responses regarding the simplicity of data management, data analysis and data reporting were more varied, with 33% of responders indicating they disagreed that the NAQS data easily integrates into the NAIWB database[28].

Participant responses to the question “Do you think the current sampling schedule is appropriate for timely detection of avian influenza” were varied, with 40% of participants agreeing that the current sampling frequency is appropriate for timely detection of avian influenzas in Northern Australia.

> *…It is difficult to answer this questions without a robust study, which will also involve uncontrollable variables given planned sampling framework will depend on many factors, including presence of birds, and ornithological expertise in different sites…*

Regarding timeliness of the system, participants highlighted that delays related to the NAQS active surveillance component of the NAIWB program were predominately related to the

[28] 6 participants provided answers to this question (Agree = 17%, Neutral = 17%, Don’t know/not relevant = 33%)

remote locations surveillance activities take place, and the resources available for surveillance activities:

> *The delay in submission due to vast distances that must be covered, in remote areas, potentially rendering samples not as reliable as if they could be analysed sooner…*

Of 20 responses, 8 (40%) participants indicated that it would be difficult for additional resources to be reallocated to accommodate additional surveys for the NAQS active surveillance component of the NAIWB program (excluding 9 participants who responded "Don't know/not relevant"):

> *NAQS staff are already overstretched especially for field surveillance & data management but AI sampling is one of our priorities so we would endeavour to make it work, likely compromising other field surveillance.*

Several participants provided examples of times the NAQS component of the NAIWB active surveillance system successfully adapted to the need for increased surveys, including changes to sampling sites, sampling types and new programs in offshore territories.

Of 23 responses, 16 (70%) participants agreed with the statement "If required, the NAQS component of the NAIWB active surveillance system could be adapted for surveillance of other avian diseases". Participants listed avian paramyxoviruses (APMs) including Newcastle disease (ND), avian poxviruses, avian cholera, infectious bursal disease, duck plague, infectious coryza, and infectious anaemia as diseases the system could potentially be used for.

Twenty-three participants (92%) provided the length of time they had been involved or interested in the NAQS component of the NAIWB surveillance system. Seven (30%) participants reported involvement for more than 10 years and seven (30%) participants reported involvement for less than one year. All participants who reported involvement or interest in the NAQS active surveillance component of the NAIWB program agreed that this component has both consistently and reliably collected and reported accurate and true data since their involvement in the program.

> *[Sampling] has evolved from catching shorebirds & ducks/geese for swabbing and serology to environmental faecal sampling - means we can get many more samples for less money and less impact on birds. There was a lull in 2013-15 where shorebirds were deemed less risk and AI sampling was lesser priority but was consistently in our operational plan*

Responses to whether the NAQS active surveillance component of the NAIWB surveillance program meets its objectives were varied. Some participants commented that passive surveillance activities and activities in southern areas were more useful for early detection of AIVs compared to NAQS active surveillance activities:

> *General Surveillance [is] more sensitive for HPAI early warning. Not enough samples collected to be early warning for HPAI in apparently healthy wild birds.*

> *[Not] sure there's been many cases of a warning of outbreak (ie very little commercial poultry in Northern Australia & southern outbreaks haven't been linked to northern LPAI findings) but is still useful to know what viruses are circulating in northern wild birds, and to not (yet) be finding evidence of spread from eg SE Asia*

Several participants indicated that 300 samples per site per calendar year is insufficient to understand the diversity of AIVs in wild birds in Northern Australia, changes over time and dynamics in different species.

Regarding failures in collection or reporting for the system, participants reported these sometimes occurred, with weather, location and presence of birds highlighted as the main reasons for failures.

The majority of participants indicated they are satisfied with the data reported by the NAQS component of the NAIWB surveillance program, and that the data is of high quality.

Twelve years of data

The extract of NAQS data from the NAIWB database includes information on collection date, bird species present at the time of collection, testing laboratories, pan-influenza A results, HA subtypes at each laboratory where typing occurs, NA subtypes at each laboratory where typing occurs, as well as comments on samples. The relevance of these variables to AI surveillance activities have changed over time.

The number of birds listed for the variable “Species” ranges from 1 – 12 different birds (over 780 different combinations between 2011 – 2022) and the variable “species abundant” ranges from 1 – 4 different birds (23 different variations between 2011-2022). For some observations, different bird species are entered in a single field, separated by a comma. Additionally, there are variations in the taxonomic level provided for birds. A list of the different birds recorded in the NAQS extract of NAIWB data for 2011-2022 is available in Appendix 3G.

The amount of data collected by the NAQS component of the NAIWB active surveillance system has increased over time, and newer data fields are therefore incomplete for earlier

samples, such as latitude and longitude. Similarly, some variables are no longer relevant. The variable “Host ID” is also empty for all samples collected between 2011 and 2022, and ‘Species abundance’ is missing for 3360 samples (22.7% of all samples). A list of all 119 variables is provided in Appendix 3H.

The laboratories and tests used for testing NAQS AIV samples have changed over time. Previously, laboratories included the Elizabeth McArthur Agriculture Institute and Agriculture Victoria, but these laboratories have not been involved in testing since 2014. Similarly, ACDP replaced BSL as a primary testing laboratory for samples collected in Qld between 2020 – 2023. Earlier samples were also tested at three laboratories, but currently a maximum of two laboratories are involved in testing, and all samples have testing performed at ACDP.

The sites and frequency of sampling for NAQS AIV active surveillance activities have also changed over time. In 2011, sampling in all jurisdictions occurred at a single site (noting data is for four months only), and in 2012 all samples were collected from two sites in each jurisdiction. Between 2013 and 2021 the number of sites sampled varied for each jurisdiction, with between one and three different sites sampled each year. In 2022, both Queensland and Western Australia sampled at over three sites. Sampling at sites has also changed. Previously, sites were visited less and so a large number of samples would be collected at each site at each visit, but frequency of sampling has now increased, resulting in few samples collected at each visit (Figure 3.7).

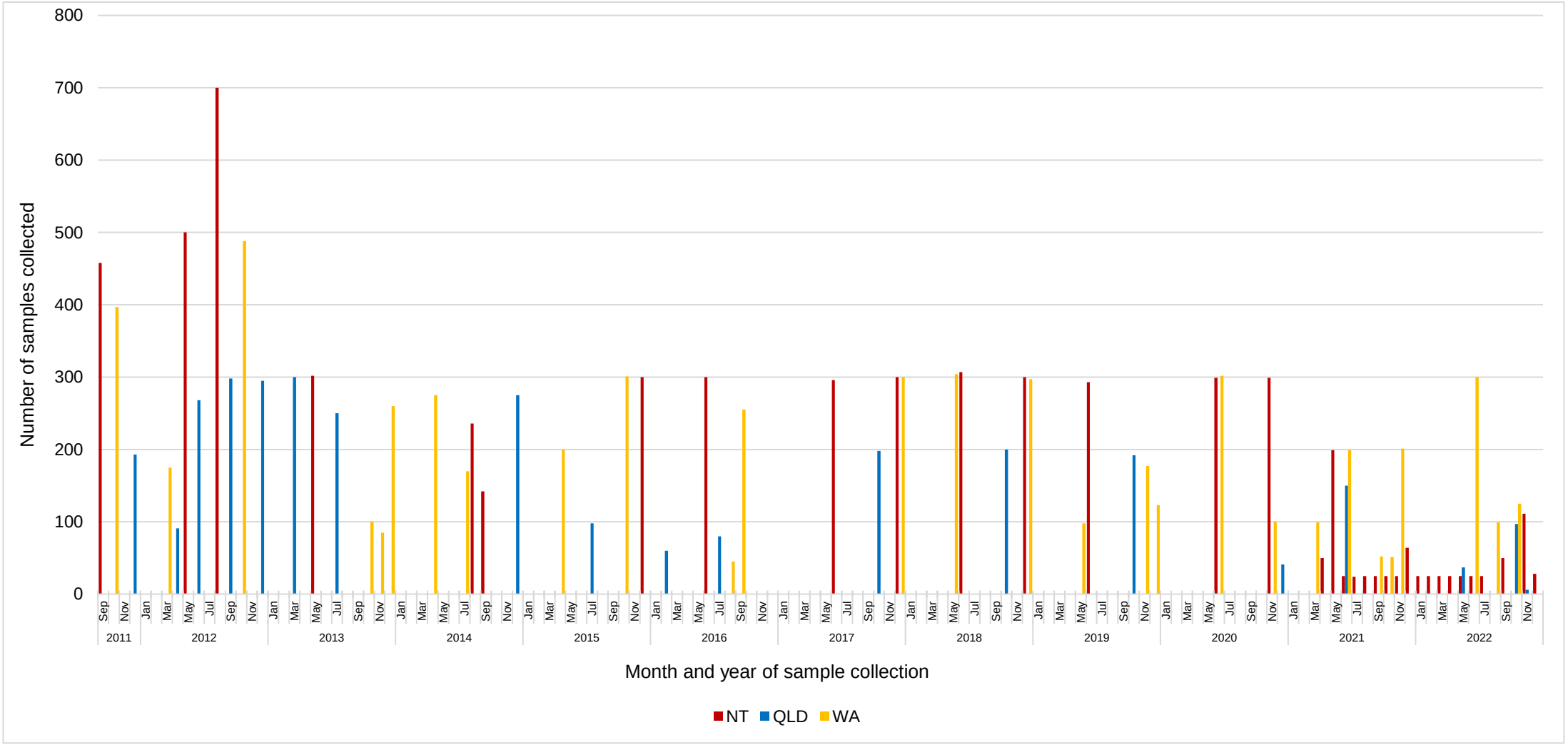


Figure 3.7 Frequency and number of samples collected for the Northern Australian Quarantine Strategy component of the National Avian Influenza in Wild Birds active surveillance program, by jurisdiction, September 2011- December 2022

Since 2020, WHA has recommended collection of 300 samples per site per calendar year for AI active surveillance activities. The majority of sampling performed by NAQS has not reached the goal of 300 samples per site per calendar year, including after 2020. For Queensland, no sites have achieved 300 samples per site per calendar year since 2014. For the Northern Territory, most sites have had at least 270 samples collected per year since 2020, and for Western Australia there has been at least one site per calendar year that has had over 300 samples collected.

Between 2011 and 2022, HA testing was not performed on 62 of the 272 samples positive or indeterminate for influenza A (23%), including when testing only occurred at one laboratory. HA typing was also unsuccessful for 119 of the samples positive or indeterminate for influenza A (44%). (Figure 3.8).

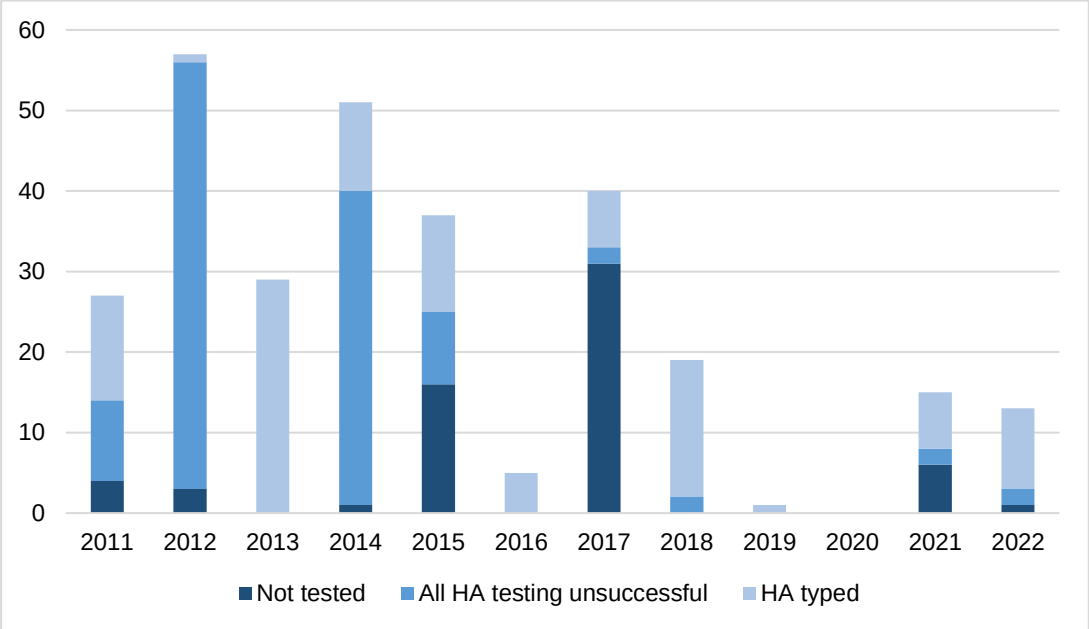


Figure 3.8. Hemagglutinin testing for samples positive or indeterminate for influenza A collected for the Northern Australia Quarantine Strategy component of the National Avian Influenza Wild Birds active surveillance program, 2011 – 2022

The data extract included entries for swabs only, including for trapped birds prior to 2014. No serology samples or results were included in the extract.

Interview with the NAQS Technical Manager

The NAQS Technical Manager highlighted that coordination of sampling and of the activities undertaken by jurisdictions and external organisations is complex for the NAQS team to manage, noting that these organisations have preferred methods and instruments to record information that differs to NAQS. The reporting from NAQS can be delayed, especially as the system evolves and is scaled up, and NAQS submissions to WHA are currently made on a bi-

annual basis rather than monthly. The NAQS team can also lose visibility of results once samples are sent to ACDP from the primary testing laboratory, which can affect the reporting of results by NAQS to WHA. Regarding sampling of 300 samples per site per calendar year, the NAQS Technical Manager noted that AI prevalence changes from year to year, and 300 may be inappropriate in a given calendar year or at a given site.

The NAQS team are equipped to pivot their work to priority areas or diseases, but successful sampling for AI surveillance is dependent on weather and the presence of birds. For some sites, the NAQS team can adapt sampling schedules and locations at short notice to ensure samples can still be collected when required. For other sites, adapting sampling schedules is more difficult. The NAQS component of the NAIWB surveillance program is currently undergoing significant changes, including upscaling to include samples collected by northern Indigenous rangers, sampling within the external territories, and sampling of seabirds. There are also discussions about adapting the AI surveillance activities performed by NAQS to other countries in the region such as Papua New Guinea and the Solomon Islands. For surveillance activities performed by external agencies (such as the Indigenous Ranger network and Parks Australia), there is less flexibility to add additional surveys onto existing workloads. Moreover, additional sample collection requires NAQS to pay for additional laboratory testing, and a large proportion of funding allocated to the NAQS component of the NAIWB surveillance activities is used for diagnostics.

The method used to record data at the time of sample collection has changed over time, from paper-based notes that are transposed into an excel spreadsheet to the recent development of an application that can capture information digitally at the time of sample collection. This application enables field data to be collated within a day, though the technical manager noted that as the application is new knowing what analysis is most meaningful to the program can create delays in analysis, and use of the application is not widespread at the time of writing this report.

The NAQS active surveillance component of the NAIWB surveillance program could be used for surveillance of other avian diseases, and in the past the NAIWB program has been used for APMs such as NDV. The NAQS Technical Manager noted that adapting the system to other avian diseases requires coordination from the laboratories to confirm appropriate and validated testing is available, as well as from WHA as there must be capacity for this information to be captured in the NAIWB database.

The NAQS Technical Manager noted that including the type of swab or media (such as eNAT®), time of day sampling occurred, and weather events could improve AI surveillance data. There is no option for different levels of data to be entered, such as sampling locations, and as a

result, many locations are combined at the time of analysis. Additionally, there is no option for collectors to explicitly enter the bird species the sample is likely to be from.

Review of NAQS and NAIWB documents

The documents reviewed provide an overview of the objectives of the NAQS AIV surveillance program, the activities included in the NAQS AIV surveillance program, and how the program has changed over time.

The NAQS documents provide a guide for sample collection that is simple to follow. The reasons for surveillance are recorded clearly and explained in these documents. The WHA NAIWB operational plan used by NAQS is also simple, providing a clear background for sampling activities and standards for NAIWB contributors to adhere to, including sampling 300 samples per site per calendar year. Recent documents on environmental sampling prepared for organisations external to NAQS also recommend taking photos of birds so they can be identified later. Documents also describe how surveillance activities previously included collecting serology and cloacal swabs as well as environmental faecal samples for AIV testing. This required birds to be trapped, which stopped being practiced from 2014. Documents indicate that when these methods were included, most positive results were from environmental faecal samples.

Discussion

Surveillance of AIVs in Australia's north is an important preparedness activity. This evaluation explored the simplicity, flexibility, stability, timeliness and data quality of the NAQS active component of the NAIWB program, to assess the usefulness of the system. The evaluation found that the NAQS active component of the NAIWB program is considered a simple and flexible system by users, however the evaluation overall highlighted there are necessary complexities to the system. Given these complexities, the system performs well to meets its objectives. As the system grows, additional resourcing is required for the continued success of the program.

The was a low response rate to the stakeholder questionnaire, limiting the reliability of the questionnaire results. Participants in the stakeholder questionnaire were overwhelmingly associated with animal health organisations, with few public health and no environmental health representatives completing the questionnaire. While NAQS is an animal health surveillance system, some AIVs have zoonotic potential and therefore AIV surveillance activities have relevance to the human health sector. The lack of participation in the stakeholder questionnaire from the public health and environmental health sectors indicates that more work needs to be done to engage these sectors with NAQS AI surveillance and the

entire NAIWB program. Due to the limited responses in the questionnaire, the importance of AIV surveillance, especially to sectors outside of animal health, could not be comprehensively evaluated in this project.

While the NAQS active component of the NAIWB program is considered simple by stakeholders, there are several complexities to the program, including coordination of internal and external staff to collect samples for influenza testing. Survey scheduling is further complicated by the weather conditions and locations of sample sites in the NAQS region, which sometimes result in survey abandonment. These complexities also create delays that compromise the timeliness of the system. Moreover, the way sample information is recorded and communicated varies, resulting in additional data cleaning and collation prior to submission to WHA, in turn delaying the timeliness of reporting. Standardisation of this across NAQS, the Indigenous Ranger Network and Parks Australia would help to simplify the system, and work is being done to expand the use of a mobile application that can be used at the time of sample collection. Once finalised, this application should be used consistently across organisations to streamline collation of data and reporting to WHA. If successful, developing a similar application for use by other NAIWB contributors could be considered. This would require an evaluation of the usefulness of the application, including how simple it is for the NAQS data collected through the application to integrate into the NAIWB database.

The NAQS program is highly flexible, including AIV surveillance activities. Additional surveys and sites can be added to the program as required, including new targets and offshore sites as exemplified by the recent addition of seabird sampling in external territories. Moreover, for some sites planned surveys can be rescheduled or alternative sites sampled if conditions are not favourable to sampling (such as flooding). The flexibility of NAQS is hindered by available resources including personnel and funding, and additional resources to support additional work would help to improve the stability, flexibility and timeliness of the system. This could include drawing on additional staff with appropriate skills within other areas of DAFF. The challenging environments NAQS operates in can also affect the timeliness and reliability of sample collection for AIV surveillance. However, stakeholders consider the system reliable despite these challenges, and many comments in the stakeholder questionnaire offered praise for the work the NAQS team delivers under difficult circumstances.

The number of samples collected per year for NAQS AIV surveillance has varied between 2011 and 2022, indicating a lack of stability with survey and sampling design. A limitation of this data was that only four months of 2011 were provided, and so the number of samples collected throughout 2011 is unknown and cannot be compared to later years. Samples from 2023 and 2024 were not included in the data extract, therefore changes to sampling in this period could

not be analysed. The NAQS surveillance activities are influenced by weather conditions, presence of birds, and perceived risk of AIVs. These conditions may have resulted in less samples collected in some years compared to others, which limits the stability of the system. The number of samples collected each year and the stability of the system could also be affected by available funding, and machinery of government (MOG) changes could influence the funding supplied to NAQS for surveillance activities. However, funding specifically for AI surveillance has been provided to NAQS for 20 years, indicating funding for the system is overall stable. It is likely that funding will continue given the risks AIVs pose for the poultry industry, and, demonstrated by the HPAI strain H5 clade 2.3.4.4b currently circulating globally, the risks HPAI can pose to wildlife and mammals including humans.

The collection of 300 samples per site per calendar year to detect AIVs was recommended in 2020. Three-hundred has been chosen as this number allows for 95% confidence of AIV being detected if there is a prevalence of 1% in a population (12). Despite the recommendation to collect at least 300 samples per site per calendar year, the majority of NAQS sites fail to meet this requirement, indicating a lack of available resourcing or abandonment of sampling due to environmental conditions or bird absence. This indicates the need for a more realistic sampling guidelines or changes to more stable sampling sites. The sample calculation of 300 samples per site per calendar year is primarily based on studies in the northern hemisphere with a focus on waterfowl rather than shorebirds. There are also several assumptions with this number, including perfect sensitivity and specificity of AIV tests and consideration of all Australian wild birds as a single, homogenously mixing population (7, 12). Provision of the sensitivity and specificity of tests from laboratories would help to determine if 300 is appropriate for detecting AIV, and clarity regarding if the “detection of AIV” refers to all AIVs, HPAIs or AIVs of a specific subtype. As suggested by a responder to the stakeholder questionnaire, further study on the appropriate sampling of wild birds in Australia may help to confirm if 300 samples per site per calendar year is suitable for timely detection of AIVs, or if this number should be changed. As bird movements and AI epidemiology varies year to year, there may be additional environmental considerations that need to be factored in when calculating appropriate sample sites for waterfowl, shorebirds or seabirds, and further investigation into these unique factors in Australia is needed.

As the demands of the NAQS AI surveillance system grows, the workload of NAQS staff increases. As a result, reporting to WHA is delayed, as evidenced but the current monthly reports being submitted on a bi-annual basis. This indicates a need for additional technical staff at the data collation and analysis level to enable workloads to be shared among more staff, or automation of the collation, cleaning, and submission of data. This would aid in improving the timeliness and stability of the system.

The animal health laboratories involved in the testing of NAQS AIV samples all perform similar testing. There is variation in protocols used, including differing CT cut-offs across laboratories that use the same PCR kit. As per the NAIWB operational plan, testing for AI samples is at the discretion of the laboratory, however consensus of CT cut-offs for laboratories that use the same protocol should be considered to improve the simplicity of the system. Regarding timeliness, routine testing of the NAQS AIV samples at laboratories can take up to two weeks to complete. While timeliness is improved for urgent samples (two business day) this may not meet the requirements of the system. The transport of samples between laboratories can also create delays with results and reporting, and improved testing capabilities at state animal health laboratories could improve this. Another key distinction between laboratories was the pooling of samples, performed at all but one laboratory. While this is at the discretion of the different laboratories (and requires additional funding not provided by DAFF) further evaluation could be done to assess the effectiveness of pooling samples. The excess reagents carried by the different laboratories indicate laboratories can readily perform testing required for the system, enhancing the system's stability.

The NAIWB database was developed over a decade ago, and since its development the data required for the system has changed over time. Many variables in the current database are obsolete and don't allow for the flexibility associated with AIV surveillance activities to be meaningfully recorded. There is currently a plan to develop a new NAIWB database, and this could include significant changes to how laboratory information and bird information is recorded to improve data quality and the sensitivity of the information. Currently, there are too many distinct laboratory variables, most of which are left empty for observations and many that are relevant to APMs rather than AIV testing. Despite the number of available variables, laboratory information for multiple tests is predominately recorded in a single variable for tests performed and test results at each lab, while other variables are left blank, indicating this format is not appropriate for the NAIWB database. A variable that allows selection of a specific laboratory test and a variable that allows selection of a specific result would help with ensuring only relevant information is collected for each observation. An option to "add new test" could be used to repeat the process for any additional testing. To further assist with data quality, a variable of "final test result" should be included, to summarise all testing across all laboratories. However, to improve the sensitivity of the information, this may also need to include a variable to capture the number of different HA types identified through testing, as wild birds can harbour multiple AIVs at the same time and samples are pooled. Similarly, multiple bird species are recorded in a single variable by varying taxonomic level. Extracting meaningful information from these variables is difficult, and having an option to add birds individually at a consistent taxonomic level would improve the quality of the data. Moreover, the data allows for the most

abundant species to be recorded, but there is no option to add the bird species the collector suspects the sample has come from, which may not be the most abundant species in all cases. Including this information would give greater meaning to AIV results. These changes would improve the data quality, simplify analysis, and allow for additional flexibility of the NAIWB database. Other information, such as weather conditions at the time of sample collection, could also be collected at the time of sampling, as this information could assist with understanding environmental conditions relevant to the epidemiology of AIVs. Given the differing considerations required for sampling of waterfowl, shorebirds or seabirds (such as migration or movement patterns, site specifications and time of collection) a distinct variable that allows selection of one of these groups for each collection should be included to highlight how each sample was collected and provide additional information on the significance of these results. As this evaluation is focused only on the NAQS component of the NAIWB program it is difficult to comprehensively assess the needs of the NAIWB database for all contributors.

The NAQS AIV active surveillance activities are designed to act as an early warning system by identifying and monitoring influenza viruses circulating in wild birds in Australia's north, with HPAIs specified as the target pathogen for these activities (33, 35). To date, the NAQS system has not identified any HPAI strains through active (or passive) surveillance activities but has provided a library of LPAI strains present in Australia's north. Many stakeholders who completed the questionnaire indicated that passive surveillance activities are more appropriate for detection of HPAI, and that due to the locations of poultry farms south of the NAQS risk sites, the usefulness of NAQS is in detection of LPAIs. As HPAI viruses can evolve from introductions of LPAI viruses into poultry, monitoring LPAIs in the north is relevant for poultry industries, particularly around areas such as Mareeba in Far North Queensland where there is some commercial poultry production. Moreover, NAQS is an early warning system that acts to detect pests and diseases before they cause significant illness, whereas passive surveillance activities are in response to illness and deaths. Relying only on passive surveillance activities, particularly in remote areas such as Northern Australia, presents a risk for unmonitored spread and mutation of AIVs. This could result in evolution of new strains that could be devastating for wildlife, poultry and potentially humans. The migration of shorebirds along the East Australian Flyway and Australia's close proximity to northern neighbours makes Australia's northern coastline vulnerable to incursion from HPAIS, including the HPAI H5 clade 2.3.4.4b virus. If the H5 clade 2.3.4.4b virus were to circulate in Indonesia or Papua New Guinea, nomadic waterfowl could become exposed to the virus. Infection with the H5 clade 2.3.4.4b virus in these birds could potentially result in movement of the virus to southern areas where poultry flocks are more abundant. As the epidemiology of AIVs evolves, using active surveillance to

detect both LPAI and HPAI strains along Australia's northern coastline and external territories is important for preparedness and understanding of AIVs in the region.

While coordinates have been collected at sampling sites in more recent years, it is difficult to gain meaning from these without use of a map or other infographic. For several sites, sampling occurs at different locations (e.g. within National Parks) and it is difficult to capture this in the current dataset. Having an additional variable so that a site can be recorded as well as a more specific location within a site (such as a specific lake within a National Park) would assist with streamlining data analysis of samples by site, while also enabling additional information on site location to be captured in the data to inform future surveillance targets.

The NAQS program does not store AIV data in a database, and once submitted to the NAIWB database the information is held by WHA, and this becomes the only source of data. Once data is submitted to NAIWB, the NAQS team loses direct access to the data and must request extracts from the NAIWB database from WHA if the team wants to perform any additional analysis or review of submitted information. While WHA can readily provide an extract, the need to request this data is an extra step for the NAQS team and relies on the availability of WHA staff to respond to a request. Developing a NAQS database for AI surveillance data that can easily integrate into the NAIWB database would increase the stability of the NAQS AIV surveillance system and improve the timeliness of analysis and of reporting to NAIWB.

The NAQS active surveillance component of the NAIWB surveillance program has undergone significant changes since AIV surveillance activities began. These changes are evident through stakeholder responses and changes to data, but there is little formal documentation describing these changes. It is therefore difficult to grasp the changes NAQS AIV surveillance has gone through since its establishment in 1992. For example, the changes in laboratories used by the system were evident through the NAQS NAIWB data extract, but there is no documented summary of when or why these changes occurred. Creating a dynamic record to document changes to sampling sites, sampling frequencies, laboratories, influenza A testing, target bird species and other components of the surveillance system would help to reduce the corporate knowledge required for analysis and reporting of NAQS AIV data. Additionally, the most recent national dossier on AIV surveillance was prepared in 2010 (15), and does not capture significant changes or outbreaks that have happened in AIV surveillance in the last 14 years. Updating of this dossier should also be completed to demonstrate these changes.

Surveillance of AIVs in Australia's north is an important preparedness activity. The evaluation found that, overall, the NAQS active component of the NAIWB program is a simple and flexible system. Given the complexities associated with conducting surveys in remote areas in

Australia's north, the system performs well to meets its objectives. As the system grows, additional resourcing is required for the continued success of the program.

Recommendations

NAQS

1. **Increase the capability of NAQS staff**

Enable multiple staff at one level to have data management responsibilities or take opportunities to automate data collation and analysis and increase epidemiological support for the NAQS team. This will improve the stability and timeliness of the system as it will allow for more than one person to have responsibility over management and reporting for the system.

2. **Increase the staffing and funding resources available to NAQS**

Allow for NAQS to mobilise quickly as additional surveys are required (including staff for data analysis and reporting, and funding for laboratory testing). This will improve stability, flexibility and timeliness of the system by allowing new areas to be sampled and results to be reported as required. This will also improve the ability of the system to meet its objectives.

3. **Standardise collection and reporting procedures**

Develop a data collection tool and maintain its usage for use by NAQS, the Indigenous Ranger Network, Parks Australia and other organisations involved in sample collection. This will improve the data quality, simplicity and timeliness of the system by streamlining analysis and reporting.

4. **Investigate appropriate sampling frameworks for different sites**

Undertake a study (including reviewing previous results from each site) to determine the most appropriate number of samples per site per year for the NAQS region to inform sampling strategy and planning. This will allow for the system to better meet its objectives of early detection of AIVs.

5. **Streamline reporting using an application such as R**

Develop a code that will streamline data cleaning and analysis for timely reporting to WHA. This will improve the simplicity, stability and timeliness of the system

6. **Create a NAQS database for surveillance data**

Allow NAQS to have greater oversight of NAQS data by creating a database. This will improve the stability, timeliness and data quality of the system.

NAIWB

7. **Restructure the NAIWB database including:**

- *Remove redundant variables such as a third laboratory, specific testing, and other variables no longer recorded in the NAIWB data.*
- *Create greater flexibility in location information that will inform sampling sites.*
- *Allow for the number of bird species present to be identified, and a new variable created for each species based on this number.*
- *Allow for a variable that records what species of bird sample is likely to be from.*
- *Create a 'final result' variable to summarise all testing a sample had performed into one variable, streamlining analysis. This will improve the data quality of the system and simplify analysis*

This will improve the data quality and flexibility of the system, simplifying analysis.

Conclusion

The NAQS active surveillance component of the NAIWB surveillance program is a complex system that performs its activities across a large and challenging environment. The system is highly flexible and adaptable, but is constrained by operational and resource limitations which also contribute to delays in reporting. The data collected by the system is accurate and valid, but the data quality could be improved by removing obsolete variables and allowing additional entries for some variables. Given the challenging environment NAQS works in, the system operates in a reasonably timely manner, however more research is needed to determine appropriate sampling for risk sites. High pathogenicity AI is a target pathogen of NAQS surveillance activities and the system also detects LPAIs, both which contribute to improving the understanding of AIV epidemiology across Australia's northern coastline. The system could be improved by increasing capabilities of staff and streamlining reporting and analysis practices.

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Appendix 3A: NAQS Animal Health Surveillance System and changes over time

Overview of changes over time

The NAQS surveillance program for AI in wild birds has changed significantly over the past two decades in the following ways:

- The number of sites and sampling frequency at these sites has increased over time, and since 2024 NAQS has started to explore sampling seabirds in external territories to test for AI
- Since 2014, all sampling has been environmental sampling and no longer includes sampling of hunter-shot or trapped birds. Collecting serology for active surveillance activities also ceased at this time.
- NAQS sampling workforce has expanded to include the Indigenous Ranger network, Parks Australia and other collaborators
- Use of eNAT® to collect samples is beginning to be used, allowing for samples to be transported without the need for cold chain requirements.

Surveillance objectives

The objectives of the NAQS AI active surveillance activities are to:

- Identify and monitor influenza viruses (including LPAI viruses) circulating in wild birds in Australia's north
- Provide early warning of HPAI viruses present in Australia, should they occur
- Maintain a library of LPAI strains present along Australia's northern coastline
- Provide guidance and training for jurisdictional governments in collection and management of AI samples from wild birds.

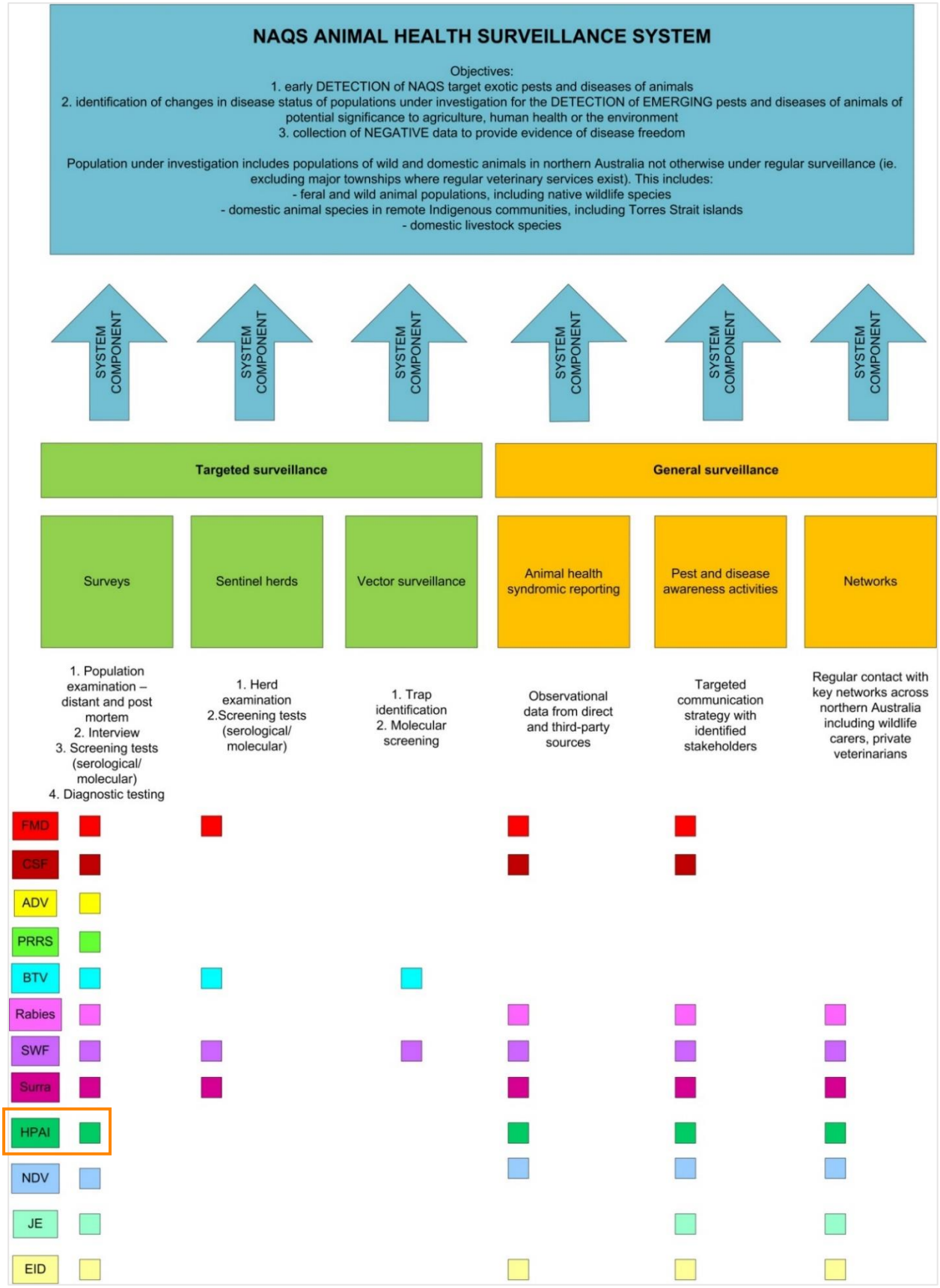

Figure 3A.1. Overview of the NAQS Animal health surveillance system

Appendix 3B: Past reviews involving the NAQS active component of the NAIWB surveillance program

Table 3B.1. Overview of past reviews involving the NAQS active component of the NAIWB program, 2011 - 2023

Year	Title	Purpose	Conducted by
2023	Review of the performance of Australia's wild bird general surveillance program for avian influenza virus	Review the performance of the passive/general NAIWB surveillance program	Em Gibson, Michelle Wille, and Marcel Klaassen
2019	Northern Australia Quarantine Strategy Risk Review	Determine overall risk for each risk area identified by NAQS	Ausvet (at the request of NAQS)
2015	Review of the National Avian Influenza Wild Birds surveillance program	Determine if the NAIWB program is meeting its objectives and if the program could be improved	Brendan Cowled and Ron Glanville (Ausvet)
2015	Contribution to the 2012 Avian Influenza in Wild Birds Surveillance Program	Outline of results from NAIWB[29] activities from July 2011 – June 2012	Tiggy Grillo
2015	Avian influenza in Australia: a summary of 5 years of wild bird surveillance	Outline of key findings from NAIWB[30] activities from July 2007 – June 2012	Tiggy Grillo and NAIWB members
2011	Administration of the Northern Australia Quarantine Strategy	Independent performance audit	Australian National Audit Office
2011	NAIWB Summary Data 2005-2010	Review data collected as part of NAIWB program including descriptive analysis	WHA (Contracted by DAFF)

[29] Presents results overall and does not distinguish results from NAQS surveillance activities
[30] Presents results overall and does not distinguish results from NAQS surveillance activities

Appendix 3C: Attributes excluded from the evaluation

Four attributes listed in the CDC Guidelines for Evaluating Surveillance Systems were excluded from this evaluation for the following reasons:

Representativeness: the NAQS system is intentionally biased to select sites based on presence of target species (32).

Sensitivity: The wild bird population size is unknown and varies with migratory patterns. The There is no system for comparison.

PVP: The wild bird population size is unknown and varies with migratory patterns. The There is no system for comparison.

Acceptability: This attribute is primarily around data collection and reporting which is managed by NAQS.

Appendix 3D: Emails sent to BVL, BSL, DDLS and ACDP

From: Gilbert, Michaela <Michaela.Gilbert@aff.gov.au>
Sent: Thursday, 16 November 2023
To:
Cc: Weerasinghe, Guy; Breed, Andrew; Iglesias, Rachel
Subject: Avian Influenza laboratory protocol - NAQS surveillance system evaluation [SEC=OFFICIAL]

Dear

I am a field epidemiology trainee placed at the Commonwealth Department of Agriculture, Fisheries and Forestry. At the National Avian Influenza in Wild Birds (NAIWB) Steering Group meeting on 9 November 2023, I spoke about an evaluation of the Northern Australia Quarantine System (NAQS) component of the NAIWB active surveillance system that I am working on as part of my training program. As part of this evaluation, I would like to describe the laboratory protocols for testing AI samples at each of the labs. This could include:

- TaqMan PCR for Influenza A
- qRT-PCR for Influenza A
- C^T cutoffs
- Differential testing
- Any H5 or H7 testing and at what point this occurs
- Information about reagents and what occurs when reagents are running low
- Transfer of samples to other laboratories for testing
- Time between sample arrival and testing
- Time testing takes
- Additional steps required for environmental faecal swab samples
- Aliquoting of samples that are sent to ACDP

Is there a laboratory protocol your laboratory has that I could have a copy of to assist me with this evaluation? I am also interested in protocols regarding the returning of samples from ACDP if this is applicable and documentation available.

Please let me know if you have any questions, I am happy to discuss further.

Kind regards,

Michaela Gilbert

MAE Scholar | One Health Section
E: Michaela.Gilbert@aff.gov.au
Department of Agriculture, Fisheries and Forestry
Office of the Chief Veterinary Officer
CQ2, 70 Northbourne Avenue, Canberra, ACT 2601
GPO Box 858 Canberra ACT 2601 Australia

Figure 3D.1: Email sent to BVL, BSL and DDLS

From: Gilbert, Michaela <Michaela.Gilbert@aff.gov.au>
Sent: Thursday, 14 December 2023
To:
Cc: Weerasinghe, Guy; Breed, Andrew; Iglesias, Rachel
Subject: Avian Influenza laboratory protocol - NAQS surveillance system evaluation [SEC=OFFICIAL]

Dear

I am a field epidemiology trainee placed at the Commonwealth Department of Agriculture, Fisheries and Forestry. At the National Avian Influenza in Wild Birds (NAIWB) Steering Group meeting on 9 November 2023, I spoke about an evaluation of the Northern Australia Quarantine System (NAQS) component of the NAIWB active surveillance system that I am working on as part of my training program. As part of this evaluation, I would like to describe the laboratory protocols for testing AI samples at each of the labs. I have reviewed the AV wild bird sample processing at ACDP (Appendix 5 of the NAIWB Operational Plan 2020-2022, diagram from page 29 included below) but wanted to clarify a few points:

- Are there additional steps required for environmental faecal swab samples beyond what is described in the operational plan?
- Is sequencing and pathotyping performed on all H5 and H7 positive samples?
- Is sequencing and pathotyping performed on other HA genes (eg. H9)? What is the C^T cutoff for this to be done?
- What is the C^T cutoff for pathotyping and sequencing to be done on the original sample compared to the isolate?
- What is the protocol for virus isolation?
- What TaqMan PCR result/s will warrant virus isolation in eggs?
- Is there a protocol for assessing virus Isolation in eggs on non-H5 / H7 samples which test positive in the Flu A (matrix gene) TaqMan PCR with a C^T value greater than 30?
- How often is the AI Australian Lineage updated?

I am also interested to know about reagents and duration of each test if there is any documentation of these that you could send through.
Please let me know if you have any questions, I am happy to discuss further.

Kind regards,

Michaela Gilbert

MAE Scholar | One Health Section
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Office of the Chief Veterinary Officer
CQ2, 70 Northbourne Avenue, Canberra, ACT 2601
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Figure 3D.2. Email sent to ACDP

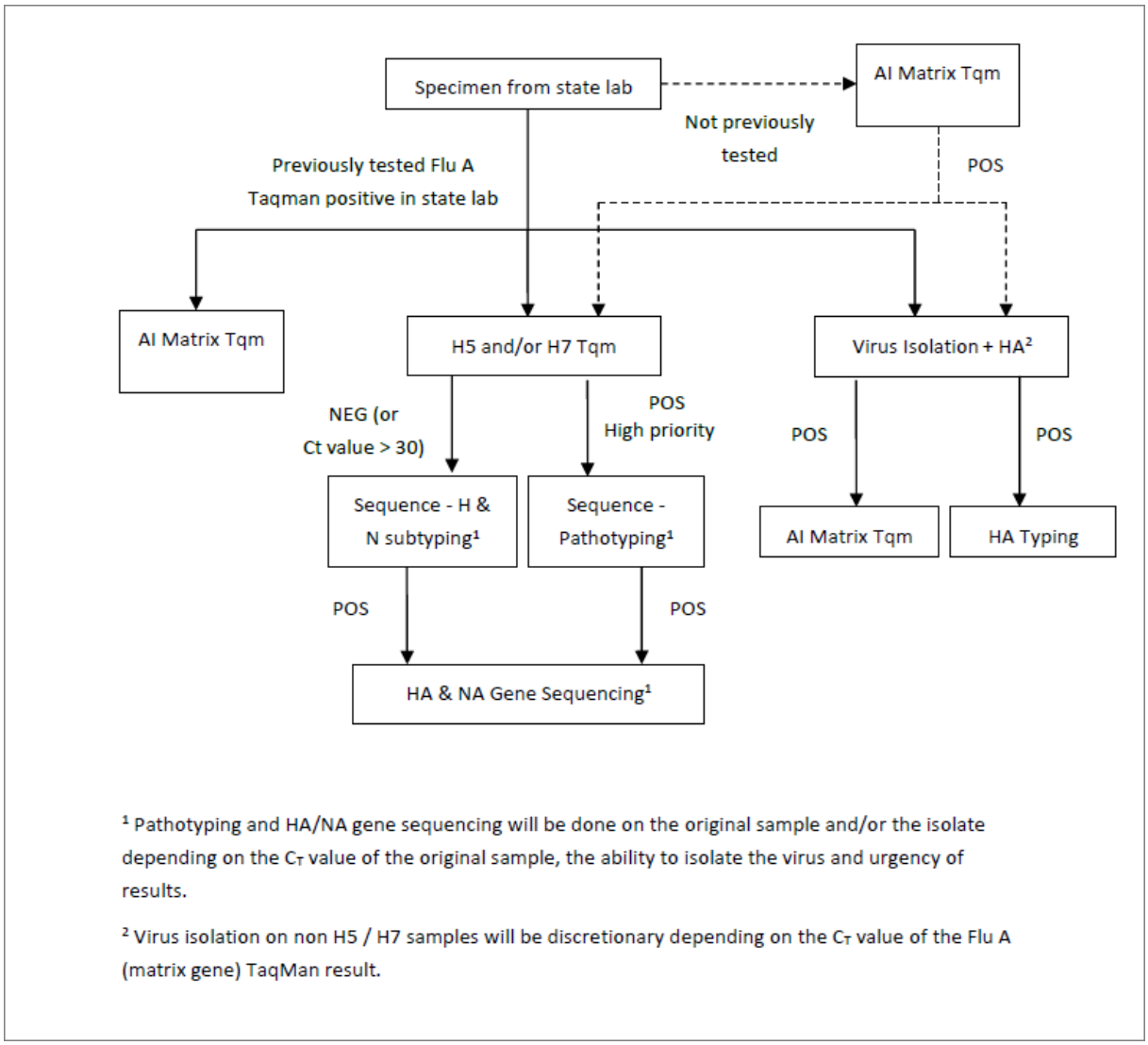


Figure 3D.3. Appendix 5 of the NAIWB Operational Plan 2020-2022, diagram from page 29

Appendix 3E: Consistency of results for samples tested at two laboratories

Table 3E.1. Results at secondary/reference laboratory for samples that tested "Positive" at primary testing laboratory

Year	Samples positive at primary laboratory	Result at secondary/reference laboratory		
		Negative	Positive	No data
2011	**27**	9	17	1
2012	**57**	7	1	49
2013	**29**	0	29	0
2014	**50**	1	3	46
2015	**24**	0	7	17
2016	**5**	0	5	0
2017	**39**	0	39	0
2018	**17**	0	17	0
2019	**1**	0	1	0
2020	**0**	0	0	0
2021	**9**	2	5	2
2022	**13**	1	11	1

Table 3E.2 Results at secondary/reference laboratory for samples that tested "Indeterminate" at primary testing laboratory

Year	Samples indeterminate at primary laboratory	Result at secondary/reference laboratory			
		Indeterminate	Negative	Positive	No data
2011	**0**	0	0	0	0
2012	**0**	0	0	0	0
2013	**0**	0	0	0	0
2014	**0**	0	0	0	0
2015	**13**	0	0	6	7
2016	**0**	0	0	0	0
2017	**1**	1	0	0	0
2018	**2**	0	0	2	0
2019	**0**	0	0	0	0
2020	**0**	0	0	0	0
2021	**6**	0	1	0	5
2022	**0**	0	0	0	0

Appendix 3F: HA testing results at primary and secondary laboratories

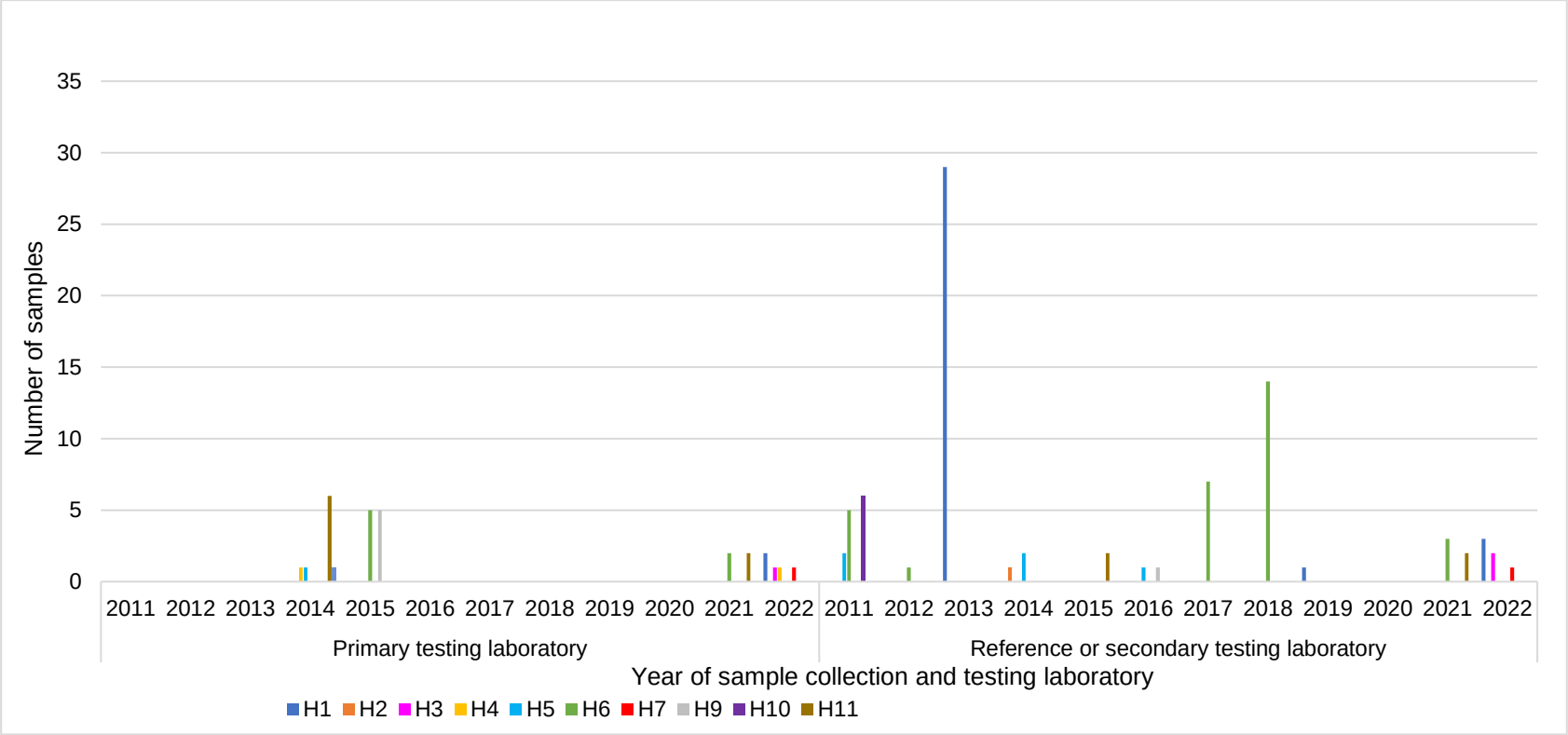


Figure 3F.1. HA subtype results[31,32] for samples positive or indeterminate to Influenza A by primary[33] and secondary[34] testing laboratory, 2011 – 2022

[31] HA typing resulted from at least one typing test at laboratory
[32] For positive or indeterminate influenza A samples that were successfully typed
[33] ACDP included as a primary testing laboratory in 2020, 2021 and 2022
[34] ACDP used as a secondary testing laboratory for all years. EMAI also included as secondary laboratory in 2011, and Agriculture Victoria also included as secondary laboratory in 2011 and 2013.

Appendix 3G: Birds listed in NAQS data 2011 - 2022

Table 3G.1. Overview of birds included in NAQS NAIWB data extract by Order, 2011-2022

Shorebirds	**Waterfowl**	**Other**
Shorebirds (Charadriiformes)	Waterfowl (Anseriformes)	Hooked-bill birds of prey *(Accipitridae)*
Masked lapwing (*Vanellus miles*)	Ducks *(Anatidae)*	Australian Pelican (*Pelecanus conspicillatus*)
Black-winged stilt (*Himantopus himantopus*)	Ducks *(Tadorini)*	Cormorant (*Phalacrocorax*)
Stilts (*Himantopus*)	Wandering whistling duck (*Dendrocygna arcuate*)	Black-necked stork (*Ephippiorhynchus asiaticus*)
Silver gull (*Larus novaehollandiae*)	Plumed whistling duck (*Dendrocygna eytoni*)	Cockatoos (*cacatuidae*)
Sandpiper (*Scolopacidae*)	Magpie goose (*Anseranas semipalmata*)	Litte corella (*Cacatua sanguinea*)
Arctic-breeding shorebirds *(Calidris)*	Pacific black duck (*Anas superciliosa*)	Red-tailed black cockatoo (*Calyptorhynchus banksia*)
Great knot (*Calidris tenuirostris*)	Pink-eared duck (*Malacorhynchus membranaceus*)	Black kite (*Milvus migrans*)
Ruddy turnstone (*Arenaria interpres*)	Grey teal (Anas gracilis)	Australian white ibis (*Threskiornis molucca*)
Reck-necked stint (*Calidris ruficollis*)	Australian shelduck (*Tadorna tadornoides*)	Eurasian coot (*Fulica atra*)
Sharp-tailed sandpiper (*Calidris acuminata*)	Radjah shelduck (*Tadorna radjah*)	Magpie lark (*Grallina cyanoleuca*)
Red knot (*Calidris canutus*)	Hardhead (*Aythya australis*)	Australasian grebe (*Tachybaptus novaehollandiae*)
Broad-billed sandpiper (*Limicola falcinellus*)	Australian wood duck (*Chenonetta jubata*)	Oriental dollarbird (*Eurystomus orientalis*)
Plovers (*Charadius*)		
Siberian sand plover (*Charadrius mongolus*)		
Black-fronted dotterel (*Elseyornis melanops*)		

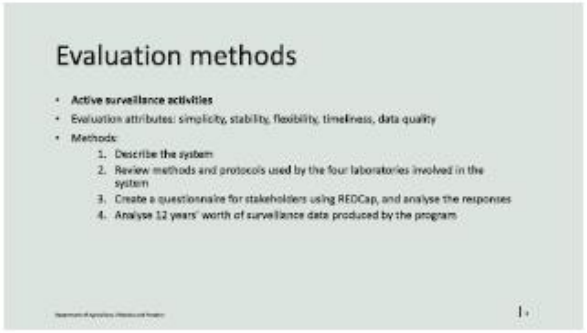
Common sandpiper (*Actitis hypoleucos*) White-winged tern (*Chlidonias leucopterus*) Terns (*Sterninae*) Common tern (*Sterna hirundo*) Stilts and avocets (*Recurvirostridae*) Whiskered tern (*Chlidonias hybrida*) Red-capped plover (*Charadrius ruficapillus*) Gull-billed tern (*Sterna nilotica*) Comb-crested jacana (*Jacana gallinacean*) Australian pratincole (*Stiltia Isabella*) Pied oystercatcher (*Haematopus longirostris*) Bar-tailed godwit (*Limosa lapponica*) Black-tailed godwit (*Limosa limosa*) Far Eastern curlew (*Numenius madagascariensis*) Ruddy turnstone (*Arenaria interpres*) Beach stone-curlew (*Esacus magnirostris*) Eurasian whimbrel (*Numenius phaeopus*) Grey plover (*Pluvialis squatarola*)		White-bellied sea eagle (*Haliaeetus leucogaster*) Glossy ibis (*Plegadis falcinellus*) Swamphen (*Porphyrio*) Royal spoonbill (*Platalea regia*) Pied heron (*Ardea picata*) Medium egret (*Ardea intermedia*) Brolga (*Grus rubicundus*) Nankeen night heron (*Nycticorax caledonicus*) Rainbow bee-eater (*Merops ornatus*) Whistling kite (*Haliastur sphenurus*) White necked heron (*Ardea pacifica*) Little egret (*Egretta garzetta*) Woodswallow (*Artamus*) Striated heron (*Butorides striatus*) Pacific Reef heron (*Egretta sacra*)

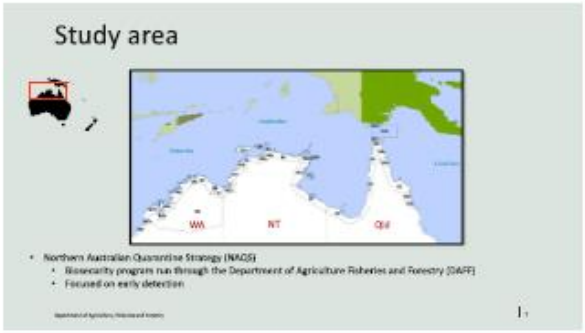
Appendix 3H: List of all variables included in NAIWB dataset 2011 – 2012

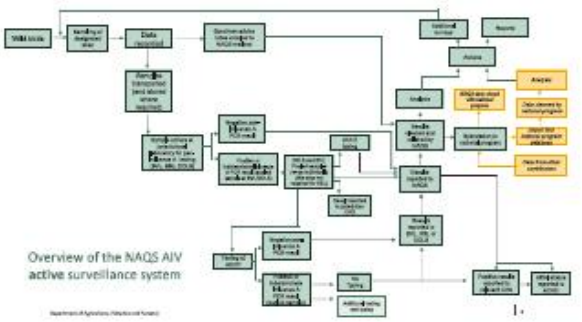
- Program name
- Collection Date
- Real date
- Host ID
- Collection ID
- LocOrgName
- OriLocLat
- OriLocLon
- Species abundance
- State
- Source
- Sample type
- Health
- Age
- Pool size
- Collection comments
- Lab1 Name
- Lab1 Sample ID
- APMVLab1-TestYesNo
- APMVLab1-EggCulture
- APMVLab1-Patho
- APMVLab1-VirusIsolate
- APMVLab1-Cleav
- APMVLab1-HNExt
- APMVLab1-Comment
- APMVLab1-Genotype
- APMVLab1-TestType
- APMVLab1-TestResult
- APMVLab1-TestCT
- Lab1 Type A PCR
- Lab1 Type A PCR CT
- Lab1 Type A PCR Rep
- Lab1 Type A PCR Rep CT
- Lab1 Cleavage seq
- Lab1 H5 test
- Lab1 H5
- Lab1 H5 CT
- Lab1 H7 test
- Lab1 H7
- Lab1 H7 CT
- Lab1 HA test
- Lab1 HA subtype
- Lab1 N1 test
- Lab1 N1
- Lab1 NA test
- Lab1 NA subtype
- Lab1 Patho type
- Lab1 Egg Culture
- Lab1 Final Virus Isolate
- Lab1 Comments
- Lab2 Name
- Lab2 Sample ID
- APMVLab2-TestYesNo
- APMVLab2-EggCulture
- APMVLab2-Patho
- APMVLab2-VirusIsolate
- APMVLab2-Cleav
- APMVLab2-HNExt
- APMVLab2-Comment
- APMVLab2-Genotype
- APMVLab2-TestType
- APMVLab2-TestResult
- APMVLab2-TestCT
- Lab2 Type A PCR
- Lab2 Type A PCR CT
- Lab2 Type A PCR Rep
- Lab2 Type A PCR Rep CT
- Lab2 Cleavage seq
- Lab2 H5 test
- Lab2 H5
- Lab2 H5 CT
- Lab2 H7 test
- Lab2 H7
- Lab2 H7 CT
- Lab2 HA test
- Lab2 HA subtype
- Lab2 N1 test
- Lab2 N1

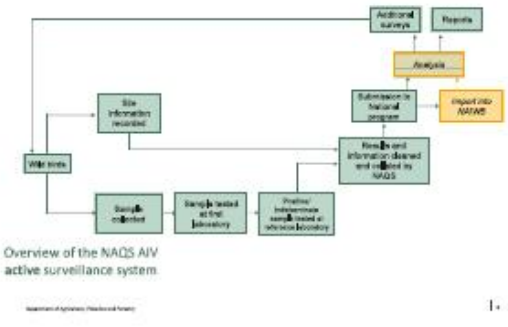
• Lab2 NA test	• Lab2 NA subtype	• Lab2 Patho type	• Lab2 Egg Culture	• Lab2 Final Virus Isolate	• Lab2 Comments
• Lab3 Name	• Lab3 Sample ID	• APMVLab3-TestYesNo	• APMVLab3-EggCulture	• APMVLab3-Patho	• APMVLab3-VirusIsolate
• APMVLab3-Cleav	• APMVLab3-HNExt	• APMVLab3-Comment	• APMVLab3-Genotype	• APMVLab3-TestType	• APMVLab3-TestResult
• APMVLab3-TestCT	• Lab3 Type A PCR	• Lab3 Type A PCR CT	• Lab3 Type A PCR Rep	• Lab3 Type A PCR Rep CT	• Lab3 Cleavage seq
• Lab3 H5 test	• Lab3 H5	• Lab3 H5 CT	• Lab3 H7 test	• Lab3 H7	• Lab3 H7 CT
• Lab3 HA test	• Lab3 HA subtype	• Lab3 N1 test	• Lab3 N1	• Lab3 NA test	• Lab3 NA subtype
• Lab3 Patho type	• Lab3 Egg Culture	• Lab3 Final Virus Isolate	• Lab3 Comments		

Appendix 3I: Slides for ISVEE presentation

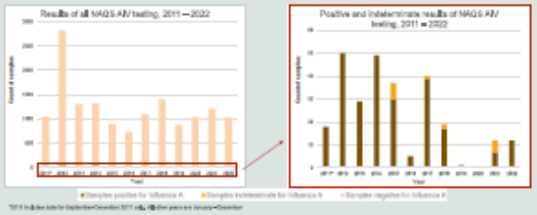








AIV results



Key Recommendations

1. **Standardise collection and reporting procedures**
   Develop a data collection tool and maintain its usage for use by NAQS, the Indigenous Ranger Network and Parks Australia. This will improve the data quality, simplicity and timeliness of the system by streamlining analysis and reporting.
2. **Investigate appropriate sampling frameworks for different sites**
   Undertake a study to determine the most appropriate number of samples per site per year for the NAQS region to inform sampling strategy and planning. This will allow for the system to better meet its objectives of early detection of AIVs
3. **Create a NAQS database for surveillance data**
   Allow NAQS to have greater oversight of NAQS data by creating a database. This will improve the stability, timeliness and data quality of the system.
4. **Increase the capability of NAQS staff**
   Improve data management skills and increase epidemiological support available for the NAQS team. This will improve the stability and timeliness of the system
5. **Amend the national database**
   Remove redundant variables, restructure how wild bird data is recorded, restructure how laboratory data is recorded. This will improve the data quality and flexibility of the system.

System achievements

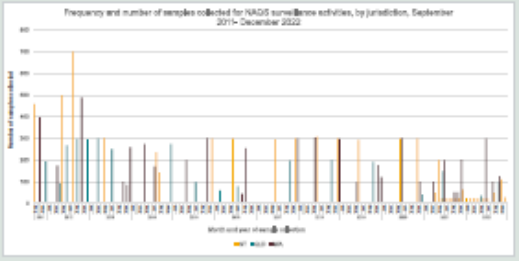
- Regarded as a simple system by those who collect and test samples and those who receive reports
- Considered reliable and consistent, given the challenges of working in remote environments
- Highly flexible
  - Sites, frequency of sampling, testing turnaround time
  - Surveillance of other viruses
- Provides library of low pathogenicity AIVs circulating in Australia's north
- System funded since 2004

Thank you to:

- Dr Guyan Weerasinghe, Technical manager of Animal Health (NAQS/DAFF)
- Dr Rachel Iglesias, Principal Veterinary Officer (OCVO/DAFF)
- Dr Andrew Bread, Principal Veterinary Officer (DAFF)
- Dr Troy Laidlow, Veterinary Officer (DAFF)
- Dr Rezanur Rahaman, Research Fellow (NCEPH/ANU)
- The NAIWB Steering Group
- Wildlife Health Australia

System limitations

- Coordination of sampling and collation of data is complex
- Majority of analysis and collation is done manually
- Data does not integrate well into national database
- Data held by national program only
- System does not collect 300 samples per site per calendar year
- Time delays
  - Reporting, sample transport
- Lack of responses from human and environmental sectors to questionnaire
  - lack of engagement/interest from these sectors
- Poor understanding of purpose of system
  - Funded to detect HPAI, limited poultry industries in area



Chapter Five:

Teaching activities

Table of Contents

Prologue

As part of the Masters of Applied Epidemiology (MAE) program, I completed the following teaching activities:

- Lessons from the Field (LFF) – Animal disease investigations in low resource settings
- Teaching to first years – One Health Response: An Introduction

This chapter provides an overview of these teaching activities.

Lessons from the Field - Animal disease investigations in low resource settings

Background

The MAE requires scholars to prepare a LFF to teach to a group of peers. My outbreak investigation of very virulent infectious bursal disease (vvIBD) in the Solomon Islands project (see Chapter 4) was my first introduction to investigating animal disease outbreaks, and so I decided to use this experience as a basis for my LFF.

Learning objectives

There were 4 learning objectives developed for this LFF:

1. Identify differences in data collection and analysis between animal disease investigations and human disease investigations.
2. Understand the challenges unique to animal disease investigations.
3. Understand the challenges of investigating outbreaks in low-resource settings.
4. Apply a One Health approach to animal disease investigations.

Case study and teleconference overview

Case study

A week before the teleconference, I provided my peers with an overview of vvIBD, some basic points about chicken production, and contextual information for an imagined low resource setting called ‘Tropical Island’, with geographic and population features similar to Guadalcanal (the largest island of the Solomon Islands). I used a subset of the data provided as part of my outbreak investigation to teach this LFF. I prepared the data by de-identifying all information, reducing the number of chickens and farms in the data, and making changes to laboratory and farm history information to simplify the teaching of the topic.

I prepared 10 questions for my peers and used the responses from my peers to inform the content of the teleconference (Appendix 5A.). These questions included asking my peers to

identify diseases in animals that have significance to humans but do not cause disease in humans, and why vvIBD, a disease that is not zoonotic, should be investigated.

Teleconference

I developed the teleconference using the responses from the case study. As many responses from my peers had focused on the ability of vvIBD to cause illness in humans I decided to bring the focus to animal health using the example of avian influenza (AI), a well-known disease with significance to One Health. AIs fall into two classifications: high pathogenicity AI (HPAI) or low pathogenicity AI (LPAI). These classifications are based on how the disease impacts poultry flocks and not on the ability of the virus to infect any other species. By highlighting this to my peers I aimed to demonstrate that diseases of animals, even those with spillover potential, are important to investigate because of how they cause disease in animals, not humans. I noted that diseases in animals can have flow-on effects for humans such as economic loss, and so are of importance to One Health. I then provided summary of the vvIBD case study and worked through my peers' responses to each of the questions, ensuring a focus on One Health in my explanations.

Personal reflections and lessons learned

Engagement with session

The LFF required me to teach my peers about a topic they were unfamiliar with. To keep my peers engaged with the LFF, I related animal disease investigations to public health investigations, as the latter is something familiar to my peers. This helped to keep my peers engaged, and demonstrated to me that relating content to audience's experiences is something I should do for any session I teach. The classification and laboratory testing questions stimulated a lot of discussion from the group, and there was also a lot of discussion about control strategies for vvIBD. These are also areas that my peers could relate to in public health, and this further demonstrated to me the importance of relating content to my audience's experiences.

One Health

Many of the case study responses from my peers focused on the zoonotic potential of vvIBD. This disease is not zoonotic and the responses indicated that I needed to communicate this more strongly. If I were to plan similar lesson for an audience with limited exposure to animal health, I would spend more time going over animal diseases and why diseases that don't cause illness in humans can still have One Health significance. The LFF highlighted to me that One Health is a concept that many of my peers have not had experience with, and I feel my LFF has aided in their understanding of One Health and of understanding animal health investigations.

Evaluation

My LFF was well received by my peers. I felt that some of my peers were slightly overwhelmed by the topic, but the feedback I received indicated that the content was interesting, and a few participants reported that they enjoyed working on something different to what they are familiar with. One participant with a clinical background commented that they felt challenged in the activity as they prefer being able to take histories from patients and asking them about symptoms, rather than relying on recounts from farmers. I used Qualtrics to collect evaluations of the LFF, and responses indicated that the lesson met the learning objectives, the content was stimulating, and my presentation was engaging (Appendix 5B).

Teaching to first years

Background

During second year, MAE scholars prepare a teaching session for first year MAE scholars. This teaching session occurs during course block one for first years, which overlaps with course block three for second years. Planning for teaching to first years began in November 2023 and with four other scholars I helped to develop a One Health teaching session titled "One Health Response: An Introduction". Collectively, we decided we would use a Japanese encephalitis (JE) case study to teach components of One Health, with a focus on the importance of collaboration between sectors.

Learning objectives

We developed 3 SMART learning outcomes that focused on building an understanding of One Health and the importance of collaboration in a response:

1. Outline the concepts of One Health.
2. Identify the stakeholders required for a One Health response to an acute incident.
3. Discuss the importance of a multi-sectoral approach in a One Health response.

Content and lesson planning overview

Lesson structure

During Course Block three, I worked with three of my peers to develop a lesson plan (a scholar previously involved in the planning did not attend course block three). To make our lesson as interactive as possible, we decided to start our lesson with questions to the class about knowledge of One Health. One of my group members suggested that our lesson should include a debate or have a stakeholder discussion to emphasise the need for collaboration of different sectors for a One Health response, and to make the lesson as interactive as possible. We decided we would divide the class of 15 into four groups of 3-4, representing public health, agriculture/animal health, environmental health and the pork industry. We discussed whether we should use a second disease example (Avian Influenza) but ultimately decided this may become too complex for an introductory session and so focused on JE only. An overview of the lesson is provided in Appendix 5C.

Previous knowledge

I assisted with the JE public health response in NSW in 2022, and during my time at the Department of Agriculture, Fisheries and Forestry (DAFF) I have become familiar with how JE affected the Australian pork industry in 2022, as described in my combined data analysis and epidemiology study project (see Chapter 2). I brought these experiences into planning the content for this lesson, particularly the animal health components. I also developed an initial

design of the lesson plan for comment by my peers and provided a presentation on JE authored by my DAFF field supervisor Rachel Iglesias for background.

My role

In the lesson planning sessions, I was responsible for developing the four fictional scenarios and talking points for each of the sectors for the group activity. In these scenarios, I hinted at the need for sectors to collaborate with each other (for example, the pork industry scenario included concern from piggery workers about vaccination eligibility, which required collaboration with the human health sector). We based the fictitious scenarios in Queensland and while the aim was to accurately reflect the responsibilities of each sector, I exaggerated some points to aid with teaching. These scenarios were reviewed by my 3 group members and are available in Appendix 5D. With another member of my group, I prepared slides detailing the response to JE in Australia and other examples of One Health in Australia. I also reviewed the slides that my group members prepared and ensured our formatting was consistent throughout the slide deck.

Lesson delivery

Lesson introduction

Our group presented the first session of the day to the first years. During the lesson, I was responsible for discussing conditions that might require a One Health approach with the first years. Some suggestions of diseases from the first years included Buruli ulcer, arboviruses and, unexpectedly, lumpy skin disease, all of which I built on. I also highlighted some other conditions including salmonellosis and antimicrobial resistance.

Japanese encephalitis in Australia

During the scenario exercise, I worked with the first years that were representing the agriculture/animal health sector to facilitate their discussions. After the group scenario, I gave an overview of the JE incursion in Australia in 2022, tying in what had occurred during the scenario activity and noting that the virus was first confirmed in pigs, and confirmation in humans was shortly after this. I used this overview to help illustrate how collaboration was essential for the response. I then summarised the *Joint National Japanese Encephalitis Virus Outbreak Response Plan* published in June 2023.

Lesson conclusion

To conclude the lesson and bring the focus back to One Health in general, I provided some additional examples of One Health collaborations in Australia. These included introducing the first years to the Human Animal Spillover and Emerging Disease Scanning (HASEDS) group, highlighting DAFF's involvement with the Communicable Diseases Network Australia, and briefly summarising the *One Health Master Action Plan for Australia's National Antimicrobial*

Resistance Strategy – 2020 and beyond. I then concluded the teaching session by presenting the One Health diagram shown at the start of the session, to relate the session back to the core One Health concepts and the learning outcomes.

Personal reflections and lessons learned

Lesson planning

Teaching to first years required me to work with a group of my peers to deliver an interactive session. I brought some of my past experience in teaching to the planning sessions and built on my skills through collaboration with my peers to deliver a session. Our group worked well together, and everyone was able to freely share ideas for the session.

Lesson delivery

During the teaching session, my teaching group and I all realised as the lesson went on that we needed to ask the first years more questions throughout the session to keep them engaged, and preparing prompts to the audience throughout a lesson is something I will keep in mind the next time I teach. We gave the first year's opportunities to ask questions regularly throughout the session, and this helped generate some interesting discussions and improved the session overall. Our lesson went by quickly, and I learned that it is important to allow for more extra time in lessons than I thought, especially if an activity is planned. In future, I will allow for additional time for group activities when planning a lesson.

Evaluation

The One Health teaching session was well-received by the first years. The MAE academic staff that were present during the session also commended our session. All first years enthusiastically participated in the scenario activity, and they asked us questions throughout the session. We used Mentimeter to collect student evaluations of the session, and these responses indicated that the session met the learning objectives, and that the session was engaging and interactive (Appendix 5E).

Appendix 5A: LFF Questions

Background

1. **What do you think are some of the key reasons for investigating this outbreak?**

2. **What are some key considerations that are needed for successful data collection?**

Data

Refer to attachment MG_LFF_vvIBD_data.xlsx for the following questions. This spreadsheet contains two tabs – one with bird-level data and one with farm-level data.

3. **What are some of the key similarities and differences you notice in this data compared to data collected in human disease investigations?**

4. **What information would you use for descriptive analysis (i.e "person", place and time)**

5. **The results for individual birds are presented in columns J (serology results) and L (PCR results) in the tab 'Bird data'. PCRs were tested as pooled samples**
    a. **On what basis would you classify a farm as 'infected'?**
    b. **What do you notice about the pooled samples? If any issues, how would you deal with these?**

6. **How would you combine the analysis of the farm-level data and individual bird data in your investigation of this outbreak?** *(Please explain how you think you would do this – you do not need to identify specific analyses or provide R code etc.)*

7. **What are some of the limitations with this data? How could these be addressed?**

8. **Please describe the key challenges you had exploring/working with this data. What are some potential solutions?**

One Health

9. **In what ways does this outbreak relate to human health and environmental health outcomes? How could this be addressed using a One Health Approach?**

10. Can you think of other animal diseases that do not cause illness in humans? Why should this disease/s be investigated?

Appendix 5B: LFF evaluation

Figure 5B.1: Responses to Lesson from the Field evaluation question "How strongly do you agree that the session met each of the learning objectives"

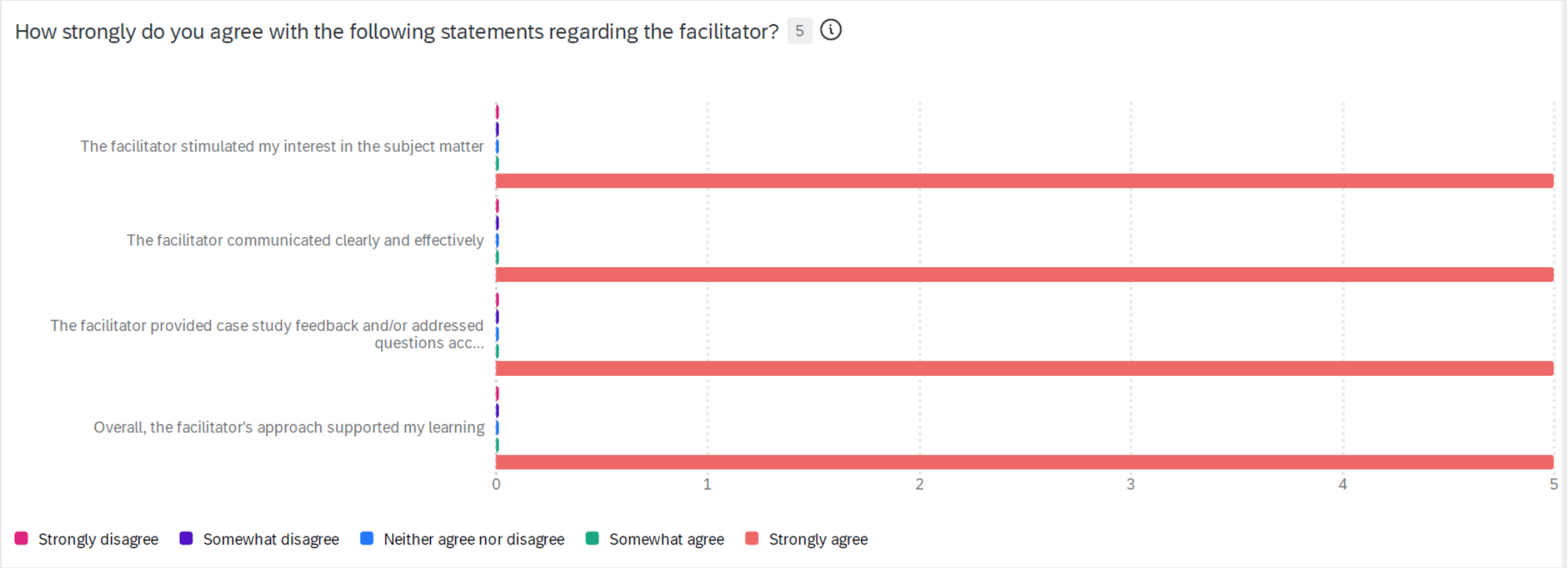


Figure 5B.2: Responses to Lesson from the Field evaluation question "How strongly do you agree with the following statements regarding the facilitator?"

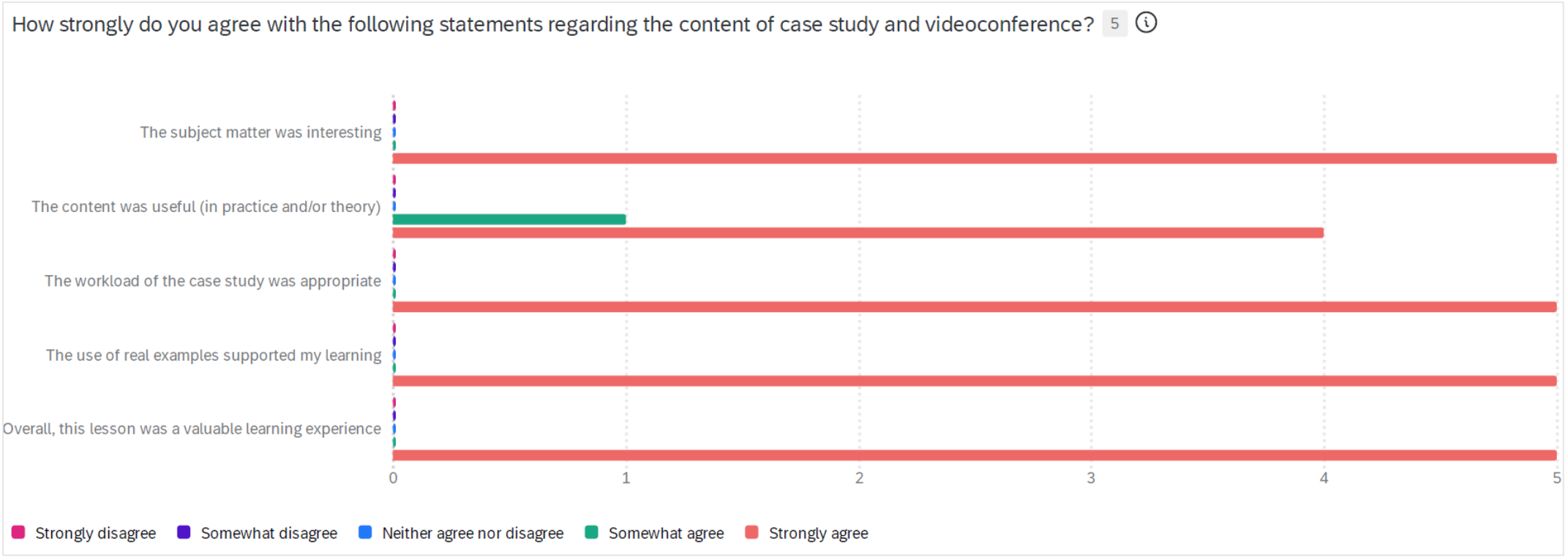


Figure 5B.3: Responses to Lesson from the Field evaluation question "How strongly do you agree with the following statements regarding the content of case study and videoconference?"

Additional comments

I think you did a fantastic job teaching animal outbreak investigations to a group of people only used to public health investigations. Really interesting case study. Great work.

Interesting discussion on the importance of a one health approach and the differences between animal and human disease outbreaks:

It was great to think about something new and really challenge my existing knowledge.

Appendix 5C – Teaching to First Years learning objectives and outline

Learning Objectives:

1. Outline the concepts of One Health.
2. Identify the stakeholders required for a One Health response to an acute incident.
3. Discuss the importance of a multi-sectoral approach in a One Health response.

Lesson Outline:

1. Introduction to One Health
    a. What is One Health?
    b. Diseases of Significance to One Health
    c. Stakeholders involved in a One Health response
2. Japanese Encephalitis
    a. Disease background
3. Scenario-based exercise
4. One Health in Australia – Japanese Encephalitis and beyond

Presentation and presenters of *One Health Response: An Introduction*

From L to R: Victoria Marriott, Michaela Gilbert (me), Marie Heloury, Elaine Ung

Appendix 5D: Lesson handouts

Handout 1. Agriculture (Queensland Department of Agriculture and Fisheries)

Responsibility: Maintaining access to markets, protecting animal welfare and managing the delivery of regional assistance to industry.

Affiliated with: The Commonwealth Department of Agriculture, Fisheries and Forestry

Scenario knowledge:

- Surveillance activities have identified positive detections of JEV in feral pigs in the Cape York Peninsula
- Other states (NSW and Vic) have reported detections of JEV in piggeries
- Southern Queensland pork industries are concerned about the risk of JEV to their industry.
- Impacts on the pork industry could impact supply of pork products at supermarkets in Australia and products that are exported.

Handout 2. Public Health (Queensland Health)

Responsibility: Protect the community from public health threats, including through surveillance, research and health promotion.

Affiliated with: The Commonwealth Department of Health and Aged Care

Scenario knowledge:

- Southern states of NSW have reported sporadic cases of Japanese Encephalitis in people
- 2 individuals presented to Robina Hospital with symptoms of encephalitis, cause unknown (Viral and bacterial panels negative).
- Individuals are unrelated to each other and come from different LGAs in Southern Queensland. One individual works at BigPig Pork.
- JEV vaccination is not currently available in Queensland (other than travel vaccines).

Handout 3. Industry (BigPig Pork Farms)

Responsibility: Multibillion-dollar industry for the Australian economy. As a primary producer, BigPig is responsible for maintaining the health of pig herds and reporting any notifiable diseases to the state agriculture department.

Affiliated with: State agriculture department (as required)

Scenario knowledge:

- Litter abnormalities have been observed in BigPig Pork farms in Southern Queensland for 2 weeks, including an increase in the number of stillbirths and number of births of underdeveloped piglets
- All standard testing has been negative to date.
- If litter abnormalities continue at the current rate there could be serious implications for BigPig to meet their market expectations.
- Staff at BigPig have voiced concerns about whether whatever is causing the litter abnormalities could affect their own health.

Handout 4. Ecology/Environment (Queensland Environment Department)

Responsibility: Protect and manage parks and forests for current and future generations, enhance Queensland's ecosystems, conserve and protect Queensland's biodiversity and threatened species

Affiliated with: Commonwealth Department of Climate Change, Energy, Environment and Water

Scenario knowledge:

- It has been an uncharacteristically wet and warm summer and mosquito abundance has been described as “very high” in Southern Queensland
- Waterbirds (such as herons) have been sighted in unusual areas, including in close proximity to large piggeries.
- Entomologists (including mosquito trappers) are concerned that they may be exposed to mosquito borne viruses.
- There is concern that Koalas (a threatened species) may be susceptible to severe illness from Japanese Encephalitis.

Appendix 5E: Teaching to first years' evaluation

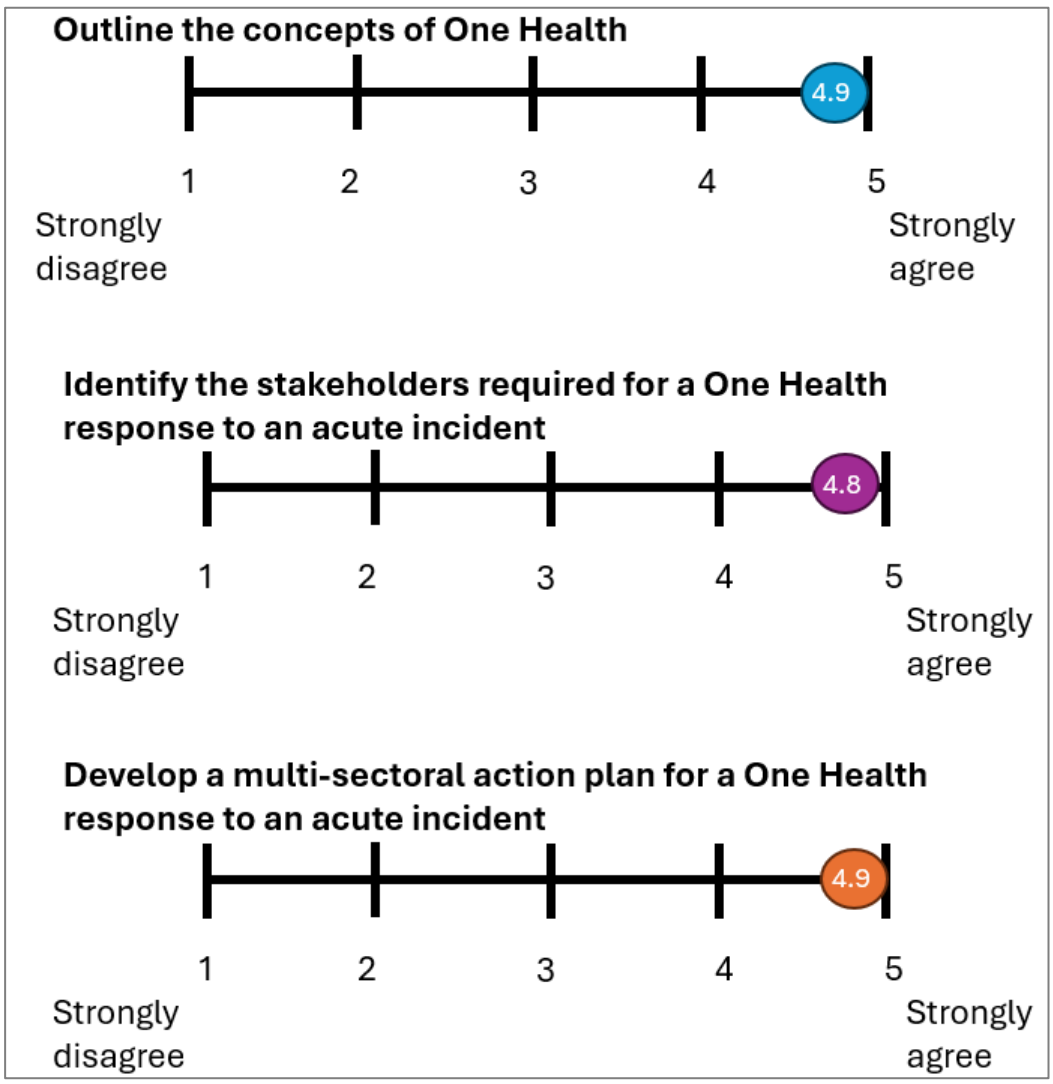


Figure 5E.1 – Averages of responses to the evaluation question "How well did the session meet the learning objectives?"

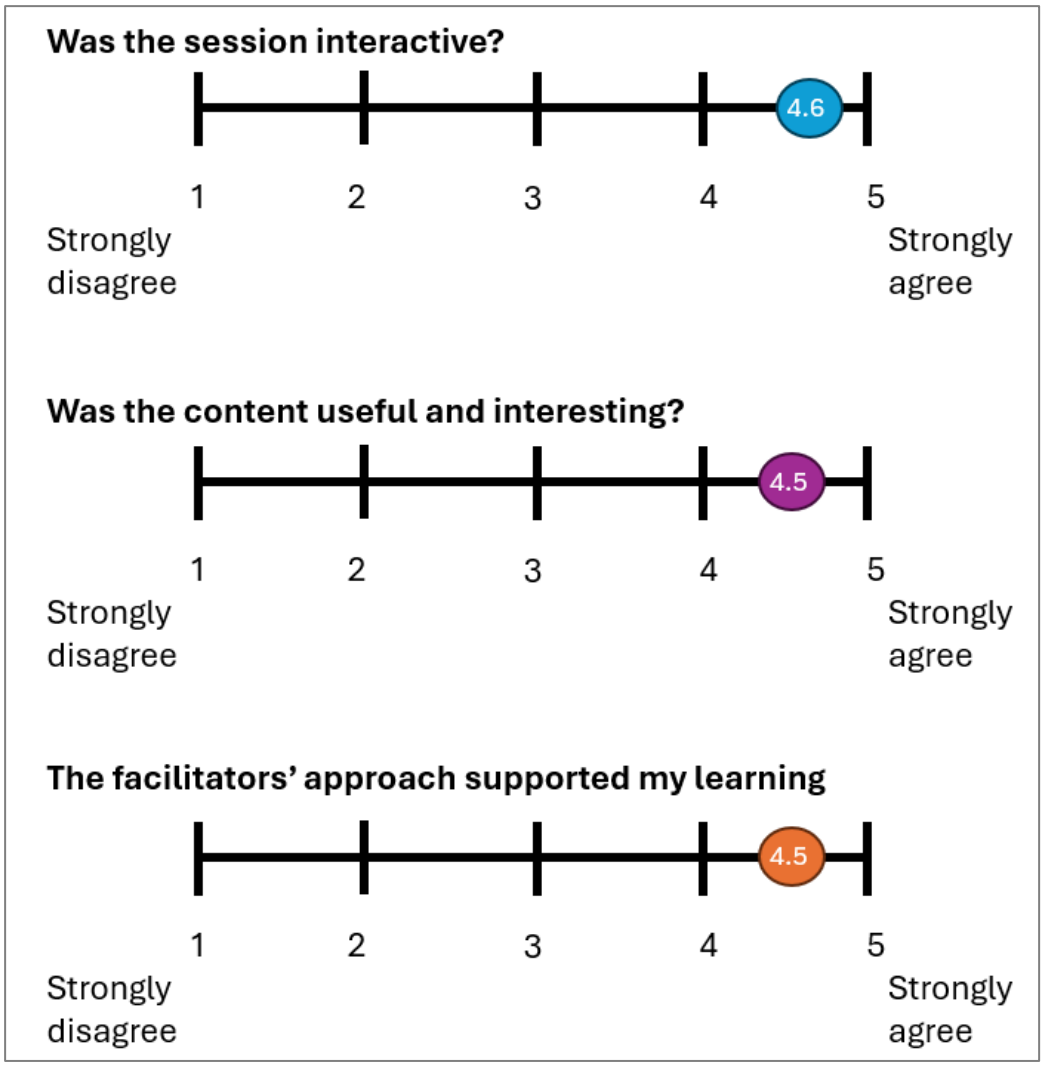


Figure 5E.2 Averages of responses to Teaching First Years Evaluation questions on session quality

Chapter Six

Other experiences

Contents

Overview

During the Master of Philosophy in Applied Epidemiology (MAE), I was involved in several activities that supported or were in addition to my core competencies. This chapter consists of the following:

- My summary for a lay audience
- Additional activities I was involved in while at the Department of Agriculture, Fisheries and Forestry (DAFF)
- Fieldwork reflections
- Additional activities I was involved in through the Australian National University.

These additional experiences further developed my understanding of One Health and my skills in field epidemiology.

Minor competencies

Lay summary

My lay summary was an entry for the June 2024 edition of the Three Chiefs Newsletter. This web-based newsletter provides regular updates from the Australian Chief Plant Protection Officer, the Australian Chief Environmental Biosecurity Officer (CEBO) and the Australian Chief Veterinary Officer (CVO) and their teams. My submission to the newsletter focused on my experience completing the MAE at DAFF, highlighting my work on Japanese encephalitis virus (JEV) in pigs for my data analysis and epidemiology project (see Chapter Two), and my very virulent infectious bursal disease virus (vvIBDV) outbreak investigation (see Chapter Four). This article is available in appendices 6A and 6B and was shared with the Australian Applied Epidemiology Network and published on the ANU MAE webpage.

DAFF experiences

The Veterinary Epidemiology and Surveillance Throughout Australia Network

I was asked to coordinate the Veterinary Epidemiology and Surveillance Throughout Australia (VESTA) network on my first day at DAFF. VESTA is a network for government animal health staff working at a national or jurisdictional level with an interest or qualification in veterinary epidemiology, surveillance or a related field. Members include veterinarians and other animal health professionals working in field, policy or laboratory roles. The coordinator role involves organising and moderating webinars, sending out announcements to network members, signing new members up to the network, and helping to foster collaboration across the network. I was supported by several other staff in this role, notably Rachel Iglesias, Emily Sellens, Rebekah Burns, Andrew Breed, Troy Laidlow, Ed Annand and Zarzeez Anindya. Through this

role, I learned about animal health activities and roles across Australia. I also developed skills in networking with members, moderating meetings, and other administrative duties associated with the network.

Wildlife Health Australia groups

DAFF works closely with Wildlife Health Australia (WHA) and as part of their stakeholder engagement strategy WHA convenes several surveillance and interest groups. These groups include representatives from different levels of government as well as from industry, university, individual experts and non-government organisations. I attended the quarterly meetings as a representative for DAFF for several of these groups. Prior to commencing the MAE, I was unaware of WHA's role in disease surveillance in wild animals and coordinating wildlife health stakeholders across the country. Wildlife Health Australia has a strong One Health focus in all of its activities.

I sat on the following WHA groups:

- The National Avian Influenza Wild Birds (NAIWB) steering group
- The Bat Health Focus Group
- The Zoos and Sentinel Surveillance Program.

The Human and Animal Spillover and Emerging Diseases Scanning group

I assisted with a stakeholder mapping activity for the Human and Animal Spillover and Emerging Diseases Scanning (HASEDS) group in early 2023. The HASEDS group is a multi-disciplinary group that prepares joint risk assessments for the CVO, the CEBO and the Chief Medical Officer, and the stakeholder mapping activity developed my understanding of One Health networks throughout Australia and abroad, including university groups and government and non-government organisations.

Cross-departmental exercises

Exercise Waterhole

In November 2023, members of the Epidemiology, Surveillance and Laboratory section in the Animal Health Policy branch coordinated Exercise Waterhole. This national exercise was designed to assess the ability of the animal health laboratory network to respond to two concurrent animal disease outbreaks – high pathogenicity avian influenza (HPAI) and lumpy skin disease (LSD). Members of DAFF facilitated the event, acting as the Operations Centre as well as acting as members of the public and other key personnel (such as couriers and district veterinarians). This included calling and emailing state and territory laboratories with scenarios to see how each laboratory would respond. I attended the exercise as an observer, but also made some phone calls to laboratories for the exercise.

Exercise freedom bird

In December 2023, I was invited to participate in an exercise run by the Department of Health and Aged Care (DoHAC) called Exercise Freedom Bird. The exercise was developed in collaboration with DAFF to explore how DoHAC and DAFF would respond to an outbreak of HPAI affecting poultry, wildlife and humans in Australia, and focused on how DAFF responds to emergency diseases such as HPAI, and at what point DoHAC may be involved. The exercise had a strong focus on collaboration between the two departments.

Fieldwork

Piggery visit

To support my investigation into JEV in pigs in Australia (see Chapter Two) I visited a piggery in NSW in August 2023. The piggery was in a mixed agricultural area in a council zone of around 6,000 people, with a small town close by. The region included cattle, sheep and canola farms. It had rained the night before my visit, and this allowed me to see how water pooled in the area as it may have done in late 2021 and early 2022.

There were stringent biosecurity rules to enter the piggery, and as I live on a regional property and keep some livestock, I had to purchase new clothes and shoes for the visit. I signed in on arrival, before showering and putting on clothes and boots provided by the farm. During the 3 hours I spent at the farm, I observed pigs at different stages of production as well as the different sheds, other facilities and effluent disposal.

Piggeries are very wet places, and surfaces are constantly washed down to remove effluent. Walking through the farm I was able to see where the grass between sheds had become waterlogged from the rain the previous night which provides an indication of how wet the area would have been at the beginning of 2022 after heavy rains that flooded river systems. The waterlogged grass reminded me of rice paddies, a known risk for JEV transmission due to their favourability to mosquito habitats. While different to rice paddies, the waterlogged long grass would also promote mosquito populations, though these are likely different to those in South East Asia I was also taken to see the effluent dams, which are quite close to the piggery and bigger than I expected. I was able to see the number and diversity of birdlife at the dams, and I could also see neighbouring farms with cattle close by, Cattle are the preferred bloodmeal for some species of culex mosquitoes.

This visit was important for me to understand the production data I was working with for my combined data analysis and epidemiology chapter. During the visit, I saw how sows and progeny pigs are managed, including what different sheds look like and how pigs are moved

around the farm, and I was able to ask questions about pork production from those working with the pigs on a day-to-day basis.

Solomon Islands

In June 2024, I travelled to Solomon Islands with members of the Pacific Engagement Program for Animal Health (PEPAH) team to support my work on vvIBDV in Solomon Islands (see Chapter Four). I was in Honiara for a week, during which time I visited several chicken farms with Livestock Officers from the Solomon Islands Ministry of Agriculture and Livestock (MAL). Due to the nature of the PEPAH team's visit, I also visited several pig farms, some of which were properties that also had chickens. I observed the management and the different housing styles for chickens first-hand and spoke to several farmers about their flocks.

Visiting the farms allowed me to identify potential biosecurity practices that could help to reduce the spread of vvIBDV in Solomon Islands context and to appreciate the challenges to implementing these interventions. For example, having a pair of slip-on shoes at the entrance to each chicken pen could help prevent the spread of vvIBDV, but during my visit I saw that young children that live on or near some farms often go in and out of the different pens as well, and getting them to wear slip-on shoes when they are often barefoot would be difficult.

My visit was just under 12 months after the PEPAH team initially travelled to Solomon Islands to assist with vvIBDV (July-August 2023), and so some of the farms we visited were not part of the initial investigations. Some farms had also come under different management since July-August 2023, and for others the owners were away when we visited. I did not assist with collecting samples from animals as I was keen to avoid contact with chickens to ensure I did not inadvertently introduce disease to my own chickens upon return to Australia. Instead, I assisted with data collection. Data was collected using ArcGIS with preprepared forms, a similar format to the forms used for the survey in July-August 2023. My involvement in the data collection helped me understand why the data I was analysing for my chapter was incomplete and sometimes of poor quality. As the data was collected at the individual bird level, the "comment" section at the end of the survey was used to enter useful information about the farm for the first sample. However, this information could be inadvertently entered in a different, empty field or omitted completely. There were several location fields (district, village, and farm name) on the form, but usually only one was filled in as an identifier along with the farmer's name. As these fields were at the top of the form, farm history and other farm information was occasionally entered here instead of in the comment section. It was also extremely hot and humid when I visited Solomon Islands, and these conditions affected my ability to enter data precisely and in a timely manner. I also needed assistance from livestock officers to translate some of my questions to farmers and given they were busy collecting samples, I kept to only

a few questions. The data entry is usually completed by a veterinarian or para-veterinarian who is involved in sample collection, so there is limited time for data to be entered accurately. This may explain why so many farms in the initial outbreak investigation were missing farm history.

I planned to present my report and data analysis to MAL staff during my visit, but many officers were out of the office at training for the week, and it was far more valuable for the MAL officers who were available to go to farms and collect samples with PEPAH staff. I also needed to make changes to the presentation after seeing sample and data collection on the ground, and I decided to instead present over Zoom at a more suitable time.

Fieldwork with Solomon Islands Ministry of Agriculture and Livestock Officers from left to right: Petrona Hauirae, Michaela (me), Sereh Kuri, Amira Taililiti, Peninah Filo (Intern) and Marion Keni

This visit was shared by the Australian Chief Veterinary Officer on social media. A copy of this post is available in Appendix 6C.

MAE experiences

EPIWATCH

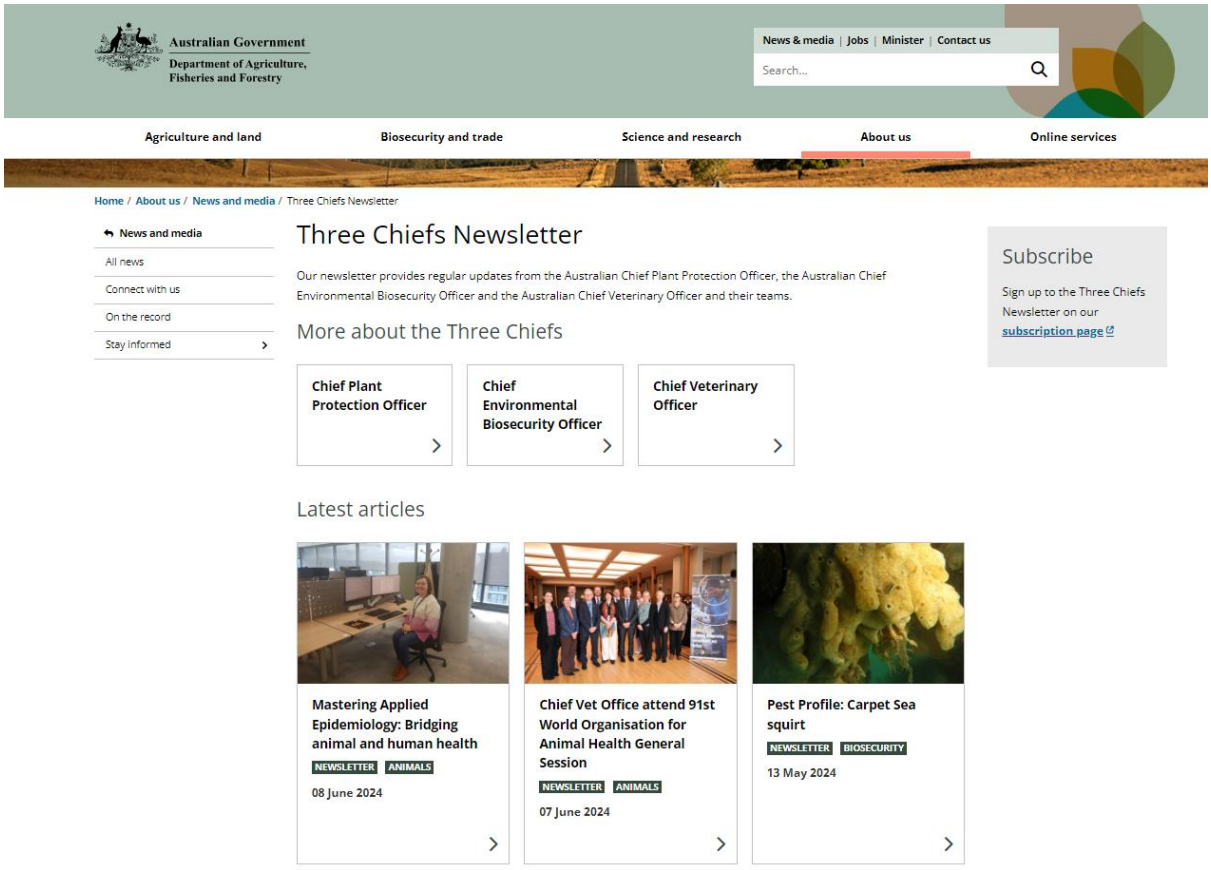
Along with several other members of my cohort and several students from the Kenya and Tanzania Field Epidemiology Training programs, I attended an EPIWATCH workshop in June 2023 hosted by the Kirby Institute at the University of New South Wales. This tabletop exercise focused on responding to a Smallpox pandemic, and also examined what could happen after the pandemic was declared over. EPIWATCH developed this scenario prior to the coronavirus disease of 2019 (COVID-19) pandemic, and I found the scenario interesting in that many

aspects were similar to what occurred in response to the COVID-19 pandemic, especially the divide between urban and regional/rural areas. Following the scenario, we prepared a 'Watching Brief' in small groups. This is a task often completed by the EPIWATCH team that uses event-based data to present an overview of a disease or syndrome and involves reviewing a list of articles flagged by the EPIWATCH system, including many not in English. This was my first time working with event-based surveillance and I found it was a good learning experience. My group prepared a brief on Monkeypox and received positive feedback from the EPIWATCH team, but we were unable to move to publication due to other commitments.

SAFETYNET Conference

In September 2023, ANU in partnership with the South Asia Field Epidemiology and Technology Network (SAFETYNET) and the Training Programs in Epidemiology and Public Health Interventions Network (TEPHINET) hosted the 1st SAFETYNET Scientific Conference (formerly known as the 11th Southeast Asia and Western Pacific Bi-regional TEPHINET Scientific Conference). The conference was scheduled close to the dates of our second course block, and so the National Centre for Epidemiology and Public Heath funded attendance for the 2023 MAE cohort. As part of this arrangement, my cohort assisted with running the conference, including timekeeping and marshalling attendees where required. Between these responsibilities, we were able to watch presentations of our choosing and participate in pre-conference activities. The theme of the conference was "Connecting the Dots: Advancing Human, Animal, and Ecosystem Health", and the plenary sessions included presentations on One Health and animal health investigations. The majority of conference presentations were focused on public health. A plenary session that stood out to me was Pawin Padungtod, who works for the Food and Agriculture Organisation (FAO) in Vietnam. Dr Padungtod spoke about African Swine Fever, including disposal of animals. He also noted that many people who work at FAO move to organisations like the World Health Organisation due to greater opportunities for work and career progression. This highlighted to me the need for greater focus on food and agricultural health to successfully implement a One Health approach to disease investigations.

Appendix 6A: Three Chiefs Newsletter launch page



Appendix 6B: Lay summary article

Mastering Applied Epidemiology: Bridging animal and human health

- NEWSLETTER
- ANIMALS
- BIOSECURITY

8 June 2024

The Department is currently hosting a Master of Philosophy in Applied Epidemiology (MAE) Scholar from the Australian National University, Michaela Gilbert, who during her two-year placement is contributing to animal health epidemiology projects across the department, including the Office of the Chief Veterinary Officer (OCVO).

Michaela Gilbert is currently completing a Master of Applied Epidemiology at DAFF. Credit: Michaela Gilbert

The MAE program is run through the Australian National University and involves two years of on-the-job training and intensive course blocks to produce experienced graduates. Scholars complete several projects to demonstrate their skills in epidemiology and submit a thesis at the conclusion of the program.

Epidemiology is the study of disease in a population with the aim of better preventing and controlling disease. A population could be a group of humans, animals or even plants. Most epidemiologists working in the department are focussed on animal health.

Understanding how disease affects animal populations is important for our industries. The Australian pork industry was impacted by an outbreak of Japanese encephalitis in 2022, but the severity of the impact varied between herds. Michaela is investigating how the disease affected different pig farms and whether any risk factors explain the different levels of disease. This will improve our understanding

of Japanese encephalitis in Australian pig populations and may help to minimise impacts of future outbreaks.

Along with assisting the Department with domestic work, Michaela has also assisted working with Department's overseas partners. In July 2023, the Solomon Islands Government sought Australia's aid to investigate an outbreak of very virulent infectious bursal disease. This important disease of chickens causes large numbers of deaths in chicken flocks, resulting in income losses for farmers. Michaela has applied her epidemiology skills to support the investigation.

Michaela's placement with the department has advanced her epidemiology skills and her understanding of One Health, and she has made valuable contributions to several important projects. Michaela's background in public health has also complemented her current work in animal health epidemiology. One Health recognises the connections between human health, animal health and ecosystem health, and Michaela's experience will help her foster cross-sector collaboration in the future. She hopes to continue working in One Health after completing the MAE.

Applications for the 2025 MAE program close 21 July 2024 and there is further information available at: Master of Applied Epidemiology Program.

Appendix 6C: Solomon Islands Fieldwork social media post

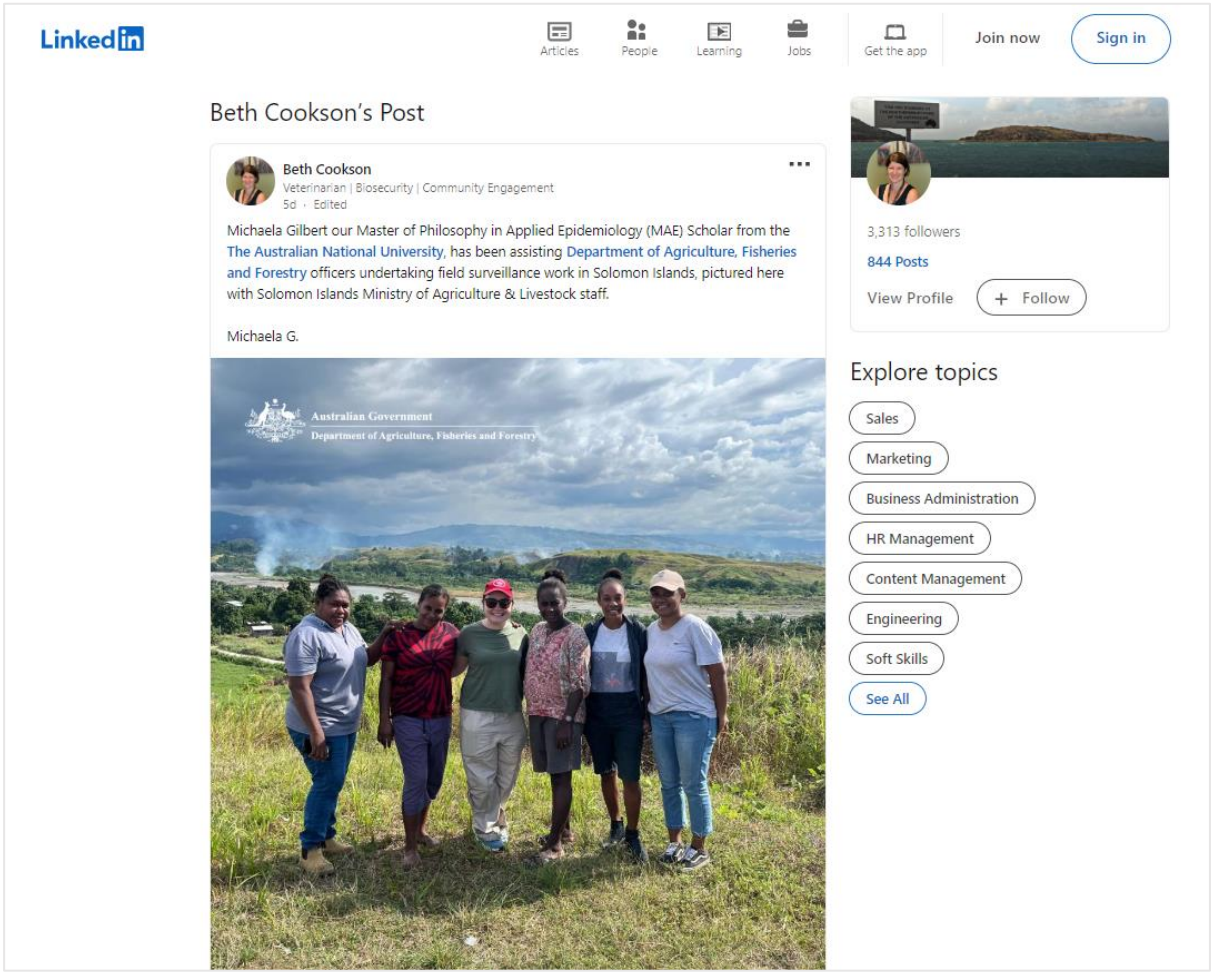


Figure 6C.1. Social media post from the Australian Chief Veterinary Officer Beth Cookson, posted 21 June 2024

From left to right: Marion, Peninah, Michaela (me), Amira, Sereh and Petrona

Bloody Ridge, Honiara