15th Mosquito Control Association of Australia Conference

The new normal: Responding to emerging and resurging mosquito risks

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Keynote Speaker Abstracts

Lessons learned after 20 years of West Nile virus in North America

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The presence of West Nile virus in the United States has progressed from unexpected to epidemic to endemic. Control of the virus through control of mosquito vectors is challenging. The number of different *Culex* vectors, varying levels of resources for surveillance and control, presence of insecticide resistance, focal and sporadic nature of outbreaks, and public resistance and lack of evidence for some vector control methods, have created a complex framework for public health vector control across the country. Lessons learned over a 20 year period include: 1) Although evidence exists that adult mosquito control can reduce transmission to humans, validation is problematic; 2) reactive mosquito control can be expensive and untimely; 3) models have demonstrated that larviciding can contribute to reduced risk of infection in humans, but real-world evaluation is lacking; 4) cyclical nature of funding for public health vector control undermines capacity building; and 5) it is time to revisit vaccination for prevention of human West Nile disease.

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An Integrated Mosquito Control Program For the 21st Century

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The Lee County Mosquito Control District (LCMCD) is a large, integrated mosquito management program serving the citizens of Lee County in Southwest Florida since 1958. It is the largest mosquito control district in terms of budget and full-time personnel in the United States. A subtropical climate, flat landscape, thousands of acres of salt marsh, and one of the fastest growing populations in the US necessitated the development of a robust control program. Lee County provides optimal breeding habitat for 54 species of salt marsh and freshwater mosquitoes which breed in large numbers from May through October with the remaining months productive during high tides and rainfall events. LCMCD uses 6 Airbus H125 helicopters to ferry inspectors, apply larvicide, and provide ultralow volume (ULV) adulticide applications to barrier islands. A fleet of five fixed wing aircraft including King Air and DC-3s provide adulticiding to inland, freshwater areas. Larvicide active ingredients include BTi, BS, methoprene, spinosad, temephos, and larviciding oil in rotation. Twelve ULV trucks treat small-area neighborhoods with permethrin + PBO or malathion. Aerial adulticide applications include naled and deltamethrin. Five trap trucks survey mosquitoes on the wing from May through October seven nights a week; CDC light traps monitor selected sites, and BG Sentinel traps monitor *Aedes aegypti* populations for our sterile insect technique (SIT) program. The SIT program was launched in 2017 on Captiva Island then shifted to Ft. Myers after Hurricane Ian. *Gambusia affinis*, a native mosquitofish, is produced for field and homeowner use. Drones have recently been integrated into our larval control activities and the district just purchased a Light Detection and Ranging unit to uncover cryptic breeding habitat. Particularly troublesome mosquitoes include *Aedes taeniorhynchus*, *Culex nigripalpus, Cx. quinquefasciatus*, *Ae. aegypti*, *Ae. albopictus*, *Mansonia* spp., *Anopheles atropos*, and *Psorophora columbiae*.

Japanese encephalitis virus - reminiscences from the past and considerations for today

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Japanese encephalitis virus (JEV) was first reported in the Torres Strait of northern Australia in 1995, when three human cases, two of which were fatal, occurred on Badu, an island in the central Torres Strait. An additional 55 people on Badu and other islands were found to have evidence of subclinical infection, and a large number of domestic pigs on nine islands were found to have seroconverted. Ten isolates of JEV were obtained, two from subclinical human sera, and eight from Cx. annulirostris mosquitoes. JEV reappeared in 1998, with two cases, one of which occurred on the Australian mainland at the mouth of the Mitchell River in southwest Cape York. The isolated were identical and shown to belong to Genotype 2. A closely related virus was isolated from mosquitoes in Western Province of Papua New Guinea (PNG), suggesting that PNG was the origin of the incursions. Further virus isolates occurred in 2000 and subsequently in 2004, but they belonged to a genetically different and belonged to Genotype 1.

JEV reappeared in Northern Australia in 2001, with a fatal case from the Tiwi Islands, and subsequently a widespread outbreak occurred the following year in Eastern Australia affecting over 80 piggeries and with over 40 human cases. Isolations of JEV were found to belong to a third Genotype, Genotype 4, that had previously only been found in Indonesia.

This presentation will discuss these outbreaks, exploring similarities and differences, and in particular, unexpected epidemiological patterns of JEV and the closely related Murray Valley encephalitis, two viruses that are believed to share vectors and vertebrate hosts.

Enter *Culex*: mosquito vectors of Japanese encephalitis virus

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The widespread outbreak of genotype 4 Japanese encephalitis virus (JEV) in Australia in 2021-2022 resulted in 45 notified human cases with 7 deaths and significant stock loss in commercial piggeries. Responses to this unprecedented event involved vaccinating at-risk populations, development of specific public health messaging, virus surveillance in mosquitoes and focal mosquito control. The effectiveness of these responses is underpinned by a thorough understanding of virus transmission dynamics in the local context, including which Australian mosquito species are vectors of JEV. This presentation will review the outcomes from field studies and laboratory-based vector competence studies undertaken to incriminate key mosquito vectors of JEV in Australia. Other factors which can impact the role a mosquito species plays in transmission, such as population dynamics, survival and host feeding behaviour, will also be discussed. As there is limited species-specific information on genotype 4 JEV infection in mosquitoes, much of what will be presented will be drawn from historical investigations, including those conducted into incursions of genotype 1 and 2 viruses into northern Australia between 1995 and 2005. Evidence suggests that the main vectors of JEV in Australia are mosquitoes of the genus *Culex*. Of these, *Culex annulirostris* should be considered the primary vector, with this species likely playing a similar role to that of *Culex tritaeniorhynchus* in JEV endemic areas of Asia. Other species, like *Culex gelidus*, *Culex sitiens*, *Culex palpalis* and *Culex quinquefasciatus* may also be important vectors when local conditions are suitable. Mosquitoes of other genera, like *Aedes* and *Verrallina*, are relatively poor vectors of JEV but could play a role in vertical transmission of the virus. Outcomes from surveillance programmes and targeted research will further elucidate what mosquito species are vectors of JEV in Australia.

Abstracts

Evaluating sugar feeding of *Aedes* in implementation of BioGel as mosquito baiting

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Two competent *Aedes* species, *Aedes aegypti*, and *Ae. albopictus* cause diseases notably chikungunya fever, dengue fever, and Zika fever. In managing the outbreak, wide application of chemical insecticides has been used to control mosquitoes leading to insecticide resistance among the species. Thus, novel baiting system innovation needs to be introduced and improvised. *Aedes* mosquitoes need sugar sources to produce energy for the requirement in flight dispersal, reproduction, and survival. In combating *Aedes* vectors, Attractive Toxic Sugar Baits (ATSBs) were previously designed by taking advantage of the requirement for mosquitoes to consume sugar. The basic concept is to use either floral and fruit nectar or artificial sugar as an attractant to entice mosquitos. In our study, the sugar baits were designed together with a hydrogel bead and proposed as a unique pesticide delivery medium in stimulating the mosquitoes to the baits. Thus, the selection of sugar preferences based on their sugar-feeding behavior needs to be explored to enhance the bait's attractiveness. A series of experiments was tested to determine and compare the best attractants of hydrogel beads which were color (purple, red and blue) and nectar-sugar solution (20% mango, 20% mango with 10% sugar, and 20% mango with 20% sugar) as liquid bait between *Ae. aegypti* and *Ae. albopictus*. The results show that*, Ae. aegypti* and *Ae. albopictus* preferred different liquid bait concentrations which were 20% mango with 20% sugar and 20% mango with 10% sugar respectively. For colored hydrogel beads without liquid bait, Ae. aegypti prefer purple color meanwhile *Ae. albopictus* prefer red color. However, both species were attracted to the red-colored hydrogel beads with liquid bait. From an overall perspective, the observed *Aedes'* preference for the baiting system can potentially be a promising new vector control approach in the combat against *Aedes* species.

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Genetic basis of vector competency for African Horse Sickness Virus (AHSV)

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African horse sickness virus (AHSV) is a double-stranded RNA virus of *Orbivirus* genus, responsible for respiratory and circulatory impairment in equids. This hemorrhagic disease severely impacts equids with approx. 80-90% mortality and currently there is no well-adopted treatment or preventive measures to control this disease. AHSV was out-of-concern for several years as the virus was endemic to Saharan Africa, but in 2020, AHSV isolate serotype-1 had been reported in south-east Asia, especially in Thailand and Malaysia, which raised concern for Australian equine industry. We will utilize ongoing vector competence experiments and field studies to focus on horse-feeding insect (with particular focus on *Culicoides*) species-of-significance to AHSV transmission. Illumina-based short and oxford-nanopore based long read DNA sequences are being assembled and scaffolded using state-of-the-art HiFi sequencing to get a chromosome-level genome assembly. While there is a scarcity of reference-level genomic resources for AHSV-transmitting insects, our genome will serve as a baseline for future blood-meal, differential expression, and genetic mitigation studies. We will extend the scope to study the post-infection transcriptome and proteome to elucidate the intra- and inter-species variations, the genetic mechanism for AHSV vector competence and identify candidate gene-targets for future genetic engineering studies. We shall briefly present ongoing multi-omics extraction protocols and virus spiking trials at PC3 facility and the sophisticated bioinformatic pipelines for multi-omics studies. We shall discuss our findings in the light of ACDP’s response to this emerging disease and indicate how this research can contribute to other arbovirus-borne disease studies.

Creation and characterisation of *Wolbachia*-infected *Aedes aegypti* strain with Bangladeshi genetic background

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Dengue cases in Bangladesh are surging as the widespread insecticide resistance in populations of the main vector *Aedes aegypti* compromises the conventional chemical-based control program. One sustainable alternative to disease control, with an increasing evidence base, is the replacement or suppression of wild mosquito populations with mosquitoes infected with the bacterial endosymbiont *Wolbachia*. Mosquitoes infected by this bacteria can have virus blocking and reproductive phenotypes that can be exploited to interrupt virus transmission. Controlled releases of *Wolbachia* infected mosquitoes cannot be performed without characterizing the effects of *Wolbachia* infection in local *Ae. aegypti* populations and exploring the probable outcomes of any subsequent releases of that strain. In order to investigate the potential of a *w*AlbB to spread through Dhaka *Ae. aegypti*, we created and characterized a *Wolbachia* infected *Ae. aegypti* strain with a Dhaka wild type genetic background and then compared a variety of fitness traits to an uninfected *Ae. aegypti* strain from Dhaka. Complete cytoplasmic incompatibility was demonstrated, with minimal apparent fitness costs. High-intensity resistance (41 – 54% mortality) to the 10 times the diagnostic dose of permethrin was recorded in the backcrossed *w*AlbB-Dhaka strain. No significant differences were observed in fertility, lifespan, and fecundity to wild type Dhaka mosquitoes. *w*AlbB infected males were found to compete successfully with wild type Dhaka males for successful mates. Eggs of *w*AlbB infected females remained viable for 16 weeks similar to Dhaka uninfected females. Overall results suggest that the *Wolbachia* *w*AlbB strain would spread successfully through local Dhaka *Ae. aegypti*.

Factors influencing community participation approaches in *Aedes* management in the Torres Strait

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Introduction: Community participation is an important part of *Aedes* mosquito management as it can reduce disease transmission risk and help sustain vector control efforts. Historically, top-down, government-led approaches have been preferred when engaging communities in *Aedes* mosquito management in high-income countries. Few studies have explored the factors influencing the choice of these approaches. This case study aims to understand the community participation approaches used in *Aedes* mosquito management in the Torres Strait, Queensland; a unique, tropical region at risk of *Aedes* mosquito-borne disease.

Methods: Fifteen semi-structured interviews were conducted with local government and state government agencies working in *Aedes* mosquito management in the Torres Strait. Six key mosquito management plans and policies were also reviewed. Thematic analysis was used to identify, analyse and attribute meaning from the data collected.

Results: A range of community participation approaches was implemented within the two main *Aedes* mosquito management programs in the Torres Strait (*Aedes Albopictus* Elimination Program and the TSIRC Environmental Health Program). The *Aedes Albopictus* Elimination Program predominantly used top-down, informing approaches, whereas the TSIRC Environmental Health Program used both top-down and bottom-up approaches. These approaches were chosen for reasons related to regulation, attitude and beliefs, and resourcing.

Conclusions: This research provides valuable insight into the community participation approaches used in a unique, tropical region of Australia, and highlights the factors influencing the choice of these approaches. In light of these findings, we argue the need for more ‘bottom-up’ empowering approaches to be considered to optimize the sustainability of *Aedes* mosquito management in the Torres Strait.

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Duration Of Efficacy of IRS With Fludora Fusion In Papua New Guinea.

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Vector control reduces malaria transmission. Long-lasting insecticidal net (LLIN) mass distribution is a core intervention of the National Malaria Control Program in Papua New Guinea (PNG). However, nets do not interrupt all transmission and additional tools are needed, such as residual spraying (RS). To build RS capacity, and assess acceptability and efficacy, we conducted a pilot RS trial with the insecticide Fludora Fusion®, a formulated combination of clothianidin (neonicotinoid) and deltamethrin (pyrethroid) in two villages in Madang Province, PNG.

As part of the trial, we assessed the residual efficacy of Fludora Fusion® to induce mosquito mortality over 8 months post spray application using WHO standard wall cone bioassays. Specifically, four houses from each village were selected randomly with different wall types (4 sago palm,4 bamboo). Two unsprayed control houses from each village were also included. Susceptible Anopheles farauti colony mosquitoes were exposed to the sprayed walls. The primary outcome was mosquito mortality at 24 hours after exposure. We also trialed a new X-Ray Fluorescence spectroscopy technique alongside the monthly wall bioassays to track the concentration of the insecticide on the walls over time.

Overall, wall bioassays resulted in close to 100% median 24h mosquito mortality for 2 months after spraying. Bioefficacy decreased to around 75% (range 10-90%) at 9 months after spraying. We observed a more pronounced decrease in bioefficacy on sprayed outdoor surfaces as compared to indoor surfaces.

Our study shows that RS kills more than 80% of mosquitoes for up to 6 months after spraying when applied indoors to wall surfaces commonly found in rural PNG settings. Reapplication of spraying must be done after 6 months. Residual spraying may thus represent a valuable addition to LLINs to control malaria and other mosquito-borne diseases in PNG.

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Molecular ecology and population genetics of Australasia's arbovirus vector *Culex annulirostris*

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The mosquito *Culex annulirostris* Skuse (Diptera: Culicidae) is an important arbovirus vector in Australasia transmitting many endemic arboviruses within Australia. This one mosquito transmits both alpha virus (Ross River virus and Barmah Forrest virus etc.) and Flavivirus (Murray Valley encephalitis virus, Kunjin, and Japanese encephalitis virus) through Australasia and the Pacific. It is part of the *Culex s*itiens subgroup that includes also *Cx. palpalis* and *Cx. sitiens*. Single locus DNA sequencing studies suggest *Cx. annulirostris* consists of a complex of two putative species and we explore this through microsatellites analysis to describe the population genetics of *Cx. annulirostris* through Australia and the Indo-Pacific. A distinct Pacific Island lineage was confirmed along with two reproductively isolated cryptic species in Papua New Guinea, the Torres Strait Islands and the northern Cape York Peninsula. Most of mainland Australia consist of two distinct but reproductively compatible lineages of *Cx. annulirostris*. A northern Australian lineage expands the through south-east coast of Queensland and NSW. A second distinct southern lineage is detected through inland NSW, Victoria, South Australia and southern WA. These two lineages appear to be reproductively compatible, and the Great Dividing Range presents a strong gene-flow barrier separating these populations. Molecular diagnostics have now been developed to distinguish the two cryptic species in Cape York and PNG and it appears from the 2022 JEV outbreak that both Australian lineages are capable of JEV transmission.

JEV – A new approach

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The Wide Bay Public Health Unit (WBPHU) is performing an extensive vector surveillance program as part of Queensland Health's state-wide response to Japanese Encephalitis Virus (JEV).

In early 2022, the WBPHU was notified of seven infected piggeries in the region. Several challenges became evident in the early-stage response, including:

piggery detections limited to sites with breeding sows

detections were lagging indicators, with the WBPHU being advised 3-4 months after the infection event no scope to test or assess the role of other amplifying hosts (wading birds & feral pigs)

To overcome these barriers and to broaden the scope of surveillance, the WBPHU initially partnered with Commonwealth Scientific and Industrial Research Organisation (CSIRO), with the aim of developing a diagnostic tool to detect JEV in both piggery effluent and bird faecal samples. This relationship soon morphed into an expanded working group with key stakeholders and a pilot project was commenced.

If successful, the project may:

overcome the challenge of detecting JEV beyond the limitations of traditional vector and serological surveillance methods dramatically reduce the JEV detection times significantly expand the surveillance target provide a better understanding of the role of other amplifying hosts, in this outbreak.

From a public health perspective, preparations have been made to respond to future cases of JEV, however the environmental health focus is to learn about this virus in our area and focus on prevention and education in high-risk areas.

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A recombinant alphavirus platform based on the Australian insect-specific Yada Yada virus

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Alphaviruses are mosquito-borne viruses that cause a range of debilitating diseases in humans and animals. These viruses have epidemic potential, with Chikungunya virus (CHIKV) alone causing millions of cases worldwide and large epidemics in recent years. In Australia, the average annual incidence for the endemic Ross River (RRV) and Barmah Forest viruses (BFV) is approximately 5400 cases, with 48% from Queensland. Both alphaviruses can cause chronic, debilitating symptoms such as arthritis and fatigue, with no current treatments or vaccines available.

Insect-specific alphaviruses (ISAs) infect only mosquitoes and cannot replicate in vertebrates. Several were recently discovered, including the first Australian ISA; Yada Yada virus (YYV). Viable clones of many ISAs have never been recovered, including YYV. In this project, ISAs were utilised to construct recombinant viruses for application as potential vaccine candidates, diagnostic tools, and to further alphavirus host restriction studies. Using RNA extracted from mosquito homogenate and circular polymerase extension reaction (CPER), an infectious clone of YYV was successfully recovered that replicated to high titres in mosquito cell culture. Purified virions were used to generate the first ISA-reactive monoclonal antibodies, which will become valuable tools for ISA research. Recombinant alphaviruses were then constructed using YYV by replacing the structural genes with that of various pathogenic alphaviruses, such as RRV and BFV. These chimeric viruses successfully replicated in mosquito cells and did not infect vertebrate cells as they retained the insect-specific host restriction of YYV. Antigenic analysis confirmed antigenic authenticity to parental pathogenic viruses, and the chimera allows structural elucidation by electron microscopy of chimeric particles. ISA chimeras represent a versatile platform for alphavirus research, including application to diagnostics, assessment in mouse models as potential vaccine candidates, and structural analyses of other alphaviruses.

Enhanced mosquito-borne disease surveillance in response to increased endemic and exotic virus activity in WA, 2021/22 – 2022/23

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A significant increase in flavivirus activity, including Murray Valley encephalitis virus (MVEV) and Japanese encephalitis virus (JEV), occurred in 22/23 following heavy wet season rainfall in northern regions of WA which created ideal breeding conditions for mosquitoes and hosts.

The first evidence of JEV activity within WA was confirmed in two feral pig samples from the Kimberley collected in October 2022. Prior exposure to JEV was also confirmed in February 2023 in three chickens from Kununurra, two from Wyndham and one from Halls Creek in the Kimberley region, in addition to one chicken from Newman in the Pilbara. Targeted mosquito surveillance in northern WA resulted in multiple detections of mosquito-borne viruses of public health importance including MVEV, West Nile virus Kunjin strain (WNV_{KUN}), Ross River virus (RRV) and Barmah Forest virus (BFV) but notably JEV has not yet been detected in mosquitoes.

There were no human JEV cases notified in WA. However, four confirmed and two probable human MVE cases were acquired in WA, including two fatal cases. The last time flavivirus activity occurred on this scale in WA was in 2011, when 9 human MVE cases were notified.

A multi-agency, one-health approach was adopted to manage the risk of JEV, encompassing animal and human health, through evidence-based surveillance and response activities. Enhanced surveillance activities included basing two officers in the north of the State in the Kimberley region, in addition to expanding existing trapping in southern WA to capture high-risk breeding sites in proximity to piggeries. A feral pig surveillance program was established and enhanced sentinel chicken surveillance continued across 21 sites throughout WA, in addition to ad hoc sampling of domestic chicken flocks outside the range of the current program.

Public health responses included collaboration with local governments on mosquito management measures and targeted public messaging on mosquito avoidance. Significant resources, including mosquito repellent and bed nets were also deployed to Aboriginal health organisations to distribute to high-risk communities.

Ross River virus disease case numbers were slightly lower than the long-term average in 21/22 and significantly lower in 22/23, which is on track to be the lowest number of cases in a financial year for over a decade. This was reflected in surveillance activities with only six RRV detections in mosquitoes over the period with the most recent in December 2021. While there were 94 Barmah Forest virus (BFV) detections in 21/22, this did not translate into increased human BFV case numbers and there were no further detections in 22/23.

Finally, the number of dengue, malaria and chikungunya cases diagnosed in returned overseas travellers to WA increased significantly over the course of 2022 and into 2023, particularly dengue cases among travellers to Indonesia, associated with increasing travel following COVID border closures.

Responding to the threat of Japanese encephalitis virus: A summary of enhanced surveillance activities in Western Australia

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Following the declaration of Japanese encephalitis virus (JEV) as a Communicable Disease Incident of National Significance in Australia, WA adopted a multi-agency, one-health approach to manage the risk, encompassing animal and human health, through evidence-based surveillance and response activities. The response involved collaboration between the Environmental Health Directorate (EHD), Communicable Disease Control Directorate (CDCD), Communications, PathWest, Department of Primary Industry and Regional Development (DPIRD), Public Health Units, Aboriginal health organisations and local government.

Enhanced surveillance activities undertaken by the EHD encompassed testing of sentinel chickens, mosquitoes and feral pigs for JEV. To facilitate this work, two officers were deployed to the north of the State to undertake targeted mosquito sampling in the Kimberley and Pilbara regions, in addition to expanding existing trapping in southern WA to include those high-risk wetlands and areas in proximity to piggeries. Sentinel chicken surveillance has continued across 21 permanent sites throughout WA, with ad hoc sampling from an additional 22 domestic chicken flocks throughout the Mid West, Wheatbelt Kalgoorlie-Leonora and Kimberley regions. EHD also established an active JEV Feral Pig Surveillance Program to further enhance surveillance efforts throughout the State.

Surveillance activities yielded WA’s first evidence of JEV activity in WA, when two feral pigs in the far north Kimberley were confirmed (via serology) to have ‘Prior exposure to JEV’. Seroconversions to the virus have also been confirmed in sentinel chickens flocks located in Kununurra, Wyndham, Halls Creek (Kimberley) and Newman (Pilbara). Despite more than 5,500 mosquito pools being tested from over 600 sites, JEV has not yet been detected in mosquitoes. However, *Culex tritaeniorhynchus*, a key vector of JEV considered exotic to WA, was detected in the east Kimberley for the first time, as a result of this work. This has significant implications for the potential transmission of JEV in the region.

SA Health Japanese encephalitis virus response

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The South Australian (SA) response to the emergence of Japanese Encephalitis virus (JEV) in SA was guided by the 'SA JEV mitigation through surveillance and control program' (the program). The program built upon existing surveillance structures and systems in place between SA Health and local councils required to support mosquito and arbovirus surveillance, prevention and control.

Routine surveillance undertaken by SA Health was expanded to include increased frequency of surveillance, extending the surveillance season to include late winter. The SA mosquito management subsidy program was expanded to include additional local government areas and funding was increased to facilitate the expanded program.

The sentinel chicken program was increased from six to ten flocks with blood sampling frequency increased from monthly to weekly. All samples were tested for JEV in addition to MVEV and WNV/KUN.

Agreements were developed to ensure efficient, timely and targeted responses to notifications of human JEV infection from Communicable Disease Control Branch and responses to notification of suspected or confirmed JEV in animals from the Department of Primary Industries and Regions (PIRSA).

Collaborative projects were undertaken with SA Water, the pork industry, Department for Environment and Water.

The SA response also included an enhanced media and communications campaign, community engagement program and vaccination program. A seroprevalence survey was undertaken to help understand the extent and nature of JEV in the community which to provide information to help plan further targeted public health interventions in response to the JEV outbreak.

Biting profile of a control naive *Anopheles farauti* population in northern Queensland

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Anopheles farauti is one of the major vectors of malaria in the Southwest Pacific region and is responsible for past outbreaks in northern Australia. This mosquito often shows behavioural adaptation to indoor residual spraying (IRS) and insecticide-treated nets (ITNs) by changing its all-night biting to primarily feeding early evening. With limited insight into the biting profile of *An. farauti* populations in areas that have not encountered IRS or ITNs, the aim of this study was to profile the biting behaviour of a malaria control naive population. The study was conducted at Cowley Beach Training Area, in north Queensland, Australia. Encephalitis virus surveillance traps were used to document the 24-hr biting profile of *An. farauti* and human landing collections (HLC) to follow the 18.00-06.00hrs biting profile. The HLC were performed at both the end of the wet (April) and dry (October) seasons. Random Forest Modelling shows that time of night is the most important variable for predicting *An. farauti* biting activity. Temperature was found to be the next important predictor, followed by humidity, collector, and season. The significant effect of time of night and peak in time of night biting, between 19.00 and 20.00 hours was also observed in a generalized linear model. The main effect of temperature was significant and appears to have a positive effect on biting activity, where biting would continually occur through the night on warmer evenings. This population's biting profile is similar to populations found in other parts of its range prior to insecticide intervention. Finally, the time of biting onset and biting end was tightly constrained which is likely underpinned by an endogenous circadian clock rather than any light intensity. This study sees the first record of a relationship between biting activity and the decreasing temperature during the night for the malaria vector, *Anopheles farauti*.

Exotic vector detections adjacent to a major Sydney cemetery: introduced or local?

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Since 2021, there have been multiple detections of *Aedes aegypti* at an air cargo receiving facility in Sydney, which is adjacent to a large cemetery. The site contains thousands of water holding vessels (e.g. flowerpots) that provides habitat for container breeding mosquitoes. There is a threat that exotic species, such as *Ae. aegypti* or *Aedes albopictus*, could become established at this site. A risk analysis was undertaken in the cemetery, which included adult trapping (immediate and long-term), and larval surveys with eDNA to examine for evidence of exotic breeding. A subsequent larval control program was conducted following ongoing detections. In the immediate adult trapping program, up to 23 traps/night were set for 8 nights (29/January to 9/February/2022), including CO_2 baited EVS traps and BG traps. These were operated adjacent to the air cargo receiving facility. A total of 1,685 adult mosquitoes were trapped, including *Aedes notoscriptus* (84%) and *Culex quinquefasciatus* (12%). In the long-term adult trapping program (22/March to 20/April/2022, and 7/November to 22/February/2023), A total of 3,417 adults were collected, including *Ae. notoscriptus* (55%) and *Cx. quinquefasciatus* (25%), with no exotics. In the larval surveys, >100 vessels were sampled, with collected larvae reared to adults in the laboratory. A total of 1,880 adults were identified, including *Ae. notoscriptus* (42%) and *Cx. quinquefasciatus* (57%), and again no exotics. In the eDNA survey, there was no evidence of *Ae. aegypti* DNA detected in the water samples. In the larval control program, 3,185 vessels were treated with ProLink® [(S)-methoprene] with post treatment surveys confirming effective control beyond 8 weeks in treated vessels. Long-term adult trapping results also showed that mosquito numbers declined after treatment. While *Ae. aegypti* was not found in the cemetery, the site contains many container breeding mosquitoes suggesting there is a high risk of the established of exotic mosquitoes.

A virtual training program on the AMCA's 'best practices for mosquito management'

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The American Mosquito Control Association received a grant from the U.S. Centers for Disease Control and Prevention (CDC) to create a Virtual Training Program on Best Practices for Integrated Mosquito Management. The topics covered will include Larval Surveillance, Adult Surveillance, Arbovirus Surveillance, Mapping and Data Management, Setting Action Thresholds, Larvicides, Adulticides, Biological and Novel Control Techniques, Monitoring and Managing Insecticide Resistance, Community Engagement, and Record Keeping and Analysing. Learners who successfully complete the entire program will receive a certificate they can display as verification of the achievement and advance their career. Although the content will be geared towards the U.S. experience, it will still be of value to practitioners in other countries, as the principles of integrated mosquito management are universal. The program is set to be launched in October 2023 and will be freely available to anyone interested in learning about science-based mosquito control.

CRISPR Gene Drives: An alternative to traditional mosquito control strategies

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Genome editing, in particular CRISPR, has been the forefront of biomedical research, allowing scientists to dive deeper into key genetic and molecular mechanisms pertaining to a range of disease. In recent years, CRISPR has allowed researchers create gene drives (GDs), a mechanism of changing the probability of inheritance of selected alleles, to supress mosquito populations in laboratory settings by targeting essential fertility or sex determination genes (Wang *et al*. 2021). Suppression of naturally occurring mosquito populations may however have detrimental effects on the ecosystems, considering the importance of mosquitoes in natural processes such as pollination. Eradication may also cause reinvasion from other populations belonging to same or different species. CRISPR has also allowed researchers to modify mosquito immune genes that can supress virus titrations (Bui *et al.* 2023). Targeting such non-lethal genes involved in host-pathogen interactions may circumvent unnecessary effects of total population suppression.

In the proposed study, we aim to identify *Plasmodium falciparum (P.f.)* refractory genes in a laboratory population of *P.f.* resistant *Anopheles farauti* mosquitoes and introduce the refractory genes into wild type mosquitoes. The *An. farauti* population in our laboratory has naturally become resistant to *P.f*. over time, providing us with an excellent model to identify potentially refractory alleles. These genes will be identified using genomics approaches and systematically introduced to and selected for in wild-type mosquitoes using CRISPR mediated GDs in a laboratory setting, providing us with a tool to reduce malaria parasite transmission.

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We don’t have time! – Balancing treatment options in a crisis

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The recent record breaking floods in central Victoria stretched resources to breaking point. In the Shire of Campaspe 90-95% of the town of Rochester was flooded. All around the Shire every piece of Channel Bench or lower lying land along the Campaspe River, Loddon River and Murray Rivers flooded (in many cases areas that do not usually flood). In relation to mosquito control activities, suitable equipment and chemical were available for most treatment activities, but not the staff to use them. Response became a balancing act, we can’t do it all so…“What can we do for the most Public Health benefit?”.

The primary focus from State Government was on adulticiding, however this is a very short-term treatment option. With limited man hours we needed to use an integrated approach looking at short and medium term effects.

To this end we undertook a risk assessment and categorised our risks to select the most suitable treatment options: Adulticide, Larvicide or Residual treatment (or a usually a combination).

An additional complication occurs in relation to land that Council does not manage or own. The risk assessment identified many locations where the land managers have not been able to undertake control activities and where Council have not been able to negotiate access to undertake activities on their behalf.

A time-varying geospatial model of the JEV wildlife reservoir in Australia

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Japanese encephalitis virus is a mosquito-borne virus endemic to southern Asia and the western Pacific that can cause life-changing disability and death in humans when spillover infections occur. Historically, the disease has only been known in Australia from occasional incursions into the Torres Strait and Cape York. However, since early 2022, the disease has been identified in scores of people, piggeries, and feral pigs across the central and eastern Australian mainland, encompassing more than 20 degree southward latitudinal expansion from previous recorded infections. The combination of vector, hosts, and environment that can maintain transmission in Australia is poorly understood. We obtained vector data from public databases and arbovirus surveillance programs from Australian States and Territories and waterbird observations from curated checklists to construct dynamic geospatial models of habitat suitability for the wildlife reservoir using static and dynamic (monthly) environmental covariate data. We modelled habitat suitability for vector and host species cohorts and discuss how these models can help estimate of the potential spatial distribution of JEV transmission risk.

Murray Valley encephalitis virus: genotypic differences in vector competence of *Culex annulirostris*

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Murray Valley encephalitis virus (MVEV) is a mosquito-borne flavivirus endemic to north-western Australia, although re-emerges periodically in south-eastern states during periods of high summer rainfall causing widespread outbreaks. While symptomatic infections are rare in humans, such cases can be lethal and frequently cause permanent neurological effects. Following extensive flooding events in late 2022, MVEV was detected in mosquitos and sentinel chicken populations in New South Wales, South Australia, and Victoria in early 2023. Subsequently, Australia has seen a rise in human cases of MVE, resulting in two deaths, including the first reported case in Victoria since 1974. With no vaccine, nor effective antiviral drugs available to treat MVEV infections, understanding the underlying transmission patterns remains crucial in controlling human infection.

Four genotypes (G1 – G4) of MVEV have been described with two known to circulate within Australia: G1 is the major type on mainland Australia and has been responsible for major epidemics of MVE in in the country. The second minor genotype (G2) comprises only ~5% of all mosquito isolations in Australia. G2 is the oldest lineage of MVEV and is restricted in its geographic distribution to north-western Australia. Here, we will artificially infect the major mosquito vector of MVEV, *Culex annulirostris*, and use molecular and histological techniques to determine the competence of these mosquitoes for G1 and G2 viruses. The study will assess the differences in the vector competence of *Culex annulirostris* for MVE viruses, which may underlie the contrasting patterns of distribution of these genotypes.

An overview of recent research on *Aedes notoscriptus*, a forgotten local mosquito

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Aedes notoscriptus, though widespread and abundant in Australia, receives relatively low research attention compared with high profile arbovirus vectors such as *Ae. aegypti*. Invasive in other countries, this local mosquito is a primary vector of dog heartworm and is known to vector arboviruses to humans in Australia. *Aedes notoscriptus* is also suspected as a mechanical vector of *Mycobacterium ulcerans*, the bacterium causing Buruli ulcer. Previous research shows that *Ae*. *notoscriptus* is a complex of divergent genetic lineages, some geographically restricted and others widespread in eastern Australia. Our recent research on the *Ae*. *notoscriptus* complex focuses on behaviour of male mosquitoes, genomic population structure at broad and local scales and alternative methods of population suppression. We find, through human-baited field collections and tent experiments, that male *Ae. notoscriptus* are not attracted to human hosts, in stark contrast to male *Ae. aegypti*, while female *Ae. notoscriptus* show similar preferences for human subjects as *Ae. aegypti*, suggesting that they are attracted to similar cues. A spatial autocorrelation analysis of genetic distances indicated genetic similarity of *Ae*. *notoscriptus* at >1 km separation, a tenfold higher distance than for a comparable population of *Ae. aegypti* from Cairns, Australia. These results suggest that *Ae. notoscriptus* is highly mobile, highlighting challenges of localised intervention strategies. Autodissemination of pyriproxyfen and *Beauveria bassiana* by adult mosquitoes was trialled in an isolated housing development community in Victoria using In2Care® traps. Far more mosquitoes eclosed from water samples collected in the control sites compared with samples collected from ovitraps in the In2Care® treatment site, indicating transfer of pyriproxyfen from the In2Care® stations to other breeding sites. In combination with community engagement and source reduction, the autodissemination method could suppress populations of *Ae. notoscriptus* in specific situations. Our results advance our understanding of the ecology and suppression of this native Australian mosquito.

Global evaluation of *Wolbachia* loss in *Ae. aegypti*

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The *w*Mel and *w*AlbB strains of the bacterial endosymbiont *Wolbachia* are being introgressed into *Aedes aegypti* populations as a biocontrol method to reduce the transmission of medically important arboviruses such as dengue, Zika, and chikungunya. Introgression of *Wolbachia* through the local *Aedes aegypti* population relies on both persistence of *Wolbachia* throughout the host life cycle and a high fidelity of maternal transmission of *Wolbachia* between generations. *Wolbachia* prevalence in *Ae. aegypti* populations have been shown to fluctuate over time but are rarely lost from populations. The cause of these fluctuations may be due to invasion of wild-type mosquitoes into release areas, reduced fitness of *Wolbachia*-infected mosquitoes relative to wildtype mosquitoes, or alternatively the loss of *w*Mel or *w*AlbB infection from mosquitoes.

*w*Mel- and *w*AlbB-country-specific release colonies are generated through a backcrossing scheme that results in mosquitoes carrying a *w*Mel or *w*AlbB infection, a country-specific nuclear genome, and an Australian mitochondrial genome. This process provides a unique way to assess *Wolbachia* loss in the field as a mosquito that has lost a *Wolbachia* infection can be identified by the presence of an Australian mitochondrial genome and no *Wolbachia* infection. To explore the fidelity of *w*Mel and *w*AlbB persistence and transmission, we used SNP genotyping to estimate the prevalence of *Wolbachia* loss by measuring *Ae. aegypti* negative for *Wolbachia* infection but carrying an Australian mitochondrial genotype. We observed *w*Mel loss ranging from 1.27 to 44.82% measured at various time intervals after *w*Mel-infected mosquito releases stopped across five diverse field sites and *w*AlbB loss ranging from 3.4 to 21.9% in one field site. Collectively, these findings suggest *Wolbachia* loss is significantly contributing to reduced *Wolbachia* prevalence at some field sites more than others and efforts should be focused on identifying potential underlying causes.

Modelling the future risk of Japanese encephalitis using geographic information system

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Climate change's effect on zoonotic disease risks is not yet well understood. Japanese Encephalitis (JE) is a viral zoonotic disease endemic in Southeast Asia and the western Pacific. The spread and transmission are associated with rain, warm temperatures, migratory waterbirds, the presence of pigs as amplifying hosts and a competent vector. In 2022, the virus reached the Australian mainland in an unprecedented outbreak spanning the eastern seaboard. This project aims to accurately identify future high-risk areas for JE transmission in a changing climate and use that information to inform preparedness policy at a state and regional level. A newly developed model, deployed in a geographical information system (GIS), was used to analyse the suitability of the state of Victoria, Australia, for the JE vector Culex annulirostris. The parameters considered included rain, temperature, elevation, and the distance to water bodies and piggeries. Future climate projections included in the model were generated using the global climate model ACCESS-CM2, driven by worst- and best-case scenarios SSP126 and SSP585. Monthly risk maps were generated for the current and projected climate in the years 2040 and 2060. The results showed that changes in climate expected for the state of Victoria will indeed influence the risk of JE since the areas suitable for the presence of C. annulirostris will expand. Data on the location of piggeries and water bodies combined with the climate projections allowed us to identify with higher precision local areas where JE has a higher likelihood of becoming endemic. When overlaid with regional jurisdictional boundaries, the analysis also identified regional/provincial governments that need to improve their infectious disease preparedness policies. In conclusion, climate change will create conditions in Victoria that could help JE to become endemic, indicating the need for greater preparedness.

Mosquito species range maps, species richness, and geolocation enabled identification keys

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Wouldn't it be great to have a computer key to Australian mosquito species on your phone that would have the flexibility to eliminate those species that are not expected to occur at your location of interest? For that you need species range maps, and some are available for Australian mosquitoes but are usually images based on expert opinion and not easily queried. Databases of georeferenced species occurrence points are also available, but species are usually unevenly or poorly sampled throughout their potential range. I report on a project to construct range maps based on occurrence records for all (n=280) morphospecies of Australian mosquitoes. I created Military Grid Reference System (MGRS) maps of Australia with 100 km square grids recorded as present or absent for each species, according to a variety of sources, including online databases and historical reports from the literature that I georeferenced. In addition to including grids that contained actual occurrence records, range maps were made more conservative/realistic by identifying grids that were likely to be positive; being grids that surround known positive grids or lay between close positive grids. The resulting range maps could be used in a key that used location, either via GPS or MGRS grid reference. These maps could complement expert opinion maps and be updated as new observations arise. Overlaying grid maps creates impressions of potential species richness, although these maps are affected by sampling effort, which is concentrated around traditional centres of medical entomology activity. It was noted that some uncommon and informal species have not been recorded for decades, suggesting that these species are overlooked or have disappeared.

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Flaviviruses alters endoplasmic reticulum-mitochondria contacts to regulate respiration and apoptosis

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During infection, DENV and ZIKV remodel the endoplasmic reticulum (ER) to form viral replication factories. They induce the elongation of mitochondria in favor of viral replication suggesting that infection modulates mitochondria functions. Early observation with DENV suggested that this concomitant with an alteration of ER-mitochondria contacts (ERMC).

Here, we performed an extensive transmission electron microscopy-based analysis to demonstrate that both DENV and ZIKV indeed alter ERMCs. We have further dissected at the molecular level how this morphological alteration is regulated by flaviviruses, and in which extent this impacts on key mitochondrial activities. Stable ERMCs rely on specialized tethering protein complexes located at the surface of both organelles, such as VAPB-PTPIP51, RRBP1-SYNJ2BP and IP3R1-VDAC1. DENV and ZIKV infection induced an overall alteration of ERMCs protein-protein interactions that was attributed to NS4B viral protein. Interestingly, flaviviral infection altered the expression profile of both RRBP1 and SYNJ2BP, suggesting that they specifically target this tethering complex. Importantly, expression knockdown of ERMC proteins increased virus replication, supporting that ERMCs are altered for the benefit of viral replication. Furthermore, DENV or ZIKV infection as well as NS4B expression markedly modulated mitochondrial oxygen consumption rate. Our metabolomic and mitoproteomic analyses revealed that this correlated with a decrease in the abundance of several metabolites of the Krebs cycle and with changes in the stoichiometry of the proteins of the electron transport chain. Most importantly, ERMC destabilization by protein knockdown showed a decrease in virus-induced apoptosis demonstrating the importance of ERMC alteration by DENV and ZIKV for a sustained replication.

Molecular detection of zoonotic arbovirus from mosquitoes in Papua New Guinea farms

J Goi

Mosquito-borne viruses pose a serious threat to public health globally. Surveillance of zoonotic arboviruses and their vectors is important to plan for their control. Japanese encephalitis virus (JEV) can cause serious disease and death. Endemic in Asia and Western Pacific countries including Papua New Guinea (PNG). Since its first detection in 1997/98 knowledge about JEV distribution and vectors in PNG has been very limited. Present study, different mosquito traps were used to collect mosquitoes from livestock farms in PNG to detect JEV and other zoonotic viruses.

Mosquitoes were collected in commercial chicken and pig farms in Central, Morobe and Western Provinces from 2019-2021 using CDC light traps (UV and incandescent) and BG traps. Mosquitoes collected were identified, pooled and sent to the Australian Centre for Disease Preparedness. Mosquitoes were further pooled, processed and tested by a panel of specific real-time RT-PCR assays.

Total of 29,920 mosquitoes were collected the surveys.18,951 Culex spp. Unfed mosquitoes were processed for PCR testing. JEV was detected in Cx. gelidus mosquitoes (n=2 pools) from a piggery in Morobe Province. Phylogenetic analysis revealed these viruses belong to JEV genotype 4 and are related to the genotype 4 strain that caused the recent large-scale JEV outbreak in Australia. Kokobera virus was detected in Cx. annulirostris Cx. quinquefasciatus and Cx. pullus from all sites sampled except Morobe Province.

Study demonstrates continued circulation of JEV in PNG. Findings also represent the first detection of JEV in PNG in *Cx. gelidus* mosquitoes and the earliest detection JEV genotype 4 in the Australasian region. This is the first time this arbovirus has been detected in zoonotic arbovirus vectors in PNG livestock farms. Our study highlights the potential for cross-border migration of JEV between PNG and Australia and need for regional collaboration to facilitate surveillance and control of this important zoonotic pathogen.

A Needle in a Haystack:
Screening Mosquito Pools for Arboviruses in FNQ

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Sentinel surveillance of arboviruses in Queensland can provide early detection of human pathogenic arboviruses that may help define and predict mosquito-borne disease risks. Wide-scale surveillance of mosquito trap collections in areas of high faunal diversity and mosquito density is a feasible, informative tool. We recently undertook a comparison of methods for detection of human pathogenic arboviruses such as Ross River and Barmah Forest virus in Far North Queensland (FNQ). Tropical Public Health Services (Cairns) set traps from March to August 2022 in 16 sites around FNQ . Trap contents were screened using routine qRT-PCR for two specific viral targets and RNASeq NGS. Mosquitoes were identified by species, morphologically, and stored in DNA/RNA Shield +0.1% Tween at -20°C until pooled for RNA extraction. Mosquitoes were pooled by species into 230 samples and homogenised. Pools were screened for qRT-PCR for pan-alpha and JEV-specific NS5 targets. Potentially positive pools were then Sanger sequenced to confirm. These 230 samples were then submitted for Total RNASeq NGS in five unique samples, depleted by mosquito rRNA via a custom depletion probe kit. Data was trimmed, paired and blasted to ncbi viral protein by diamond and mapped by MEGAN. Reads were then mapped to reference genomes with Geneious. Out of these five data sets, two were positive for BFV. Complete genomes were also obtained for a number of insect-specific viruses including ones not previously found in Australia. qRT-PCR results, to directly compare with RNASeq, provide a number of hits for arboviruses but less sequence length due to primer design. Considering the labour hours and consumables required for wide-scale qRT-PCR, RNASeq NGS is proving to be cost-effective screening method for mosquito arboviral surveillance.

Japanese encephalitis emergence in Australia: transmission pathways and populations at risk

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Japanese encephalitis (JE) is the leading cause of viral encephalitis in Asia. In Australia, only six locally transmitted human JE cases were reported between 1995 and 2021; five from northern Queensland and one in the Northern Territory. Thus, JE was considered to be largely a disease of travellers. In 2022 and 2023 there was a dramatic range expansion of the virus across the southern Australian states (34 confirmed human cases, 10 probable cases and 6 deaths). Large numbers of domestic piggeries (>80) and feral pigs from across Australia were also identified as JE exposed or infected. In early 2023, the closely related, and ecologically similar Murray Valley encephalitis virus (MVEV) was also circulating widely in the same flood-prone areas associated with the JEV expansion. Human MVE deaths have subsequently been recorded in Victoria and the Northern Territory. We discuss the climatic drivers of these disease outbreaks, and speculate on key hosts, vectors and transmission pathways. We also reflect on the potential mitigation of those transmission pathways including the implications of, and options for, mosquito control and mass immunisation programs for the human population. Such vaccination strategies will be necessary if outbreaks continue in the immunologically naïve Australian population. In an effort to address issues of human vaccine availability, efficacy and cost, we present data on the immunogenicity of reduced JE vaccine doses delivered by the intradermal route in adults and children. We also examine whether JE vaccination confers cross-protection to MVEV. These studies will inform vaccination policy should Australian JEV or MVEV outbreaks persist or worsen. The research will be especially valuable if questions of vaccine supply and expense threaten Australia’s ability to immunise its most at-risk populations.

Could sugar provide protection from mosquito-transmitted viral infections?

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Mosquitoes are known to transmit a wide variety of viruses, parasites and other pathogens. Mosquito cell surfaces are covered by a dense array of sugar molecules (glycans) and lectins (glycan-binding proteins). These sugar molecules found on midgut glycoproteins are often exploited by microbial pathogens as recognition sites for attachment and pathogenesis. We hypothesise that the differences between the surface sugar structures in mosquitoes of different species could be a factor affecting vector competence. In this study, we characterised the surface glycan structures and specificity of glycan-binding proteins of various mosquito cell lines derived from Ae. albopictus, Cx. quinquefasciatus, Cx. tarsalis and An. gambiae using lectin and glycan arrays, respectively. Our data demonstrates key differences in the range of glycans displayed on different mosquito cell lines. The glycan binding profiles also differ among the different cell lines. As a prerequisite to functional studies, this study provides useful information to a better understanding of the molecular interactions including the carbohydrate structures for transmission blocking strategies. The role of these specific glycan-lectin interactions between mosquito cells and viruses are now being further elucidated with exciting findings on novel intervention strategies in the pipeline. Overall we show that sugar may provide the key to developing the next topical anti-viral treatment for arboviral infections.

NSW Arbovirus Surveillance and Mosquito Monitoring Program 2022-2023

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The NSW Arbovirus Surveillance and Mosquito Monitoring Program is conducted from Spring to Autumn each year when mosquitoes are most active in NSW. The Program was expanded in 2022-2023 with more surveillance sites and Japanese encephalitis virus (JEV) testing in response to detections of JEV in January and February 2022. There were no detections of JEV in the Program in the 2022-2023 season, however there were numerous detections of other flaviviruses, Murray Valley encephalitis virus (MVEV) and Kunjin virus from January to April 2023. MVEV and Kunjin virus DNA was identified in mosquito collections from 4 January to 12 March 2023 (20 collections). Seroconversions for MVEV and Kunjin virus were detected in sentinel chicken flocks from blood collections from 12 January to 17 April 2023 (38 separate instances of seroconversion). MVEV and Kunjin detections were primarily in the Riverina region of southern NSW, in an area that from the JEV detections in early 2022 was considered of high JEV risk. The MVEV and Kunjin detections followed the number of *Culex annulirostris* mosquitoes collected peaking in the week-ending 7 January 2023. Substantial flooding in inland NSW in 2022 through to early 2023 accompanied by large congregations of breeding water birds, hosts of MVEV and Kunjin virus, likely contributed to the increase in these flaviviruses in the environment.

Exotic vector detection adjacent to major Sydney cemetery: a highly suitable habitat

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Exotic to New South Wales (NSW), *Aedes aegypti* and *Aedes albopictus* pose a significant biosecurity and public health threat to Australia. In the case of *Ae. aegypti*, the establishment of insecticide resistant strains that are widely prevalent overseas would make control more challenging. Predictive models show *Ae. albopictus*, if introduced, could establish along the entire east coast of Australia[1]. Thus a competent vector of exotic viruses including dengue, yellow fever, chikungunya, and Zika would be present in major urban centres.

A major potential pathway of exotic mosquitoes arriving into Australia is via first points of entry (FPoE). This includes airports, seaports, and cargo handling facilities, better known as Approved Arrangement (AA) facilities[2]. At such sites, an exotic mosquito surveillance program is conducted by the Department of Agriculture, Fisheries and Forestry (DAFF) with concomitant response strategies within these sites.

Between December 2021 and February 2023, there were 10 detections of *Ae. aegypti* at a single AA facility in Sydney. This facility is located immediately adjacent to vegetated land conducive to mosquito harbourage, and a cemetery with thousands of water holding containers that could provide habitat for container-inhabiting mosquitoes. Thus there is a genuine risk that exotics could become established at this site.

A multiagency response led by NSW Health to the exotic mosquito detections was required. This included urgent insecticide application and enhanced surveillance within the AA facility, risk assessment and planning as well as a comprehensive vector survey of the site and surrounds, regular adult trapping within the cemetery, and a larval control program. This presentation will summarise these responses, highlighting the challenges, and will inform future planning and responses, improve policies in addressing the gaps in exotic mosquito responses, and minimise the risk of the establishment of exotic vectors around comparable settings in NSW.

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Mosquito-specific viruses: novel agents to control the transmission of arboviral pathogens

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Biological control is a proven approach to controlling arboviral diseases; exemplified by the release of *Wolbachia* bacteria in populations of *Aedes aegypti* to control dengue. However, *Wolbachia* are not readily available to control arboviruses that are not transmitted by *Aedes aegypti*. Insect-specific viruses (ISVs) are benign viruses that are part of the natural biota of mosquitoes, maintained in a transmission cycle from female insects to their offspring. A range of ISVs can also induce resistance to infection by human arbovirus pathogens in mosquitoes, a phenomenon referred to as superinfection exclusion. We will present progress on a National Health & Medical Research Council funded project to develop a range of ISVs as biological control agents. The aims of the project are to 1. Establish persistent infections of ISVs in a range of vectors, 2. Test the vector competence of the ISV infected vectors, 3. Determine the mechanisms of transmission interference by ISV and 4. Evaluate ISV population invasion potential in semi-field cages. Intrathoracic microinjection of *Aedes aegypti* with the lineage II insect-specific flavivirus (ISF) Binjari virus (BinJV) established somatic tissue infections. Intense and disseminated infections were observed in the progeny of these mosquitoes using immunofluorescence analysis of whole mosquitoes, however the filial infection rate has been variable, ranging from 0 - 42%. Inoculations of *Aedes aegypti* and *Aedes vigilax* with the lineage I ISF Parramatta River virus (PaRV) produced isolated infection loci in somatic tissue. These early results have prompted a systematic approach to the evaluation of ISV susceptibility across vectors and strategies to increase vertical transmission rates.

Australian *Culex annulirostris* mosquitoes are competent vectors of JEV genotype IV

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Japanese encephalitis virus (JEV) is the causative agent of Japanese Encephalitis disease, which is an infection of the central nervous system that can be fatal. It has broad distribution across Asia and parts of Oceania and has shown an ability to spread to new regions. JEV has five genotypes (GI-V), the least prominent and most divergent being GIV and GV.

In 2022, Australia experienced its largest outbreak of JEV on the mainland resulting in 42 human cases across 5 states and territories including 7 fatalities. Sequencing determined the outbreak to be caused by GIV, which had previously been restricted to Indonesia. *Culex annulirostris* mosquitoes are known vectors of JEV, however no data was available on whether this species was a competent vector of JEV GIV.

In response to this outbreak, we conducted a 14-day vector competence trial of JEV GIV outbreak strain in Australian *Cx. annulirostris* mosquitoes. We assessed viral load in midguts at 4 days post exposure (dpe) and 14 dpe in saliva and carcass samples. qPCR results showed 100% midgut infection rate, 100% carcass dissemination rate, and 93% saliva transmission rate. Dissemination of virus throughout the mosquito body at 14 dpe, including the ovaries and salivary glands, was supported by histological analysis of mosquito cross-sections. These results prompted us to conduct a study on vertical transmission of JEV GIV in *Cx. annulirostris* to assess risk of re-emergence by this pathway. We determined that transovarial transmission did occur as eggs were positive for virus by qPCR, however no trans-stadial transmission to adults was observed.

Our study determined that Australian *Cx. annulirostris* mosquitoes are efficient vectors of JEV GIV and was the first completed vector competence trial on this outbreak strain.

Arbovirus Surveillance and Mosquito Borne Diseases in Queensland: 2021/2022 - 2022/2023

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Prior to 2022, the Queensland arbovirus surveillance network comprised three components: 1) human case notification, 2) the Arbovirus Sentinel System which targets flaviviruses in remote locations, and 3) the Peri-urban Alphavirus Surveillance Program. Human notifications combine laboratory and clinical data stored in the Notifiable Conditions System. Both the Arbovirus Sentinel System and Peri-urban Alphavirus Surveillance Program use sugar-based techniques to detect virus RNA in expectorate of mosquitoes collected in CO_2-baited traps. Following the emergence of Japanese encephalitis virus (JEV) in 2022, mosquito-based surveillance was expanded to include whole mosquito processing via the bespoke JEV responsive surveillance program. Further, the Peri-urban Alphavirus Surveillance Program was extended to include testing for JEV.

Ross River virus accounted for the greatest number of mosquito-borne disease notifications in both 2021-2022 and 2022-2023, but numbers were generally below the 5-year mean for each quarter. The number of notifications of Barmah Forest virus infection was consistent with the average number reported in previous years. An increase in overseas acquired infections (including malaria, dengue and chikungunya) was observed from the beginning of 2023, consistent with increased international travel.

JEV was detected for the first time outside northern Queensland in February 2022, with confirmation of an infected commercial pig herd in the Goondiwindi region. Notably, 5 human cases (2 confirmed and 3 probable) of JEV were notified between March and May in 2022. JEV was also detected from pools of mosquitoes on four occasions collected as part of the JEV response. Aside from evidence of exposure or infection in feral pigs from NAQS surveillance, JEV activity has not been detected south of Cape York since July 2022.

Despite notifications in other states, no confirmed locally-acquired Murray Valley encephalitis virus (MVEV) or West Nile (subtype Kunjin) virus (WNV_{KUN}) cases were notified in Queensland during the reporting period. However numerous detections of MVEV and WNV_{KUN} were documented via mosquito surveillance in 2023.

The emergence of Japanese encephalitis virus in Australia, 2022: a Queensland perspective

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Japanese encephalitis virus (JEV) activity was first notified in southwestern Queensland on 26 February 2022, with confirmation of infection in a commercial piggery in the Goondiwindi region. To date, a total of 5 human cases (2 confirmed and 3 probable) have been notified in Queensland, with one fatality reported. All human cases had likely exposure in the Southwest, Darling Downs or Western Downs regions of southern Queensland. Infections in humans were notified in March, April and May, while infections in commercially-farmed pigs were notified across February-July. JEV was detected in mosquitoes collected in the Wide Bay region from one trap in March and three traps in April. Surveillance of feral pigs by the Northern Australian Quarantine Strategy from June 2022 onwards has demonstrated exposure in feral pigs in several locations across northern Queensland.

The response to JEV in Queensland has been multi-faceted, with a focus on vaccination roll out, public and clinician messaging and communications, mosquito-based surveillance, operational training and engagement across government agencies. Whilst a Multi-Agency Threat Assessment Team was convened in early stages of the JEV outbreak, the Queensland Japanese Encephalitis Virus Taskforce continues to be the primary forum for engagement across government agencies to drive strategic JEV response.

Building Vector Control Capacity to Reduce Malaria Transmission in Papua New Guinea

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Papua New Guinea (PNG) has the highest prevalence of malaria in the Western Pacific region, and despite renewed focus on bednets and malaria case management, malaria transmission is increasing. Key underlying causes include insufficiently resourced public health infrastructure and insufficiencies with current vector control products. Malaria vector bionomics in PNG are also complex with a heterogenous distribution of vector species across the country, and a considerable amount of outside and early evening biting occurring when bednets are not protecting people. Vector control is crucial to the prevention of malaria and complementary vector control tools are needed reduce transmission. To address this situation the NATNAT project, **N**ewly **A**dapted **T**ools **N**etwork **A**gainst mosquito-borne disease **T**ransmission is testing and optimising new and existing vector control tools in PNG. A new vector control testing facility has been established in Madang Province enabling product evaluation under laboratory and semi-field conditions. Recently, the NATNAT team completed a 2-year indoor residual spraying (IRS) field study on the north coast of PNG. Preliminary analyses show a clear reduction in mosquito biting rates as a result of the IRS, and high community acceptance. In addition, we are conducting several pilot studies to explore use-cases, effectiveness and acceptability of larval source management and spatial repellent interventions. The program will also provide evidence on the acceptability and health systems barriers and enablers to uptake of vector control tools to strengthen policy and implementation networks with the PNG National Department of Health and other stakeholders.

Impact of mosquito control programs on Australian stingless bees

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Mosquito control programs are often asked to respond to questions about the impacts of their control tools on native fauna. Recently, Australian beekeepers have expressed concerns about the effects of commonly used larvicides on the health of stingless beehives (e.g., *Tetragonula carbonaria*). In this talk, we will present results from a series of field experiments designed to quantify stingless bee foraging behaviour and toxin acquisition following exposure to mosquito control products (i.e., *Bacillus thuringiensis israelensis*) in coastal treatment environments. Data from these and lab experiments will provide a realistic assessment of pesticide exposure at potential contact points between stingless bees and mosquito control programs. Once published, the study will provide mosquito control units with a key reference to use in support of their activities.

Development of an all-in-one metabarcoding approach to mosquito and arbovirus surveillance

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Next-generation sequencing (NGS) techniques such as DNA metabarcoding have the potential to revolutionize vector and arbovirus surveillance. Here, we describe the development and application of a DNA metabarcoding approach to a large sample of light trap collections from Southeast Queensland, Australia. The metabarcoding approach confirmed the presence of all morphologically identified mosquito species as well as commonly misidentified species and difficult to differentiate biting midge species (Culicoides). The general agreement between the proportion of NGS reads and species abundance allows users to reliably infer the overall composition of bulk light trap collections in the absence of morphological identification. Importantly, identification of a variety of vertebrate blood-meal hosts was observed, including humans and a variety of known reservoir host species for Ross River virus and other endemic diseases. Lastly, a diversity of insect-specific and medically important viruses, including RRV, were detected. The results support the use of DNA metabarcoding as an efficient, sensitive vector and arbovirus surveillance tool and means of elucidating spatially and temporally variable vector-host relationships.

What do high school students know about mosquitoes and mosquito-borne diseases?

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A de-identified Knowledge, Attitudes, and Practices (KAP) survey was administered to student participants (Year 10-12) in the STEM Champion Mozzie Hunter citizen science initiative. The aim of the KAP survey was to assess student knowledge in the general areas of mosquito biology, endemic mosquito-borne diseases, use of personal protective measures, and awareness and participation in citizen science. The results of the survey have highlighted critical gaps in student knowledge and, specifically, areas where local and state health agencies can improve public health awareness messaging relating to mosquitoes and mosquito-borne diseases to young people. The survey also revealed areas of success, such as a high awareness and use of personal protective measures to reduce mosquito bites and the threat of disease. Critically, the survey revealed an almost universal lack of participation in and unawareness of citizen science. Such information suggests poor engagement and targeting of young people in current citizen science activities. Surveys of this nature are rare, but critical, as they can help guide and improve future engagement and educational activities targeting specific demographic groups.

Scoping review of Japanese Encephalitis disease transmission models

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The Japanese Encephalitis (JE) outbreak in south-east Australia in 2022 was a large and unexpected expansion in geographic distribution of this disease, causing >40 reported clinical infections in people, and severe losses on many commercial pig farms. Transmission models have used environmental, host and vector data to determine factors that influence the size and duration of JE outbreaks in host species such as pigs, and the risk to people. The objective of this study was to identify model parameters and structures to inform development of JE transmission models in Australia. This review will identify gaps in the information required for JE transmission modelling, and therefore, provides a foundation from which data for JE disease transmission models can be collected.

Methods:

We conducted a PRISMA-ScR-guided systematic scoping review of published population-based infectious disease transmission models for JE. Databases searched included Google Scholar, PubMed, ProQuest, Scopus, and Web of Science with search terms (“Japanese encephalitis” OR JEv) AND model AND (spread OR transmission). Records were each screened by at least two reviewers, and data extracted from eligible records. Collated data included the context, type and purpose of the model, parameters used, vector and host species included, and climatic variables incorporated in the model.

Results:

Of the 573 papers identified from searches of peer-reviewed databases, 2.6% (n=15) were eligible following screening and eligible for data extraction. Analysis is ongoing, and a review of data extracted, including gaps identified, will be presented.

Conclusion:

Given the public health risk and impact on the Australian commercial pig industry, there are information gaps that need to be addressed to develop evidence-based models for JE prevention and control.

Metagenomic sequencing reveals extensive diversity of RNA viruses in Western Australian mosquitoes

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Mosquitoes harbour a wide diversity of microorganisms, including insect-specific viruses and viruses of public health importance. In recent years, metagenomic approaches have enhanced the study of these widely diverse and complex virus populations in field-collected mosquitoes. We used metagenomics to characterize the infectome in mosquitoes trapped as part of the WA arbovirus surveillance program.

Firstly, we performed high-resolution metagenomic sequencing on six mosquito species associated with medically important viruses: *Aedes vigilax*, *Culex annulirostris*, *Cx. australicus*, *Cx. globocoxitus*, *Cx. molestus* and *Cx. quinquefasciatus*. We identified 41 RNA and one DNA viral species from 19 families, including 13 novel viruses. *Culex* mosquitoes exhibited a significantly higher diversity of viruses than *Aedes*, and no virus was shared between the two genera. We observed heterogeneous distribution of viruses between geographical regions and between closely related species suggesting the possible role of geography and host species in shaping virome composition. *Wolbachia* bacteria were detected in three members of the *C. pipiens* complex, excluding *C. globocoxitus*.

Secondly, we characterized viruses from cytopathic effect (CPE) positive tissue culture supernatants obtained by inoculation of mosquito homogenate, in which flaviviruses and alphaviruses were excluded via fixed-cell ELISA using virus-specific monoclonal antibodies. We characterized whole genomes of 91 RNA viruses belonging to 11 species from five viral families. The viruses included Gan Gan virus, associated with mild human disease, and Batai, Wallal and Warrego viruses, known to cause animal disease. We also identified one Murray Valley encephalitis virus and a Ross River virus, both known to cause human disease and a possible limitation of the screening ELISA in this study.

Follow-up epidemiological investigations are needed to determine whether other viruses detected infect humans or other animals. In summary, we have used an unbiased approach to expand and understand the diversity of RNA viruses and other microorganisms in WA mosquitoes.

Museum genomics resolves cryptic species in the arbovirus vector Culex annulirostris

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The common banded mosquito *Culex (Culex) annulirostris* Skuse, 1889 is a primary vector of Murray Valley encephalitis, Japanese encephalitis virus (JEV), Ross River virus and other arboviruses in Australia and elsewhere in Australasia. Although previous sanger-based molecular studies reported putative cryptic species in *Cx. annulirostris*, diagnostics and taxonomic resolution was hampered because the link between genotype and phenotype was not preserved due to destructive sampling techniques and a lack of authoritatively identified reference specimens. We successfully identified specimens held in Australian museums or reference collections dating back to 1951 by using genome skimming from only one or two legs to sequence the complete mitochondrial genome and ribosomal operon. Phylogenetic analysis of the mitogenome confirmed the presence of at least three cryptic species within *Cx. annulirostris*, corroborated by morphological analysis of non-destructively sampled voucher specimens, with an additional cryptic species identified after combing our dataset with previously published COI data. We diagnose four cryptic species, comprising: *Cx. annulirostris* sensu stricto widespread throughout Australia, with smaller populations throughout Timor-Leste and Australasia; *Culex (Culex) somerseti* Taylor, 1912 stat. rev. (herein resurrected from synonymy within *Cx. annulirostris*), predominantly eastern Australian; and two undescribed species from Papua New Guinea and north Queensland, Australia, and another from the Solomon Islands. Ancestral state reconstruction revealed recent migration of two independent *Cx. annulirostris* populations from Australia to Papua New Guinea and Timor-Leste, respectively. We also resolve the validity and placement of manuscript names from museum collections and the literature. Adoption of museum genomics will enable improved biosecurity diagnostics of native and exotic species, tracking of historical incursions of vectors using historical collections, and working towards describing the remarkable 170 unnamed mosquito species documented in Australia. Epidemiologists can now complete vector competence testing on each revised species to identify their specific role in disease transmission, including the recent outbreak of JEV in Australia.

REAPER: Mosquito *in vivo* virus targeting to control viral transmission

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Vector-borne diseases pose a significant global health burden.

Current strategies to control mosquitoes and the viral diseases they spread rely primarily on the use of insecticides. These measures can have significant adverse effects on local ecology, and mosquitoes are becoming increasingly resistant. Next generation biocontrol tools, such as genome engineering, offer an environmentally safe, customisable, and scalable option in which to control mosquito populations. To this end, we have established a mosquito population control method whereby viral RNA Expression Activates Poisonous Effector Ribonucleases (REAPER), leading to mosquito death if infected with specific viruses.

Here, we have directly manipulated *Aedes aegypti* mosquito genome to express Cas13 and guide RNAs targeting chikungunya virus. When a mosquito takes an infected blood meal, guide RNAs binds to viral RNA leading to Cas13-mediated cleavage of essential mosquito RNA. This activation of REAPER essentially senses the presence of specific viruses and can lead to premature mosquito death via non-specific targeting of cellular RNA. This novel technique is the first instance of Cas13 mediated biocontrol developed for disease vectors.

Our results show that after infection with chikungunya virus, REAPER is effective at causing death in infected mosquitoes. At 14 days post blood meal with CHIKV, there is a 30% decrease in mosquito survival compared to controls. Viral load of each infected mosquito was also assessed, and a significant decrease ($p=0.004$) in the amount of virus present was shown in REAPER mosquitoes.

We show that REAPER is a large step forward in mosquito control, with future work being performed to translate it into different viral targets and mosquito species. Next generational population control strategies such as shown in this study are an increasingly viable tool that could be deployed during viral outbreaks.

Entomologists, do they really matter? Xenomonitoring for Lymphatic Filiariasis Surveillance in Samoa

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Molecular xenomonitoring (MX) is the process of testing mosquitoes for filarial DNA using the polymerase chain reaction and has recently shown positive results as a complement to human studies for monitoring the success of mass drug administration of anti-filarial pharmaceuticals. Recent studies have highlighted the need for a better understanding of whether classifying mosquitoes into species groups improves the usefulness of MX results, especially in counties where entomological expertise is limited and speciation of the mosquito population requires substantial expertise. During molecular xenomonitoring conducted in Samoa throughout 2018 and 2019 (McPherson et al., 2022), mosquitoes were sorted by sex and species by qualified entomologists and trained technicians and pooled by both species and the location in which they were caught. We aimed to determine whether separating the mosquitoes into different species prior to aggregating into pooled samples would alter the estimated infection prevalence of the overall sample.

We compared MX results for the main vector of lymphatic filariasis, *Aedes polynesiensis* and all species combined, and found that temporal trends in the mosquito infection prevalence were similar between regions. We found that for the purposes of detecting temporal trends in the presence of lymphatic filariasis, sorting by either mosquito genus or by mosquito species had no significant effect on our results. While this observation requires further investigation, it is a promising result for the possible simplification of the MX technique and its future implementation as a significant and useful complement to human surveys post-MDA surveillance.

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Detection of Murray Valley encephalitis virus in Victoria and screening technologies

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A high level of mosquito activity was observed during the 2022-2023 mosquito season, which was preceded by significant rainfall events across Australia. During the 2022-2023 mosquito season, molecular whole mosquito trap processing methods for rapid detection of viruses resulted in multiple detections of Murray Valley encephalitis virus (MVEV) across the state of Victoria. MVEV causes Murray Valley encephalitis, a rare but significant disease of humans. These are the first detections of MVEV since 2011 when it was detected in sentinel chicken flocks positioned along the Murray River. High throughput sequencing methods allowed for whole genome sequences to be generated. Comparison of the new sequences to historical sequences placed the recent MVEV detection in genotype G1, which is the dominant genotype observed in Australia. Within, the G1 genotype the virus was placed into sub-lineage G1A. This is the first time the MVEV G1A has been detected outside of northwest Australia.

Zika Mozzie Seeker update: Innovative citizen science sustains invasive *Aedes* mosquito surveillance

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South East Queensland is at constant risk of invasion by 'Zika mozzies' (*Aedes aegypti* and *Ae. albopictus*) from Queensland distributions and international pathways. Belated detections risk covert outbreaks of exotic diseases (Zika, dengue or chikungunya) in our large cities. Since 2017 Metro South Health has partnered with its' community and Queensland Health's diagnostic laboratory to deliver 12 synchronised rounds of Zika Mozzie Seeker (ZMS). This citizen science platform links eggs from DIY ovitraps to a molecular diagnostic for species identifications. Collectively the results deliver expansive, ephemeral networks (n = 166 suburbs) that increase entomologic confidence that Zika mozzies have not invaded the region.

ZMSs success is underpinned by community engagement and innovative ICT infrastructure. The Metro South Health electronic coordination hub connects eggs collections to a sensitive PCR methodology developed by Queensland Health's Forensic and Scientific Services. Each PCR can rapidly detect the genetic material of a single 1st instar *Ae. aegypti* within 5,000 non-target mosquitoes. To date (R 1-11) a consistently high rate (58%) of participation (n = 4,210; mean 382.76 per round) was achieved by supporting registrants (n = 7,312, mean 665 per round) with electronic communications (SMS and email). All eggs (approx. 466,000, mean 42,400 per round) have tested negative (n = 141 PCR cohorts). Each participant is notified of their result by SMS. Each round concludes with an egg abundance heat-map being posted on the ZMS website.

Keeping SEQ free from Zika mozzies during the count-down to the Brisbane Olympics is challenged by climate change, increasing urbanization, and biosecurity pressures. ZMS aligns with government strategies to promote citizen-science in Australia and WHO recommendations for novel community-based *Aedes* mosquito monitoring plans. Integrating residential surveillance (ZMS) with commercial surveillance programs (Rapid Surveillance for Vector Presence) offers a new normal for enhancing 'early-warning' programs for invasive urban mosquitoes.

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Optimal methods to collect bloodfed mosquitoes at Australian piggeries

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The unexpected expansion of Japanese encephalitis virus (JEV) transmission into southern Australia exposed our poor knowledge of the ecology at piggeries. The complex transmission dynamics of JEV involve multiple vector and vertebrate species that vary in their contributions to transmission. One of the best ways to identify the links between JEV vectors and vertebrate hosts is to analyse the bloodmeals of mosquitoes in an area with JEV transmission. Bloodfed mosquitoes are notoriously difficult to collect because they quickly digest their bloodmeals and are usually not attracted to CO_2 while digesting. Many bloodfed collection methods exist and we tested resting boxes, battery-powered aspiration, barrier screens, and CO_2 attractant traps at Australian piggeries and their nearby wetland areas. We address the pros and cons of each method and which method yielded the highest number of bloodfed mosquitoes. We also provide an overview of the mosquito ecology at piggeries: their breeding habitats, species composition, presence within pig sheds, and overall abundance. Our results inform larger studies that will take place in 2024 and 2025.

Mosquito-borne disease surveillance across Victoria for the 2021-22 and 2022-23 seasons

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Victoria experienced average rainfall during 2021-22 with slightly above average rainfall for the southwest of the state only. Temperatures were above average across the State with very much higher than average temperatures for Melbourne and the northwest. This led to the development of mosquito populations to high levels during the peak of summer 2021-22. Mosquito surveillance identified 17 positive trap detections for Ross River virus (RRV) and a further 2 detections of Barmah Forest virus (BFV). This translated to 108 confirmed human cases of RRV and 5 human cases of BFV for the 2021-22 mosquito season.

After the detection of Japanese encephalitis virus on a commercial piggery in March 2022, testing of mosquitoes was enhanced to include detection of Japanese encephalitis virus (JEV). During the 2021-22 season, seven detections of JEV in trapped mosquitoes were detected across six Local Government Authorities. 10 c0nfirmed human cases of JEV were notified in 2021-22.

Heavy rainfall commencing on the 15 October 2022 across land that had previously received substantial rainfall during the winter months led to flash flooding across much of central and Northern Victoria. The 2023 Victorian outbreak of Murray Valley encephalitis was heralded by the detection of Murray Valley encephalitis virus (MVEV) in trapped mosquitoes in early January with detection continuing on an almost weekly basis to the end of March with a total of 48 positive MVE traps over the season). A total of eight human cases of MVE (including 6 fatalities) were recorded throughout the season. Mosquito surveillance also detected 8 positive traps for West Nile virus (Kunjin) although this did not lead to human cases being identified. JEV was not detected in any mosquitoes tested throughout the 2022-23 season, with only one human case notified to the department. Mosquito surveillance and testing for alpha and flaviviruses has been demonstrated to provide an important early warning system on which response activities were based.

Enhanced Japanese encephalitis virus surveillance across piggeries in Victoria.

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In response to detections of Japanese encephalitis virus in Victoria during early 2022, the Victorian Department of Health in collaboration with the Department of Energy Environment and Climate Action (DEECA) initiated an enhanced surveillance program across Victorian commercial piggeries in late 2022. The aims of the study were to investigate the presence/absence of JEV in commercial piggeries and provide an assessment of early warning detection systems (mosquito testing and saliva testing) to limit the potential for human cases of disease.

The Department undertook surveillance across six participating farms including mosquito trapping and sampling of oral fluids from piggeries that had either had previous detections of JEV or were in areas considered at risk for JEV transmission based on previous detections or human cases of JEV.

Enhanced surveillance occurred from December 2022 to June 2023. Japanese encephalitis was not detected in mosquito populations or oral fluids during the surveillance period. However, information on mosquito abundance and species composition will be important in determining the receptivity of piggeries and impact of surrounding infrastructure and landscape features into the future.

In vivo characterisation of the Australian Japanese encephalitis virus genotype 4, NSW2022.

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Introduction: Japanese encephalitis virus (JEV) is a mosquito-borne flavivirus of clinical importance, with symptoms in humans ranging from febrile illness to severe encephalitis. JEV is the leading cause of viral encephalitis in Asia, and there is now a threat that the virus may become endemic in Australia. In February 2022, a rare genotype of JEV, genotype 4, caused unprecedented and widespread outbreaks in all mainland eastern states of Australia, leading to 42 confirmed human cases and 7 deaths. A virus isolate was obtained from a stillborn piglet brain in New South Wales in February 2022 (NSW2022), which requires *in vivo* characterisation.
Methods: Adult (6-48wks) C57BL/6J, IFNAR-/- and IRF7-/- mice were infected with JEV isolates from 3 different genotypes (genotype 4 NSW2022, genotype 3 Nakayama, and genotype 2 FU) and the phylogenetically related Murray Valley encephalitis virus (MVEV). Mice body weight loss, disease score, survival, viremia, and brain and spleen titres, and histology were obtained.
Results: C57BL/6J and IRF7-/- mice modelled JEV and MVEV neuroinvasive infection with virus replication identified primarily in the cortex, thalamus, and hippocampus. Histopathological lesions such as perivascular cuffing, neuron vacuolation and vasogenic oedema faithfully recapitulated those identified in post-mortem human brains. IFNAR-/- mice produced a lethal viraemia, without neuroinvasive infection. Neuroinvasive infection leading to mortality of C57BL/6J and IRF7-/- mice was significantly rarer for NSW2022 compared to FU and Nakayama.
Conclusion: The Australian outbreak genotype 4 isolate of JEV, NSW2022, may be less neurovirulent in mice compared to other genotypes of JEV. IFNAR-/- mice are a model of viremia and mortality, while C57BL/6J and IRF7-/- mice is a model of neurovirulence. This is the first comprehensive *in vivo* analysis of the NSW2022 isolate.

Supporting vaccination and surveillance options for the pig industry and one health

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With the widespread detection of Japanese encephalitis virus (JEV) in piggeries throughout Queensland, New South Wales, South Australia, and Victoria in 2022, there is a critical need to assess and develop new surveillance options, conduct assessments of JEV vaccines registered internationally and improve antibody diagnostics methods.

At the Australian Centre for Disease Preparedness (ACDP, CSIRO), we are undertaking several projects to pursue these challenges to support industry and government priorities.

Central to this objective, a pig infection (*in vivo*) model has been established using the 2022 JEV circulating strain. Using the developed JEV infection model, we are assessing JEV vaccines used internationally to determine if the outbreak virus strain "genotype" affects vaccination suitability. The defined reagents generated in this study are improving our ability to diagnose JEV in pigs using antibody tests.

We are exploring new surveillance methods to expedite detection of JEV on farms, with projects to assess the suitability of sample collection at the pig herd level.

This presentation will provide an up-to-date summary of the work being undertaken at ACDP to inform early warning surveillance approaches, vaccination strategies and serological diagnosis in pigs.

Reprioritisation of surveillance to respond to changes in risk of arrival and establishment of exotic mosquitoes into New Zealand

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The post-border national saltmarsh mosquito surveillance programme began in 2005 to complement the eradication programme of *Aedes campthorynchus* (Southern saltmarsh mosquito) in New Zealand. The programme was designed to support freedom of *Aedes campthorynchus* and to prevent re-invasion of *Aedes campthorynchus* given that there and been approximately $70 million investment in eradication. Drivers including climate change, global range extensions of vectors and changes in trade have highlighted the need for re-prioritisation of the surveillance programme to not only consider saltmarsh habitat, but to include other types of habitat to detect exotic mosquitoes in New Zealand. GIS tools and a pathway risk analysis were utilised in the re-prioritisation of the surveillance programme.

A new chimeric vaccine (ISVac-JEV) and rapid diagnostic assays for Japanese encephalitis virus in pigs

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Mosquito-borne flaviviruses cause a significant global health burden and have potential for pandemic emergence. The unprecedented outbreak of disease caused by Japanese encephalitis virus (JEV) in 2022, affecting humans, pigs and horses underscores the unpredictability of arboviral incursions. Upscaling of vaccine and diagnostic capabilities during outbreaks can be slow and expensive. Here we describe the rapid production of a chimeric flavivirus vaccine and diagnostic antigens for the Australian 2022 genotype IV outbreak strain of JEV using the recombinant platform based on the Australian insect-specific flavivirus, Binjari virus (BinJV). We showed that the chimeric BinJV-JEV virus particles were antigenically indistinguishable from the parental virus and grew to high titres in insect cells while remaining replication defective in vertebrate cells.

JEV can cause substantial reproductive losses and infertility in farmed pigs, resulting in large economic losses. Infected pigs can also play a major role in the JEV transmission cycle posing a “One Health” problem. However, there are currently no veterinary vaccines for JEV available in Australia to prevent infection of animals. When the BinJV-JEV chimera was assessed as a vaccine candidate (named ISVac-JEV) for efficacy in young pigs, more than 90 percent of the vaccinated animals were protected against infection after JEV challenge.

The chimeric BinJV-JEV particles were also successfully applied to a range of diagnostic assays, including a virus neutralization test, a lateral flow assay and various ELISA formats, including the development of an assay to serologically differentiate naturally infected animals from those that are vaccinated (DIVA).

The chimeric viruses created during this project represent a versatile, non-infectious (for vertebrate cells), high-yield technology for generating chimeric flavivirus particles with low biocontainment requirements. ISVac-JEV is also the first vaccine candidate designed from a genotype IV strain of JEV.

Factors and mechanisms responsible for vertical transmission of insect-specific flaviviruses in mosquitoes

A Peterson, J Harrison, R Hall, H Malmanche, J Peters, L Hugo

A novel approach to flaviviral arbovirus control is through the use of phylogenetically similar insect-specific flaviviruses (ISF) to induce “superinfection exclusion” in mosquitoes by utilizing a primary virus infection to inhibit a similar secondary virus infection. While superinfection exclusion has been shown to reduce arbovirus dissemination in the mosquito, ISFs must be vertically transmitted in persistently-infected mosquito populations to have long-lasting impacts on mosquito control.

This project aims to elucidate some of the unknown mechanisms that facilitate ISF vertical transmission (VT) in mosquitoes by investigating (i) the initial infection method of the ISF in the mosquito; (ii) determining the viral genome components in ISF VT; and (iii) determining the role of mosquito host developmental protein vitellogenin in ISF VT.

VT rates of ISFs have been assessed by inoculating mosquitoes via four methods: intrathoracic injection, infected sugar water, infected blood meal, and infected cell exposure. Surprisingly, different methods of initial infection has led to different rates of VT. Optimizing VT of ISFs would allow for ISFs to spread across populations of mosquitoes and aim to reduce infection of arboviruses to humans.

Viral factors may also enhance the rates of ISF VT. ISFs have high rates of vertical transmission whereas arboviruses do not, in spite of their phylogenetic similarities. We hypothesise that key structural proteins may play a role in ISF vertical transmission. Future studies will endeavour to identify the viral proteins that are associated with vertical transmission rates via the creation of chimeric viruses between vertebrate-infecting flaviviruses and ISFs.

Moreover, identified viral proteins may also interact with mosquito developmental proteins such as vitellogenin and mediate transovarial ISF transmission in mosquitos. Together, this work will provide insight into the factors and mechanisms that mediate ISF vertical transmission to create ISF establishment in mosquito populations to control the dissemination of pathogenic arboviruses.

2022/2023 Mosquito season in the Northern Territory

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The rainfall patterns in the NT during the past two years have been variable. While the southern part has received above average rains across two wet seasons, much of the northern half had well below average rainfall through 2021/22 but above average rainfall through 2022/23.

While the size, periodicity and distribution of rainfall has a very large influence on mosquito populations and mosquito-borne disease transmission across the NT, high tides that infiltrate coastal swamps and salt marshes play an important role in coastal areas.

In this NT round-up adult mosquito surveillance data, virus isolation data, sentinel chicken flock serology and human disease case data across two mosquito seasons is used to explore the effects of seasonal weather on mosquito-borne disease in the NT.

Human cortical brain organoid models of the Australian Japanese Encephalitis virus genotype 4 isolate

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JEV is a mosquito-borne neurotropic virus and is the leading cause of viral encephalitis in Asia, with up to 100,000 symptomatic infections and 25,000 deaths each year. The unprecedented and widespread emergence of JEV genotype 4 in Australia puts approximately 750,000 Australians at risk of infection and disease. In rare cases, virus enters the brain via the blood brain barrier and infects neurons leading to cell death and uncontrolled inflammation. The molecular basis for JEV tropism to neurons (neurocytotropism) is not known, nor is the mechanism of JEV-induced apoptosis or encephalitis. Human cortical brain organoids (hBOs) are derived from induced pluripotent cells that self-assemble into 3D tissues that mimic structures of the brain. We infected hBOs with JEV from 3 different genotypes (genotype 4 NSW2022, genotype 3 Nakayama, and genotype 2 FU) and the phylogenetically related Murray Valley encephalitis virus (MVEV). This was compared to the non-neurotropic Yellow Fever virus (YFV 17D) and the attenuated Imojev chimeric virus vaccine which comprises the JEV premembrane (prM) and envelope (E) structural genes on the YFV 17D backbone. The three JEV strains replicated to higher titers than MVEV, YFV 17D and Imojev, with clear virus replication on the outer surface of the hBOs. Organoids infected with JEV shrunk, indicative of cytopathic effect, while infection with YFV 17D and Imojev did not cause CPE. These hBO systems provide a tractable *in vitro* model of JEV infection and cell death that can be used for detailed mechanistic studies.

Japanese encephalitis virus – the Australian pig industry experience 2022

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The Australian pork industry comprises around 3 million pigs including 275,000 breeding sows. Production is predominantly spread across pig-producing regions in Queensland, New South Wales, Victoria, South Australia and Western Australia. The industry supports approximately 36,000 jobs, and families and communities across the country.

Prior to 2022, Japanese encephalitis virus (JEV) had never been detected in domestic pigs in Australia. In February 2022 JEV genotype IV was detected simultaneously in geographically and operationally unrelated piggeries in Queensland, New South Wales and Victoria, followed within days by detections in South Australia. These detections represented an extraordinary expansion in the known range of JEV. By June 2022 over 80 piggeries and 60% of the national industry had been impacted. Affected producers sustained unprecedented breeding and piglet losses impacting pig health, producer welfare and livelihood, and national fresh pork supply.

Human health, animal health and pork industry expertise collaborated nationally to respond to the incursion. Management at affected piggeries focussed on mosquito surveillance, JEV vaccination and mosquito bite prevention for at-risk personnel, and provision of guidance on mosquito control. Effective mosquito control was challenged by the need to balance interventions of choice against regulatory, food safety, logistical and environmental risks and constraints posed by the agricultural food production context.

JEV has not been detected in piggeries in Western Australia and no new detections of JEV in Australian piggeries have occurred since October 2022. Significant knowledge gaps pertaining to the ecology and epidemiology of JEV in Australia remain an ongoing constraint to better understanding and mitigating the JEV risk to pigs and associated risks to public health.

This presentation describes the lived experience of JEV in 2022 from the perspective of the Australian pig industry.

Attitudes towards personal protection against mosquito borne diseases in the PNGDF

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Mosquitoes are major vectors of various pathogens such as viruses, parasites and nematodes, some of which can cause significant human disease and even death. Preventing exposure to mosquito bites is imperative for the prevention of mosquito borne diseases, especially when vaccine and prophylactic treatments are not available. Personal mosquito protection measures such as repellents, mosquito coils, mosquito nets and the wearing of appropriate clothing can be an effective method for protection against mosquito bites and therefore its use can reduce the risk of transmission of mosquito borne disease. Mosquito borne pathogens like *plasmodia*, dengue virus, Japanese encephalitis, and lymphatic filariasis are present in Papua New Guinea and with limited large-scale chemical control efforts in the country, education and compliance around personal protective measures against mosquito bites are crucial for the prevention of disease.

We present observations of the attitudes and behaviours of members of the Papua New Guinea Defence Force (PNGDF) from surveys and questionnaires conducted in 2019 in Wewak and Vanimo and 2023 in Lae, Papua New Guinea. Survey participants (members of the PNGDF) were questioned on which mosquito bite prevention measures they use and given options to select from common items such as bed nets, insect screens and mosquito repellent, select ‘no protection used’ or record the utilised protection methods in a free-text box. Data from this study enables staff members of the Australian Defence Force Malaria and Infectious Diseases Institute to draw correlations on disease prevalence and personal protection compliance of the surveyed PNGDF members. This information is also crucial to enable effective training and capacity building to enhance the Force Health Protection of members of the PNGDF.

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PacMOSSI: The Pacific Mosquito Surveillance Strengthening for Impact Program

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The Pacific has experienced unprecedented outbreaks of dengue, chikungunya and Zika virus alongside ongoing malaria and lymphatic filariasis transmission. In response, PacMOSSI was created with the goal to support Pacific Island Countries and Territories (PICs) to strengthen vector surveillance and control to prevent, contain and control mosquito-borne diseases and improve the health and wellbeing of Pacific communities. PacMOSSI is a partnership between 21 PICs and international institutions with a focus on building south-to-south networks. PacMOSSI was designed to comprehensively build capacity across all aspects of vector surveillance and control, while at the same time being adaptive to the greatest needs of the partner countries. The range of activities include: needs assessment, online and face-to-face training, data management, citizen science demonstration projects, operational research grants, technical support, and alignment of vector strategic plans with WHO recommendations. PacMOSSI is a holistic program building capacity by training and facilitating translation into practice by providing essential infrastructure to implement best practice vector surveillance and control. PacMOSSI fulfils an essential role in the region by supporting effective implementation of PIC Ministry of Health led vector surveillance and control.

Wolbachia mosquito releases and dengue in north Queensland, 12 years on

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In the first decade of the millennium north Queensland faced an ever-increasing threat of dengue. Over 20 outbreaks were recorded between 2000 and 2010, sparked by dengue virus importations in travellers and resulting in over 2,000 confirmed locally-acquired cases of which up to 20% were hospitalised and two patients died. In 2011, the first releases of *Wolbachia*-carrying *Aedes aegypti* mosquitoes were undertaken in two small communities in Cairns, north Queensland. These first releases showed that *Wolbachia* were able to establish and persist in local mosquito populations, that the mosquitoes had a reduced ability to transmit dengue. Over the next eight years *Wolbachia* releases were progressively undertaken across the majority of dengue-prone areas in north Queensland, including the major population centres of Cairns and Townsville and other communities. These releases necessitated the development of new ways of undertaking releases, including the distribution of over 140,000 *Wolbachia* mosquito release kits to householders and schools who then grew and released their own mosquitoes throughout their communities. By 2019, *Wolbachia* releases had been undertaken across an area of 300 km^2 and covered more than 320,000 people. Ongoing monitoring of mosquito populations across these areas have shown high persistence of *Wolbachia* in mosquitoes in all areas, without the need for re-releases of mosquitoes. Against the previous decade's backdrop of increasing numbers of dengue outbreaks, the progressive coverage of areas with *Wolbachia* between 2011 and 2019 has shown a concomitant decline in locally-acquired dengue cases despite ongoing introductions of dengue virus from overseas travel-acquired cases. Interrupted time series analysis of dengue case notifications data demonstrates near-elimination of local dengue transmission in locations where *Wolbachia* has been established, with an overall reduction of more than 97% (95% confidence interval: 90-99%) in the incidence of locally-acquired dengue in *Wolbachia*-treated populations of north Queensland.

Models for ~~dummies~~ mozzies

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Mathematical models are fun (and useful). Trust me.

I know. You've read the title and already thought twice about whether you stay for this rapid-fire presentation on mathematical models. But if you don't know what a 'model' is, this presentation is for you. Modelling can so often seem like a black box, but their uses are evolving and their applications in mosquito research are increasing.

Understanding the different types of models available and their strengths and limitations is critical for policymakers and public health officials in developing effective strategies to control and prevent mosquito-borne diseases. By combining these models with data on the biology of the vectors and the epidemiology of the diseases, we can gain insights into the transmission dynamics of mosquito-borne diseases and develop strategies to reduce the burden of these diseases on human health.

Here I introduce you to mosquito related problems that models are commonly used to solve, highlight critical limitations with different methods, and offer insights into new and emerging approaches.

Is it a bird? Is it a pig? No, it’s not superman

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People are often surprised to learn that vector-borne diseases need more than just a vector for transmission. In fact, the majority of these pathogens are zoonotic, meaning a vector has to feed on a susceptible host before it can transmit a pathogen to a human. Japanese encephalitis virus (JEV), the causative agent of a debilitating disease in humans and swine, is no exception. In Asia where JEV is endemic humans are largely considered dead-end hosts and there are two cycles thought to maintain the pathogen in transmission; a pig-associated rural domestic and a bird-associated wild cycle. Following an outbreak of JEV in naïve parts of Australia there has been significant interest in identifying the potential hosts and assessing ongoing transmission risks. But where do we begin?

Here I will explore current knowledge of potential JEV through an Australian context. Examining both native and introduced species, this work draws on the ecological factors that influence host suitability, including host competence, vector-host interactions, migratory patterns and seroprevalence studies. I present the findings as a traffic light system with species we know that are suitable hosts, species that may be suitable hosts, and those that are dead-end hosts. There are many unknowns in an Australian context for JEV transmission, and I highlight the most important gaps to close. By understanding the potential host species and their roles in JEV transmission, we can enhance preparedness, mitigate risks, and safeguard public health in Australia.

Aedes aegypti elimination program, Tennant Creek, Northern Territory.

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The dengue mosquito, *Aedes aegypti*, is not established in the Northern Territory, except for in the remote town of Tennant Creek. Here, an elimination campaign has been underway since its detection in February 2021, where an initial survey determined the mosquito to be present throughout the town. The elimination campaign involves property inspection and treatment methods largely following those used in previous NT elimination programs, with numerous rounds of treatments conducted, and a wet season without any detections required before elimination can be declared (Whelan *et al*. 2020). In this presentation, we describe the methods and techniques used to suppress and eliminate the dengue mosquito in an attempt to return the NT to its dengue free status and discuss the challenges faced. Methods include breeding source reduction, receptacles treatments with various insecticides and a chlorine/detergent solution to destroy egg. Adult trapping is conducted to aid in locating larval breeding sites. To ensure the dengue mosquito does not spread to other areas in the NT, particular attention has been paid to regular inspection and treatment of transport hubs. A key challenge faced has been the exceptionally wet weather, with extensive local rainfall for January and February this year.

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Climate, mosquitoes and Ross River virus notifications in NSW, 1991-2021

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Ross River virus (RRv) is Australia's most common, endemic mosquito-borne disease. RRv transmission is influenced by various factors including mosquito abundance, reservoir host populations, climatic, environmental, socio-economic factors and human behaviour. The aim of this study was to describe spatial and temporal patterns of RRv disease in NSW over thirty years (1991 to 2021) alongside climatic and entomological data.

De-identified RRv notifications data from 1991 to 2021 were obtained from the NSW Notifiable Conditions Information Management System. Climatic data were obtained from SILO, a database of Australian climate data hosted by the Queensland Government, and entomological data were obtained through the NSW Arbovirus Surveillance and Mosquito Monitoring Program (ASMMP).

RRv human notifications data were analysed by broad bioclimatic regions (inland, coastal and Sydney metro), at the local level (local government and local health district), and by age and sex. Weekly and annual notifications by geographic region and annual incidence rates were calculated and described. Seasonality of RRv disease notifications was also described.

A range of climatic variables were explored in relation to RRv notifications over the study period including maximum and minimum temperature, number of rainfall days, intensity of rainfall, and humidity. The study also described entomological data from the ASMMP in relation to RRv notifications including total weekly counts of mosquitoes across all species trapped at NSW trapping locations, total weekly counts of competent mosquito vectors of RRv in NSW (*Culex annulirostris* and *Aedes vigilax*) and viruses isolated from trapped mosquitoes.

Findings from this study can help to identify high risk areas in NSW for RRv disease and provide evidence for where enhanced RRv surveillance and management should be targeted. Findings can also help to determine variables to incorporate into early warning systems to improve prediction of RRv disease in NSW.

NSW response: Japanese encephalitis virus

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In NSW, there were 14 confirmed cases of Japanese encephalitis virus (JEV) reported in 2022. All but one of these cases were confirmed in January or February 2022.

The outbreak triggered the NSW government to implement a range of activities to help prevent future cases of JEV. This included enhanced human and animal surveillance (including pig, mosquito and sentinel chicken surveillance), public communications on mosquito bite prevention, procurement and distribution of insect repellent to high-risk areas and distribution of available vaccines to target populations. NSW Health also hosted webinars and distributed information on JEV to health professionals, councils and veterinarians, and provided funding via local health districts to local councils to support vector control at mass gatherings and public areas, develop mosquito warning signage and develop local mosquito management plans. Many of these activities were supported by a one-off grant of $5.5 million that was provided by the Commonwealth Department of Health and Aged Care as part of a package of funding for JEV response activities across Australia.

Since October 2022, there have been no known confirmed cases of JEV in NSW, and there were no detections of JEV in mosquitoes trapped and sentinel chickens participating in the NSW Arbovirus Surveillance and Mosquito Monitoring program (ASMMP). However, there have been 6 confirmed cases of Murray Valley encephalitis (MVE) in NSW in 2023. There were 21 separate seroconversions of MVE and 16 separate seroconversions of Kunjin virus in sentinel chickens and 18 flavivirus detections in mosquitoes (18 MVEV, 2 Kunjin) in the 2022/23 arbovirus season in NSW.

SA Health arbovirus round-up

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The 2021-22 and 2022-23 South Australian (SA) mosquito seasons were influenced by La Niña weather events, resulting in three consecutive years of above average spring and summer rainfall across most of SA. Both seasons saw high rainfall that resulted in major flooding events, in the far north of the state in 2021-22 and along the River Murray in 2022-23. In early 2022 a cluster of nine human Japanese encephalitis cases (two fatal) were notified in SA. Climatic influences combined with the emergence of Japanese encephalitis virus (JEV) indicated that the risk of local transmission of flaviviruses was at its highest since 2010-11.

Commonwealth JEV funding allowed for considerable expansion of the local council mosquito management subsidy. The SA sentinel chicken program was also expanded and Health Protection Programs, SA Health (HPP) was also able to hire three regional based mosquito surveillance and control officers. These regional officers conducted routine surveillance programs and responsive surveillance and control in high-risk council areas not participating in the subsidy program.

The number of arbovirus infections reported in SA during the 2022-23 season was 50% lower than the 2021-22 season but similar to the number reported for the 2020-21 season. In the 2022-2023 season the majority of cases likely acquired their infection along the River Murray which is consistent with previous years. In the 2021-22 season, cases were more widely distributed over the state, including the Eyre Peninsula and Kangaroo island. This was not seen in the 2022-23 season.

In addition to the heightened risk of arbovirus, both seasons saw exotic mosquito incursions at first points of entry that required surveillance and control activities to be undertaken by HPP.

Applying the FPIC standard: Indigenous mosquito management opportunities in the Torres Strait.

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Aedes albopictus and *Aedes aegypti* are invasive mosquitoes found in the Torres Strait, associated with spread of dengue fever during outbreaks within local communities. Indigenous participation and stakeholder engagement are central to any program designed to mitigate the threat of mosquito borne disease in the Torres Strait. During 2022, the CSIRO and its capability partners Square Circle and i2i Global undertook a series of conversations with Torres Strait Islander and Aboriginal stakeholders from local, state and federal government organisations. We utilized the international standard of FPIC, or Free Prior and Informed Consent, developed by the United Nations in a collaborative design process with local communities and leaders, which privileges Indigenous knowledge, ownership and voices with a commitment to capacity development, and seeks to involve stakeholders in implementation and develop capacities in line with their self-defined needs. This process also ensures stakeholder accountability while establishing dynamic relationships through which program design, implementation and Monitoring, Evaluation and Learning can take place and is contingent, conditional and iterative as a way of maintaining social licence.

Conversations with Indigenous stakeholders explored interests in next-generation mosquito control and pathogen suppression technologies such as *Wolbachia* and genetic engineering. During conversations we discussed the potential for an invasive mosquito control program that could have positive impacts beyond the immediate public health benefits, including opportunities for an Indigenous-led business to implement the program as well as employment, training and education. This presentation will discuss the FPIC process employed, outcomes of conversations to date and future goals of Indigenous co-design of any potential project.

Co-circulation of multiple medically relevant arboviruses in Brisbane mosquitoes

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Introduction: Arbovirus surveillance of wild-caught mosquitoes is an increasingly affordable and sensitive means of monitoring virus emergence, distribution and transmission dynamics. A variety of diagnostics for screening mosquito pools for human pathogens is available and in common use worldwide, but most provide a very limited information about the circulating virus lineages. Metatranscriptomics is becoming increasingly affordable and sensitive for integrating complete viral genome sequencing into the surveillance framework. This enables untargeted, large-scale, high-throughput arbovirus studies from diverse samples.

Aims: We employed different molecular approaches to screen wild-caught mosquitoes for arboviruses and associated metatranscriptomics in routine surveillance activities.

Methodology: Mosquitoes were collected in CO_2-baited light traps deployed across five Brisbane urban parks from March 2021 to May 2022. Mosquitoes were identified and separated into pools of ≤200 specimens per species, location, and collection date. RNA was extracted and screened for flaviviruses and alphaviruses using genus-specific primers targeting the NS5 and nsP4 regions, respectively. A subset of samples detected as having arboviruses by quantitative reverse transcription PCR (qRT-PCR) were also submitted for library preparation using a mosquito-specific ribosomal RNA depletion method and sequenced on the Illumina NextSeq 550.

Results: A total of 54,670 mosquitoes, representing 26 species were captured and processed in 382 pools. A total of 30 arbovirus detections in 28 pools was obtained using qRT-PCR. Twenty of these samples were further investigated by metatranscriptomics and the results of 85% of qRT-PCR analysis of samples were confirmed. Both methods detected four medically relevant arboviruses: Barmah Forest, Ross River, Sindbis and Stratford viruses.

Conclusion: Augmenting qRT-PCR with metatranscriptomics provided full-length genomes of the arboviruses circulating in Brisbane. Metatranscriptomics could yield greater insights into virus evolution and transmission pathways for specific virus genotypes. The technique is increasingly affordable, sensitive and is suitable for incorporation into routine arbovirus surveillance.

The landscape epidemiology of the Japanese encephalitis outbreak in piggeries in 2022

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The widespread activity of Japanese encephalitis virus (JEV) in previously unaffected regions of eastern and southern Australia in 2022 represents the most significant local arbovirus emergency in almost 50 years. Japanese encephalitis virus is transmitted by mosquitoes and maintained in wild ardeid birds and amplified in pigs, the latter of which suffer significant reproductive losses as a result of infection. The landscape of JEV outbreak risk in mainland Australia is almost entirely unknown, particularly in the eastern and southern parts of the country where the virus has not been previously documented. Although other areas with endemic JEV circulation in the Indo-Pacific region have demonstrated the importance of the wild waterbird-livestock interface in agricultural-wetland mosaics, no such investigation has yet determined the configuration of pathogenic landscapes for Australia. Moreover, the recent emergence in Australia has followed substantial precipitation and temperature anomalies associated with the La Niña phase of the El Niño Southern Oscillation. This study investigated the landscape epidemiology of JEV outbreaks in Australian piggeries recorded between January and April of 2022 to determine the influence of ardeid habitat suitability, hydrogeography, hydrology, land cover and La Niña-associated climate anomalies in demarcating risk. Outbreaks of JEV in domestic pigs were associated with ardeid species richness, hydrological flow accumulation, and landscapes comprising agricultural land use, fragmented grasslands, and seasonal wetlands, which were specifically characterised by their temporary surface water accumulation during the 2021 La Niña period. This study has identified the composition and configuration of landscape features that delineated risk for piggeries during the 2022 emergence of JEV in Australia. Although preliminary, these findings can inform actionable strategies for the development of new One Health JEV surveillance specific to the needs of Australia.

Developing a strategic response plan for exotic mosquito incursions in NSW

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Exotic mosquitoes, especially *Aedes aegypti* and *Aedes albopictus*, pose a threat to NSW. In addition to the risk of local transmission of exotic pathogens, such as dengue, chikungunya, and Zika viruses for which there are no current vector species in NSW, there is a potential economic burden for local authorities to control mosquito populations to reduce the risk of local pathogen transmission from infectious travellers and mitigate severe nuisance-biting impacts. Exotic mosquitoes may be introduced to NSW through First Points of Entry (i.e. international airports and seaports) and Approved Arrangements (i.e. freight handling facilities), as well as other pathways.

A response to the detection of an exotic mosquito incursion will require a rapid multiagency response with strong community engagement strategies. To facilitate a coordinated response, a sub plan to the NSW State Emergency Response Plan was proposed with NSW Health as the lead agency. This approach clearly defines the roles and responsibilities of local and state stakeholders such as the NSW Health public health network, NSW Department of Primary Industries, National Parks and Wildlife Service, local councils and commonwealth agencies. Given a response would be highly localised, the stakeholder mix would be specific to a region but the framework of response presented in the sub plan would be adaptable across the state and scalable where necessary.

Following the development of the draft sub plan (including a framework for operational response informed by an expert advisory panel) and in principle support from the NSW State Emergency Management Committee, extensive consultation was undertaken with key stakeholders including national experts experienced in exotic mosquito response and management. There has been overwhelming support for the sub plan but some key legislative and operational challenges have been identified that must be resolved to ensure effective implementation of plan should it be required.

Open-source Citizen Science data for surveillance of mosquitoes and vector-borne disease

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Community participation in scientific data collection (including Citizen Science) is widely used to gather biodiversity information. Application to mosquito surveillance has become commonplace. The iNaturalist online platform hosts a wide range of biological observations collected globally, including mosquitoes. The Mozzie Monitors project on iNaturalist gathers and reports mosquito observations and has been established for Australia, Southern Africa, Brazil and, most recently, the United Kingdom and Ireland. Whilst data records for these projects only exist for a handful of years, the increasing popularity of iNaturalist has yielded sufficient observations to enable comparative analyses of mosquito fauna. Such analyses can answer questions about the effectiveness of citizen science in describing mosquito diversity and seasonality, along with exotic mosquito detection. Here we report on the performance of iNaturalist in describing mosquito diversity and seasonality in Australia. For the recently established United Kingdom and Ireland project, we compare iNaturalist records with established long-term datasets, and examine gaps in the mosquito surveillance record. Given that iNaturalist reports citizen science observations of wildlife, including potential mosquito-borne disease reservoirs, it might have utility for prediction and early warning for disease outbreaks. To that end, we tested whether iNaturalist records of known Japanese encephalitis (JE) virus vectors and reservoir hosts during the recent outbreak of that virus could be used to predict transmission. Significant associations between water bird observations and JE cases were detected, indicating possible utility of Citizen Science for studying disease ecology. The respective roles of online observation platforms and fixed-point trapping programs will be discussed.

Who’s biting who in the zoo? Implications for mosquito-borne disease risk in a unique urban landscape

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Sydney Zoo is a recently opened zoological park located within the Western Sydney Parklands, Bungarribee. The zoo is surrounded by bushland, parklands, and wetlands that provide habitat for a wide range of mosquito species. There is an abundance of local vertebrate wildlife within the parklands including feral deer and native bids and marsupials. Mosquito populations were sampled using carbon dioxide baited light traps at six locations within the zoo and six locations within the adjacent parklands. Sampling was undertaken on five occasions between February and April 2022. All mosquito specimens were identified to species and counted according to trap site and collection date. Pooled samples were tested for the presence of arboviruses. Blood fed specimens were tested to identify vertebrate blood source. A total of 2,630 mosquitoes was collected representing 21 species. The most common mosquito within the zoo was *Culex quinquefasciatus*, making up 69.9% of total specimens collected. The most common mosquito in parklands was *Anopheles annulipes*, making up 32.5% of total specimens collected. Other key pest and vector species were collected within and outside the zoo including *Aedes procax*, *Aedes notoscriptus*, *Culex annulirostris* and *Mansonia uniformis*. No arboviruses were detected in mosquitoes collected from either location. There was a total of 52 blood fed specimens collected representing 11 mosquito species. The majority of blood fed specimens were *Culex quinquefasciatus* (42.3% of blood fed mosquito specimens) with most collected from within the zoo. Notwithstanding humans (13.5% of blood fed mosquitoes), a total of 19 vertebrate species (including native animals, feral animals, and exotic animals housed within zoological park) were identified from DNA extracted from blood fed mosquitoes. It was noteworthy that *Culex quinquefasciatus* was recorded feeding on a number of mammal species, highlighting the potential role in arbovirus transmission in other urban or peri-urban settings.

Rapid Surveillance for Vector Presence (RSVP): COVID-19 impacts on an 'early-warning' program

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Aedes aegypti and *Ae. albopictus* occur in Queensland and threaten invasion of South East Queensland (SEQ; approx. 70% of Queensland's population) through international ports and domestic pathways. In SEQ any detection of a stochastic incursion will trigger eradication campaigns to mitigate future risks of cryptic transmission of exotic viruses (dengue, Zika or chikungunya). Routine surveillance can minimize delays in detection but is often logistically difficult and vulnerable to disruption by competing organizational priorities. Since 2017 Queensland Health has offered 'Rapid Surveillance for Vector Presence' (RSVP) to councils in and near SEQ to expedite presence/absence surveillance of these two mosquito species. Multiple ovitraps form 'runs' (e.g., x 10) and eggs are aggregated into batches (max. 5,000 eggs) to identify the species using a polymerase chain reaction (PCR) testing methodology developed by Queensland Health's Forensic and Scientific Services.

RSVP delivers a 'new normal' through partnership arrangements that are negotiated each year between Queensland Health and volunteer councils. Metro South Health provides the program management hub through a Medical Entomology team. Queensland Health subsidizes the costs of PCR analyses and funds an RSVP Support Officer (0.4 FTE) to; engage councils, coordinate the highly variable ovitrap rosters to comply with a delimited PCR quota, and perform laboratory processing duties (quality assurance, quantifying egg estimates per ovitrap, batching eggs into PCR cohorts and data management).

We briefly review how COVID-19 disruptions created large temporal and spatial holes in RSVP delivery to degrade a regional early-warning capability. This experience is a timely reminder for health authorities to embed strategic solutions that maintain early-warning networks for invasive urban species despite competing public health demands (e.g., COVID-19 or Japanese encephalitis responses). 'Ring fencing' of key staff and integrating surveillance with citizen science platform(s) can minimize organizational risk of delayed detections that will compromise eradication campaigns.

References:

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Japanese encephalitis Outbreak: Mosquito surveillance by three SE Queensland public health units

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Recognition of an undetected Japanese encephalitis virus (JEV) outbreak emerged in Australia in March 2022, following the confirmation of infected pigs in Queensland. Subsequent JEV activity (in pigs and humans) across multiple Australian states culminated in the declaration of a *Public Health Incident of National Concern*. Hot spots for JEV activity in Queensland occurred in Wide Bay and Darling Downs (immediately north and west of SE Queensland, respectively). Federal funding was made available to Queensland Health (Oct 2022) to enhance mosquito surveillance for JEV vectors through a competitive-based process between public health units.

We briefly discuss the rationale for our joint application in the contiguous region of Gold Coast, Metro South, and West Moreton. Our proposal recognised that the pre-existing arbovirus surveillance program may not provide indicator sites for JEV early-warning due to the absence of inland SEQ trap sites, sites along the western suburban fringes, nor the NSW border. Our bid equipped and partially staffed an ambitious supplementary arbovirus monitoring program (18 traps per week per PHU) to service the potential for unmonitored eastward invasion pathway(s) into the most populated area of Queensland (70% of Qld) and unmonitored sections of coastal wetlands.

We compare and contrast the trapping regimes across the three jurisdictions that has increased confidence that potential alternate JEV pathways into SEQ have not gone undetected. We discuss the spectrum of emergent managerial, operational and governance issues (e.g., human resources, methodology, procurement of equipment and consumables, communication, and reporting arrangements) to build and service our arbovirus surveillance programs on short notice. We provide recommendations on refinements to make a robust arbovirus surveillance in this region into a sustainable ‘new normal’.

Molecular Epidemiology reveals the rapid spread of Japanese encephalitis virus in 2022 throughout eastern and southern Australia

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An unprecedented outbreak of the mosquito-borne Japanese encephalitis virus (JEV) occurred in south-eastern Australia in 2022. The outbreak was caused by a novel lineage of JEV genotype IV, which first emerged in Northern Australia in 2021, and has since then resulted in 45 human cases, including 7 deaths, and over 80 infected piggeries. We sequenced and analysed 166 whole genomes of JEV from field trapped mosquitoes (n=9), humans (n=2), a cultured JEV isolate (n=1) and farmed (n=136) and feral pigs (n=19). The majority of outbreak sequences clustered into two genetically distinct lineages (designated clades A and B), separated by three formative single nucleotide polymorphisms, which were circulating between April February 2021 and August 2022. Both lineages were detected in human, mosquito and pig samples, while only clade B was detected in the limited number of human samples sequenced. We conclude that clades A and B and were likely to have been in circulation prior to the outbreak. A lack of spatial-temporal phylogenetic and phylodynamic structure found suggested a rapid dispersal of the outbreak strain in a largely naïve host population.