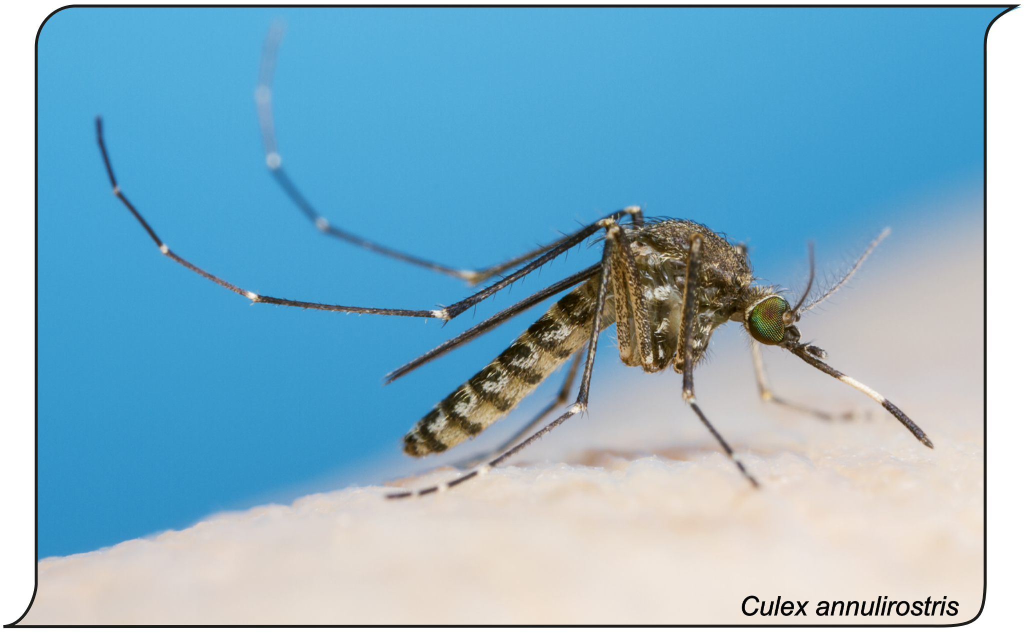
THE NEW SOUTH WALES
ARBOVIRUS SURVEILLANCE &
MOSQUITO MONITORING PROGRAM

2018-2019 Annual Report



Culex annulirostris

**S. DOGGETT, J. HANIOTIS,
J. CLANCY, C. WEBB & C. TOI**
Mosquito & Arbovirus Surveillance Laboratory,
Medical Entomology Department, CIDMLS,
ICPMR, Pathology West,
Westmead Hospital, Westmead, NSW 2145.

**L. HUESTON, H. LIM, L. McINTYRE,
J. GOODWIN, & D.E. DWYER**
Arbovirus Laboratory, Clinical Virology, CIDMLS,
ICPMR, Pathology West,
Westmead Hospital, Westmead, NSW 2145.

S. TOBIN & V. SHEPPARD
Communicable Disease Branch, Health Protection
NSW Health, North Sydney, NSW 2059.

SHPN (HP NSW) 190737
ISBN 978-1-76081-311-6

EXECUTIVE OVERVIEW

- **For the 2018-2019 season**, the NSW Arbovirus Surveillance Program: (i) monitored mosquito populations and undertook surveillance of arbovirus activity through virus isolation in the NSW inland, coastal regions and Sydney area, (ii) monitored flavivirus transmission through the testing of sentinel chickens across inland NSW. Surveillance operated over mid-October to mid-May.
- **The climatic conditions** leading up to 2018-2019 was a spell of extremely dry weather over July to September 2018. While precipitation levels were average for the last three months of 2018, conditions became extremely dry for the first six months of 2019. Neither the Forbes nor the Nicholls hypotheses were suggestive of a potential MVEV epidemic for the 2018-2019 season.
- **For the inland**, 7,998 mosquitoes were trapped and this was one of the lowest collections for the history of the program, and around one quarter of the previous season collection of 33,257. There was one EHV detection from Griffith. There were no seroconversions in the sentinel chickens from any location.
- **Human notifications from the inland** of RRV and BFV totalled 181 (177RRV & 4BFV), which was slightly less than that of the previous season and around half the previous nine season average of 343 (311RRV & 32BFV). The statistical local areas that produced the highest notifications for RRV from the inland was Griffith (12), while the Far West (179/100,000) had the highest notification rate. There were no human cases of infection with MVEV of KUNV.
- **As of August 2019**, the Forbes hypothesis is not suggestive of a possible MVEV epidemic for 2019-2020 and such an outbreak seems unlikely with the ongoing drought. The El Niño-Southern Oscillation is currently neutral, suggesting normal rainfall patterns for the remainder of the year.
- **For the coast**, 48,214 mosquitoes were trapped, which was close to the total of the previous season. There were seven isolates from the coast, including 3RRV from Ballina, 1RRV & 1STRV from Ourimbah, 1BFV from Port Macquarie, and 1BFV from Tweed Heads.
- **Human notifications from the coast** totalled 412 cases, including 347RRV and 65BFV, and this was below the previous nine season average of 627 (438RRV & 189BFV).
- **Sydney** experienced a dramatic increase in mosquito numbers upon the previous season with almost 180,000 trapped compared to the previous season of 31,000. Over 50,000 mosquitoes were collected at Picnic Point alone, and over 45,000 from the new site at Duck River, along the Parramatta River. There were 20 arboviral isolates, including: 1EHV from Blacktown; 2RRV, 8EHV, 1KOKV & 1STRV from the Georges River sites; 1EHV from the Hills District, 4STRV from Parramatta; and 1RRV & 1STRV from Sydney Olympic Park. Human notifications were slightly below average with a total of 62 (61RRV & 1BFV).
- **Detections of exotic mosquitoes at Sydney Airport continue**. This included multiple detections of *Aedes aegypti* trapped at the Sydney International Airport and an approved arrangement facility (that handles freight), and a single detection of an *Aedes albopictus* the Sydney International Airport. Responses included regular teleconferences initiated by the NSW Ministry of Health, enhanced surveillance at the airport, vector surveys, insecticidal applications, and ongoing larval treatments.

TABLE OF CONTENTS

NSW ARBOVIRUS SURVEILLANCE AND MOSQUITO MONITORING PROGRAM 2018-2019

INTRODUCTION

The aim of the Program is to provide an early warning of the presence of Murray Valley encephalitis virus (MVEV) and Kunjin (KUNV) virus in the state, in an effort to reduce the potential for human disease. In addition, the Program compiles and analyses mosquito and alphavirus, especially Ross River (RRV) and Barmah Forest (BFV), data collected over a number of successive years. This will provide a solid base to determine the underlying causes of the seasonal fluctuations in arbovirus activity and the relative abundance of the mosquito vector species, with the potential to affect the well-being of human communities. This information can then be used as a basis for modifying existing local and regional vector control programs, and creation of new ones.

METHODS

Background

Arbovirus activity within NSW has been defined by the geography of the state, and three broad virogeographical zones are evident: the inland, the tablelands and the coastal strip (Doggett 2004, Doggett and Russell 2005). Within these zones, there are different environmental influences (e.g. irrigation provides a major source of water for mosquito breeding inland, while tidally influenced saltmarshes along the coast are highly productive), different mosquito vectors, different viral reservoir hosts and different mosquito borne viruses (e.g. MVEV and KUNV occur only in the inland, while BFV is active mainly on the coast, and RRV is active in both inland and coastal areas). As a consequence, arboviral disease epidemiology often can be vastly different between regions and thus the surveillance program is tailored around these variables.

Arbovirus surveillance can be divided into two categories: those methods that attempt to predict activity and those that demonstrate viral transmission. Predictive methods include the monitoring of weather patterns, the long-term recording of mosquito abundance, and the isolation of virus from vectors. Monitoring of rainfall patterns, be it short term with rainfall or longer term with the Southern Oscillation, is critical as rainfall is one of the major environmental factors that influences mosquito abundance; in general, with more rain come higher mosquito numbers. The long-term recording of mosquito abundance can establish baseline mosquito levels for a location (i.e. determine what are ‘normal’ populations), and this allows the rapid recognition of unusual mosquito activity. The isolation of virus from mosquito vectors can provide the first indication of which arboviruses are circulating in an area. This may lead to the early recognition of potential outbreaks and be a sign of the disease risks for the community. Virus isolation can also identify new viral incursions, lead to the recognition of new virus genotypes and identify new vectors. Information from vector monitoring can also reinforce and strengthen health warnings of potential arbovirus activity.

Methods that demonstrate arboviral transmission include the monitoring of suitable sentinel animals (such as chickens) for the presence of antibodies to particular viruses (e.g. MVEV and KUNV within NSW), and the recording of human disease notifications. Sentinel animals can be placed into potential 'hotspots' of virus activity and, as they are continuously exposed to mosquito bites, can indicate activity in a region before human cases are reported. Seroconversions in sentinel flocks provide evidence that the level of virus in mosquito populations is high enough for transmission to occur.

The monitoring of human cases of arboviral infection usually has little direct value for surveillance, as by the time the virus activity is detected in the human population, often not much can be done to control the viral transmission. Via the other methodologies, the aim of the surveillance program is to recognise both potential and actual virus activity before it impacts greatly on the human population, so that appropriate preventive measures can be implemented. The recording of human infections does, however, provide important epidemiological data and can indicate locations where surveillance should occur.

These methods of surveillance are listed in order; generally, with more rainfall comes more mosquito production; the higher the mosquito production, the greater the probability of enzootic virus activity in the mosquito/host population; the higher the proportion of virus infected hosts and mosquitoes, the greater the probability of transmission and thus the higher the risk to the human population. The NSW Arbovirus Surveillance and Mosquito Monitoring Program undertakes the first four methods of arbovirus surveillance and the results for the 2018-2019 season follow.

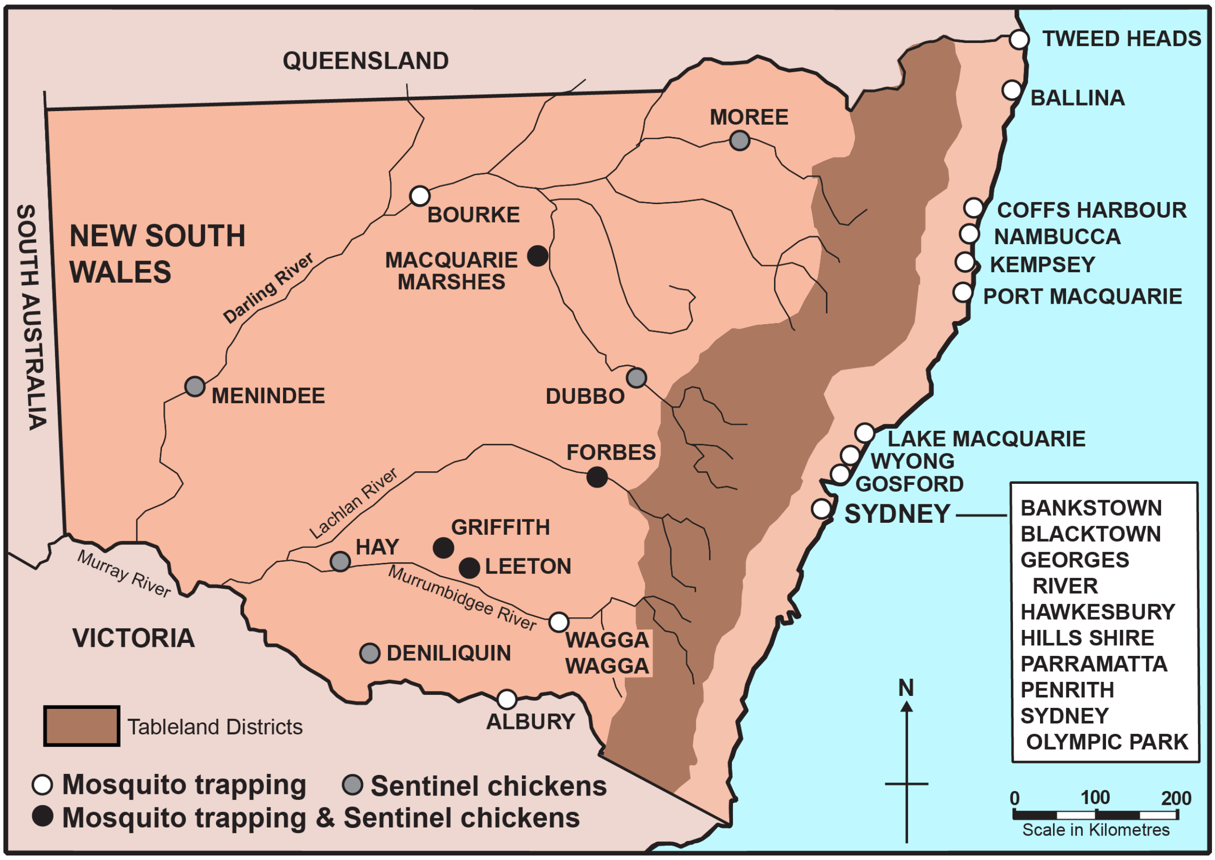


Fig 1. Mosquito trapping locations and Sentinel Chicken sites, 2018-2019.

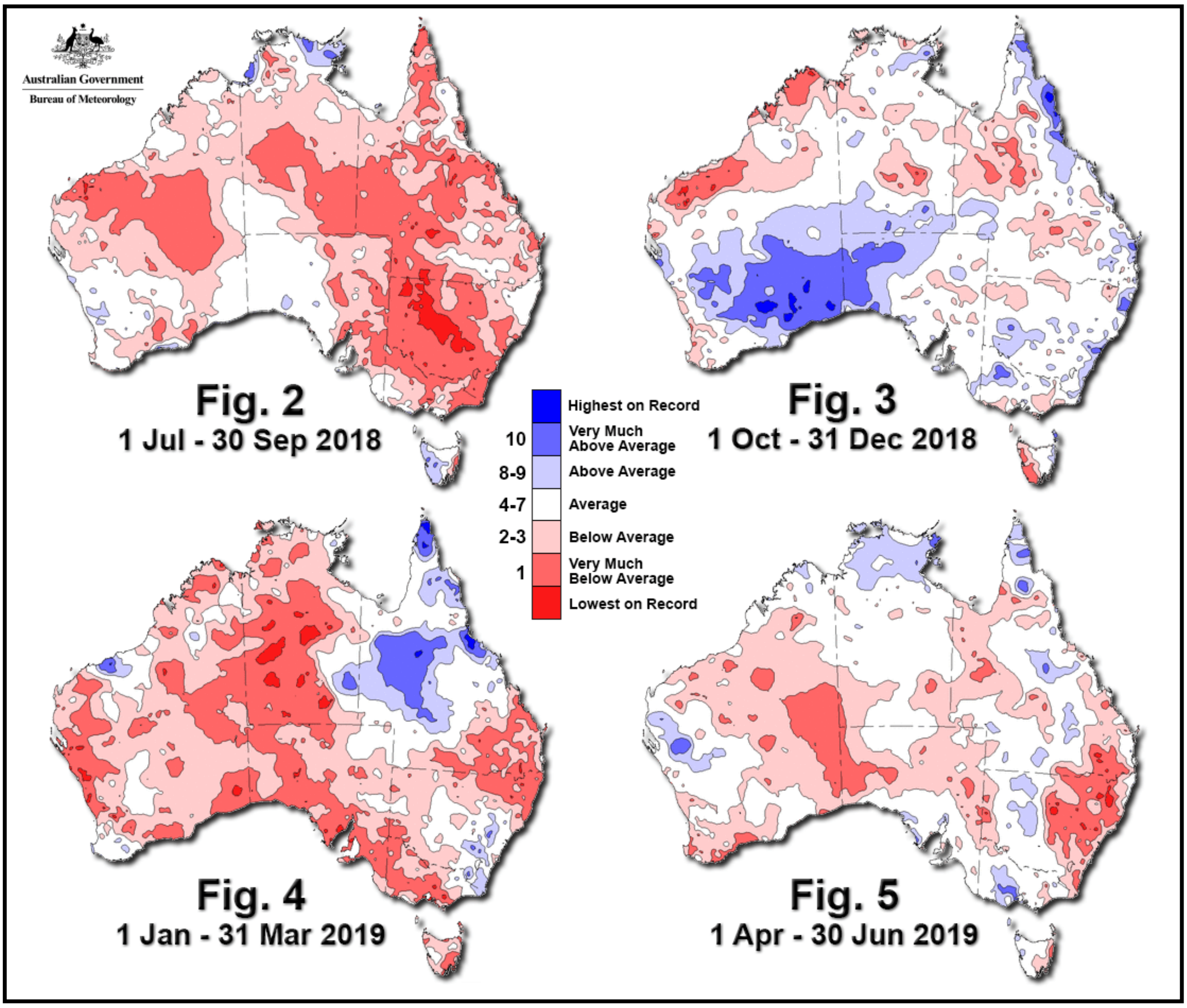
NSW Health Pathology

MONITORING LOCATIONS

For 2018-2019, mosquito-trapping sites were operated at 7 inland, 9 coastal and 8 Sydney locations. Chicken sentinel flocks were located at 9 locations (Fig 1).

WEATHER DATA

Mosquito abundance is dictated principally by rainfall patterns and irrigation practices in inland regions, while in coastal regions tidal inundation along with rainfall is important. Temperature and/or day-length are often critical in determining the initiation and duration of mosquito activity for species in temperate zones. Hence, the monitoring of environmental parameters, especially rainfall, is a crucial component of the Program.



Figures 2-5. Australian Rainfall deciles for the three month periods, Jul-Sep 2018, Oct-Dec 2018, Jan-Mar 2019 & Apr-Jun 2019. The stronger the red, the drier the conditions. Conversely, the stronger the blue, the wetter the conditions. *Modifed from the Australian Bureau of Meterology, 2019*.

The first quarter of 2018 (January to March, not shown) produced below average rainfall patterns for almost the entire state. The second quarter (also not shown) was even drier with very much below average precipitation for many parts of NSW. This dry spell continued with very much below average for almost all the state and some central regions experiencing record low precipitation (Figure 2). The final quarter of the year (Figure 3) had close to average rainfall patterns. However, conditions soon became dry again with well below average rainfall for many parts of NSW during the first six months of 2019 (Figures 4 & 5).

Maximum temperatures for the last half of 2018 were slightly (1-2^o) above average for both inland and coastal regions. The first three months of 2019 continued to be above average with maximum temperatures of 2-3 degrees above normal for northern regions of the inland, and 1-2 degrees above for the coast. By the second quarter of 2018, temperatures were again 1-2 degrees higher than the norm across the state.

MVEV Predictive Models

Two main models have been developed for the prediction of MVEV epidemic activity in south-eastern Australia: the Forbes' (1978) and Nicholls' (1986) hypotheses.

Forbes associated rainfall patterns with the 1974 and previous MVEV epidemics, and discussed rainfall in terms of 'decile' values. A decile is a ranking based on historical values. The lowest 10% of all rainfall values constitute decile 1, the next 10% make up decile 2, and so on to the highest 10% of rainfall constituting decile 10. The higher the decile, the greater the rainfall.

The Forbes' hypothesis refers to rainfall levels in the catchment basins of the main river systems of eastern Australia. These include:

- The Darling River system,
- The Lachlan, Murrumbidgee & Murray River systems,
- The Northern Rivers (that lead to the Gulf of Carpentaria), and
- The North Lake Eyre system.

The hypothesis states that if rainfall levels in these four catchment basins are equal to or greater than decile 7 for either the last quarter of the previous year (e.g. October-December 2017) or the first quarter of the current year (January-March 2018) and the last quarter of the current year (October-December 2018), then a MVEV outbreak is probable. By comparing the relevant quarterly rainfall amounts with historical decile 7 years, it is possible to obtain a ratio; a figure of 1 or greater indicates that rainfall was above the historical decile 7 average (Table 1). Rainfall was below decile 7 in all but one of the catchment basins for the last quarter of 2017, was above decile 7 in only one catchment basin in the first quarter of 2018, and below decile 7 in all of the catchment basins for the last quarter of 2018, thus the Forbes' hypothesis was not fulfilled for 2018-2019 (Table 1). Additionally, decile 7 or above rainfall did not occur in any of the catchment basins during the first quarter of 2019. Therefore according to Forbes', there should be a lower risk of an MVEV epidemic for the upcoming 2019-2020 season.

Table 1. Rainfall indices for the main catchment basins of eastern Australia as per Forbes' hypothesis, relevant to the 2017-2018 and 2018-2019 seasons.

Catchment Basin	Oct-Dec 2017	Jan-Mar 2018	Oct-Dec 2018	Jan-Mar 2019
Darling River	0.93	0.52	0.71	0.68
Lachlan/Murrumbidgee/ Murray Rivers	1.15	0.70	0.87	0.96
Northern Rivers	0.81	1.07	0.70	0.78
North Lake Eyre system	0.75	0.69	0.56	0.82

The Nicholls' hypothesis uses the Southern Oscillation (SO) as a tool to indicate a possible MVEV epidemic. Typically atmospheric pressures across the Pacific Ocean tend to be low on one side of the ocean and high on the other. This pattern then oscillates from year to year. Nicholls noted a correlation between past outbreaks of MVEV and the SO (as measured by atmospheric pressures at Darwin) for the autumn, winter, and spring period prior to a disease outbreak. For the autumn, winter, and spring periods of 2018, the SO values were respectively: 1009.27mm, 1011.80mm and 1010.90mm (indicated on Figure 6 by the yellow arrows and Table 2). The graph on the right has been updated from the originally published figure to include those MVEV active years between 2000 and 2012 (added to the MVEV tallied black columns), and includes the values for the years 2000-2001, 2007-2008, 2010-2011 and 2011-2012. The SO values leading up to the 2003-2004

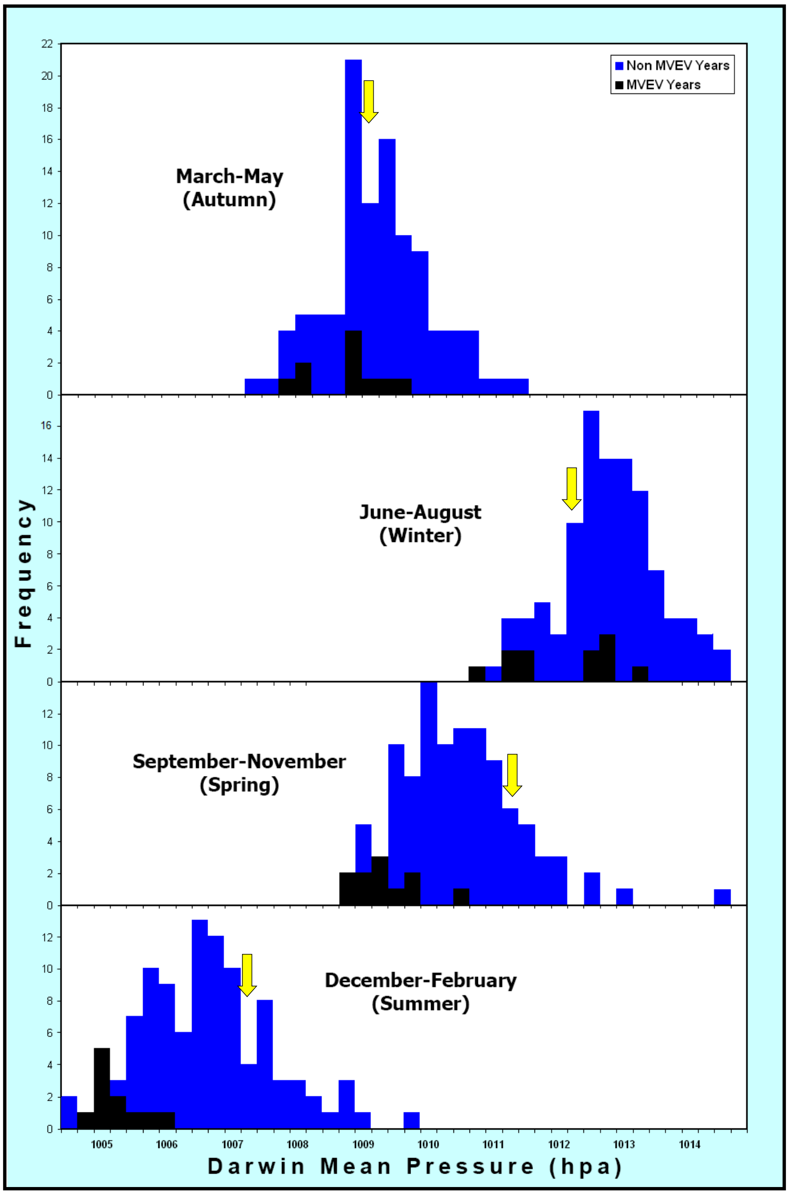


Figure 6. The SO by seasons prior to MVEV active years, according to Nicholls (1986), updated up to Spring 2018. The black bars represent the pre-MVEV active seasons. The yellow arrows indicate the respective SO values relevant to the 2018-2019 season.

season were not included as there was only one detection of MVEV, which may have resulted from over-wintering mosquitoes.

As of August 2019, the autumn Nicholls' value is 1010.27mm and the winter value is 1012.08mm. Only the winter value is within the range of values for past MVEV outbreak years, however the ongoing dry conditions suggests that an MVEV outbreak would be unlikely for 2018-2019.

Currently (as of August, 2019), the El Niño–Southern Oscillation (ENSO) is neutral, suggesting average rainfall for the remainder of the year.

Table 2. The seasonal atmospheric pressures (in mm) according to Nicholls' hypothesis, relevant to the 2018-2019 season.

	Autumn 2018	Winter 2018	Spring 2018
2018 Value	1009.27	1011.8	1010.90
Past MVEV seasons	<1009.74	<1012.99	<1009.99

It is important to note that the Forbes' hypothesis was calculated on environmental conditions experienced during major MVEV epidemic seasons and the models do not propose to predict low to moderate level activity. Thus, negative MVEV models do not necessarily indicate an absence of MVEV activity. Also, these climatic based models do not take into account unusual environmental conditions such those experienced during the summer of 2008, whereby a low pressure cell that began in northern Australia moved through to the south and possibly facilitated the movement of MVEV into NSW (Finlaison *et al*., 2008). A similar phenomenon may have occurred during the 2010-11 season, whereby a low pressure cell that formed from Tropical Cyclone Yasi and moved into Victoria bringing intense rainfall, coincided with major MVEV and KUNV activity (Doggett *et al*. 2011). Nor do these models take into account virus existing in cryptic foci in south-eastern Australia.

MOSQUITO MONITORING

Methods

Mosquitoes were collected overnight in dry-ice baited Encephalitis Virus Surveillance (EVS) type traps. They were then sent live in cool, humid Eskies via overnight couriers to the Department of Medical Entomology, Institute of Clinical Pathology and Medical Research (ICPMR), NSW Health Pathology, Westmead, for identification and processing for arbovirus isolation. The mosquitoes were identified via taxonomic keys and illustrations according to Russell (1993, 1996), Dobrotworsky (1965) and Lee *et al*. (1980 – 1989). A brief description of the main mosquito species for NSW appears in Appendix 2.

Mosquito abundances are best described in relative terms, and in keeping with the terminology from previous reports, mosquito numbers are depicted as:

- 'low' (<50 per trap),
- 'moderate' (50-100 per trap),
- 'high' (101-1,000 per trap),

- 'very high' (>1,000 per trap), and
- ‘extreme’ (>10,000 per trap).

All mosquito and arboviral monitoring results (with comments on the collections) were compiled into a weekly report, which was disseminated to stakeholders and included on the NSW Health web site.

Results

Overall, 187,397 mosquitoes representing 53 species were collected in NSW during 2018-2019, with the total being just over fifty percent higher than the previous season (with a total of 113,132 mosquitoes), but considerably lower than the number trapped in 2016-2017 (299,239). *Culex annulirostris* was the most abundant and most important of the inland mosquito species during the summer months, whereas *Aedes vigilax*, *Culex sitiens, Aedes notoscriptus*, *Culex annulirostris, Coquillettidia linealis*, *Aedes procax*, and *Verrallina funerea* were the most numerous species on the coast. A full summary of the results on a location-by-location basis is included in Appendix 1. A brief description of the most important vectors is provided in Appendix 2.

Inland

The total of 7,998 mosquitoes comprising 19 species was one of the lowest collections on record from the inland and around one quarter that of the previous season collection of 33,527. *Culex annulirostris* was the dominant species trapped at most sites and comprised 67.6% of the total inland collections. *Anopheles annulipes* (14.6%) was the next most common species followed by *Culex quinquefasciatus* (11.2%).

Coastal

In total, 48,214 mosquitoes comprising 43 species were collected from coastal NSW and close in number to the previous season’s collection (48,660). The most common species collected were *Culex sitiens* (24.8%), *Aedes vigilax* (19.8%), *Verrallina funerea* (14.6%), *Aedes notoscriptus* (12.0%), *Aedes multiplex* (9.1%), *Culex annulirostris* (4.9%), and *Aedes procax* (2.4%).

Metropolitan Sydney

A total of 179,395 mosquitoes, comprising 36 species, was collected from metropolitan Sydney and this was almost six times the previous season’s total collection of 30,945. *Aedes vigilax* (83.2% of the total Sydney mosquitoes trapped) was the most common species, followed by *Culex annulirostris* (4.0%), *Aedes notoscriptus* (3.8%), *Anopheles annulipes* (2.3%), *Coquillettidia linealis* (2.2%), and *Culex quinquefasciatus* (1.4%).

ARBOVIRUS DETECTIONS FROM MOSQUITOES

Methods

Viral detection involves modern molecular techniques for identifying viral nucleic acid.

For viral nucleic acid detection through molecular analysis from the mosquito grinds, the homogenates were screened for alpha (BFV, RRV, and SINV), and flaviviruses

(MVEV, KUNV, EHV KOKV, and STRV) using a series of multiplexed fluorogenic Taqman real-time RT-PCR assays, with modifications (Pyke AT, *et al*. 2004, van den Hurk AF, *et al*. 2014). Viral RNA was extracted using the EZ1® Virus Mini Kit (Qiagen), and amplified on the Corbett™ Rotor-Gene 6000. In the case of identifying flavivirus ‘unknowns’, a general screen using a pan-flavivirus PCR was performed (Moureau G*, et al.* 2007). For other unidentified virus from cell culture, a Pan-TBMV (Trubanaman, Buffalo Creek and Murrumbidgee virus), Pan SGV (Salt Ash and Gan Gan virus), and PCRs specific for Umatilla virus (UMAV), Wongorr virus (WGRV), Liao Ning virus (LNV), Wallal virus (WALV), Warrego virus (WARV), Beaumont virus (BEAUV), Whataroa (WHAV), and North Creek virus (NORCV) were used. Positive amplification of any one of these viruses was confirmed by Sanger Sequencing at the Australian Genome Research Facility (AGRF). The test sequence was compared by alignment against a database via the National Centre for Biotechnology information (NCBI) using the Basic Local Alignment Search Tool (BLAST).

In numerous locations across the state as part of an ongoing evaluation in surveillance technologies, honey-soaked FTA® cards (Flinders Technology Associates filter paper) were placed in the EVS traps (see discussion in greater detail below). The processing and screening for arboviruses from FTA cards were done using the protocol by Hall-Mendelin *et al.* 2010. Similarly, Taqman real-time RT-PCR detection procedures were used for virus detection from FTA card eluates described above for virus detection in mosquitoes.

Arboviral detection methodologies from the trapped mosquitoes continue to be validated within the surveillance program. As the FTA cards produced few arboviral detections, an alternative method was employed late in the season. Instead of grinding mosquitoes in pools of 25 for cell culture, as undertaken in previous years, whole mosquito collections from the one trap were pooled and tested. The basic procedure was:

- Mosquitoes (up to 500) placed into one 50ml urine pot,
- 20 x 5mm glass beads added,
- 3-5mls sterile PBS added,
- Ground for 20mins in MOSAVEX,
- PCR as above.

A short description of the various viruses and their clinical significance is detailed in Appendix 3. Positive virus results were sent to the Communicable Diseases Branch and the Environmental Health Branch of NSW Health and to the relevant Public Health Unit.

Results

From the mosquitoes processed, there were 28 detections, including 2BFV, 7RRV, 1KOKV, 11EHV and 7STRV (Table 3). From the inland, 1EHV was made from Griffith. From the coast, Georges River produced 12 isolates (2RRV, 1KOKV, 8EHV & 1STRV), while another six isolates (1 RRV & 5 STRV) were made from the sites along the Parramatta River.

Table 3. Arboviral isolates from NSW, 2018-2019.

LOCATION – Site	Date Trapped	Detection Method	Virus
BALLINA – North Creek Road	23/Apr/2019	Whole trap grind	Ross River
BALLINA – Pacific Pines	23/Apr/2019	Whole trap grind/FTA Card	Ross River
BALLINA – Pacific Pines	17/Apr/2019	Whole trap grind	Ross River
TWEED – Piggabeen Road	2/Apr/2019	Whole trap grind	Barmah Forest
PORT MACQUARIE – Stevens Street	2/Apr/2019	Whole trap grind	Barmah Forest
GEORGES RIVER – Alfords Point	14/Mar/2019	Whole trap grind	Stratford
CENTRAL COAST – Ourimbah	13/Mar/2019	Whole trap grind	Ross River
PARRAMATTA – Duck River	12/Mar/2019	Whole trap grind	Stratford
PARRAMATTA – Duck River	25/Feb/2019	Whole trap grind	Stratford
GEORGES RIVER – Alfords Point	20/Feb/2019	Whole trap grind	Ross River
SOPA – Haslams Creek	18/Feb/2019	Whole trap grind	Ross River
PARRAMATTA – Duck River	18/Feb/2019	Whole trap grind	Stratford
GEORGES RIVER – Deepwater	12/Feb/2019	Whole trap grind	Ross River
GEORGES RIVER – Deepwater	12/Feb/2019	Whole trap grind	Edge Hill
GEORGES RIVER – Picnic Point	12/Feb/2019	Whole trap grind	Edge Hill
SOPA – Newington	12/Feb/2019	Whole trap grind	Stratford
GEORGES RIVER – Alfords Point	6/Feb/2019	Whole trap grind	Edge Hill
CENTRAL COAST – Ourimbah	4/Feb/2019	Whole trap grind	Stratford
GRIFFITH – Lake Wyangan	29/Jan/2019	Whole trap grind	Edge Hill
GEORGES RIVER – Alfords Point	24/Jan/2019	Whole trap grind	Edge Hill
PARRAMATTA – Duck River	23/Jan/2019	Whole trap grind	Stratford
HILLS – Glenorie	23/Jan/2019	Whole trap grind	Edge Hill
GEORGES RIVER – Picnic Point	23/Jan/2019	Whole trap grind	Edge Hill
BLACKTOWN – Ropes Crossing	22/Jan/2019	Whole trap grind	Edge Hill
GEORGES RIVER – Picnic Point	16/Jan/2019	Whole trap grind	Edge Hill
GEORGES RIVER – Alfords Point	10/Jan/2019	Whole trap grind	Edge Hill
GEORGES RIVER – Picnic Point	9/Jan/2019	Whole trap grind	Edge Hill
GEORGES RIVER – Picnic Point	9/Jan/2019	FTA card	Kokobera

FTA Card = Sugar based surveillance. Whole trap grind = all the mosquitoes are ground (or a subsample of the larger collections) and tested for arboviral nucleic acid.

SENTINEL CHICKEN PROGRAM

Location of flocks

The 2018-2019 season began on 3rd November 2018 with the first bleed and ended on 30th April 2019 with the last. A total of nine flocks each containing up to 15 Isa Brown pullets was deployed, with one flock each at Deniliquin, Dubbo, Forbes, Griffith, Hay, Leeton, Macquarie Marshes, Menindee, and Moree (Figure 1).

Methods

The NSW Chicken Sentinel Program was approved by the Western Sydney Local Health Network Animal Ethics committee. This approval requires that the chicken handlers undergo training to ensure the chickens are cared for appropriately and that blood sampling is conducted in a manner that minimises trauma to the chickens. The chickens are cared for and bled by local council staff and members of the public. Laboratory staff are responsible for training the chicken handlers. A veterinarian (usually the Director of Animal Care at Westmead) must inspect all new flock locations prior to deployment to ensure animal housing is adequate. Existing flocks are inspected approximately every two years. The health of each flock is reported weekly, and is independently monitored by the Animal Ethics Committee via the Director of Animal Care.

Full details of the bleeding method and laboratory testing regimen were detailed in the 2003-2004 NSW Arbovirus Surveillance Program Annual Report (Doggett *et al*. 2004).

Results are disseminated via email to the relevant government groups as determined by NSW Health and are placed on the NSW Arbovirus Surveillance website. Confirmed positives are notified by telephone to NSW Health and Communicable Diseases Network, Australia.

Results

The season began with 135 pullets. A total of 2,545 samples was received from the ten flocks in NSW over the six-month period in 2018-2019. This represented 5,090 ELISA tests (excluding controls and quality assurance samples), with each specimen being tested for MVEV and KUNV antibodies. There no seroconversions in the sentinel chickens.

NOTIFICATIONS OF LOCALLY-ACQUIRED ARBOVIRUS INFECTIONS

All arboviral infections detected in humans are notifiable under the *NSW Public Health Act 2010*. When a person tests positive for an arboviral infection pathology laboratories notify public health authorities who assess the notification against agreed surveillance case definitions and take appropriate actions using NSW Health disease control guidelines.

Annual reports (by calendar year) of notifiable vector-borne diseases (VBD), including locally acquired arbovirus infections, are available on the NSW Health VBD reports website.

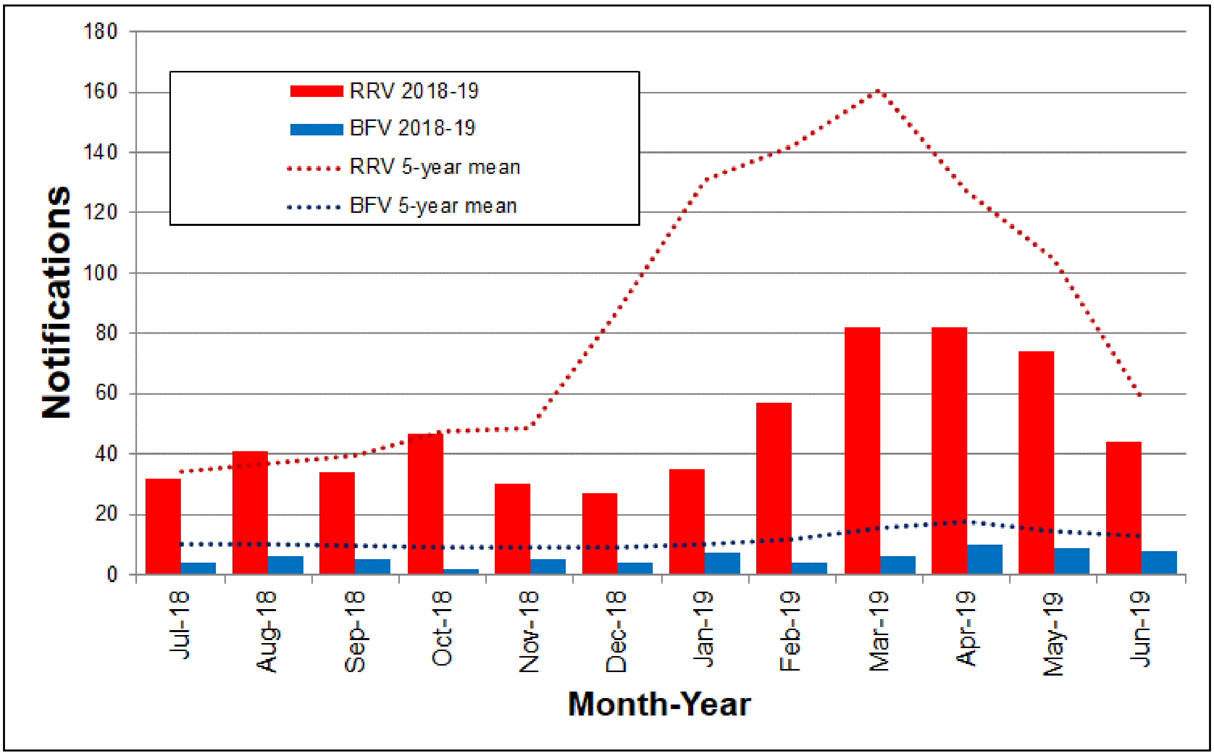
The two most common locally-acquired arbovirus infections notified in NSW are infections with Ross River virus (RRV) and Barmah Forest virus (BFV).

In the 2018-2019 financial year there were 585 notifications of RRV infection in NSW residents (Table 4), a small (2%) decrease compared to the previous year (599 notifications). There were 70 notifications of BFV infection (Table 4), which is 35% lower than the previous year (93 notifications). There were no notifications of other locally-acquired arbovirus infections in NSW during 2018-2019.

Monthly BFV notifications were low throughout the year, with a peak of 10 notifications in April 2019 (Figure 7).

RRV notifications were generally lower than the five-year mean throughout the year (Figure 7). Notifications were highest in the autumn months of 2019, peaking in March and April 2019 with 82 notifications each. This followed the typical seasonal pattern of RRV activity.

Figure 7. Barmah Forest virus and Ross River virus infections in NSW residents: notifications by month of onset for the 2018-2019 financial year, compared to the 5-year monthly means for the period from July 2013 to June 2018.



BFV and RRV notifications by place of residence of the case are presented by NSW local health district (LHD), by geographic region (Coastal, Inland, and Sydney metropolitan) and by Australian Bureau of Statistics (ABS) statistical area level 2 (SA2). See Appendix 5 for definitions of the Coastal, Inland, and Sydney metropolitan regions. Due to incomplete address information, a handful of cases (approximately one per year) could not be allocated to a region. Population rates based on the Australian Bureau of Statistics estimated resident populations. Population projections for LHDs in 2018-19 based on data from the NSW Department of Planning and Environment. The place of residence of a case may not, however, be where the infection was acquired.

Notifications of BFV and RRV infection by LHD are shown in Table 4. The highest number of notifications for BFV infection were in the Northern NSW, Mid North Coast and Hunter New England LHDs, with few notifications in other LHDs. The highest population notification rates were in the Northern NSW and Mid North Coast LHDs.

RRV notifications were highest in the Hunter New England, Western NSW and Northern NSW LHDs, while RRV population notification rates were highest in the Far West and Western NSW LHDs.

Table 4. Barmah Forest virus and Ross River virus infections in NSW residents: notifications and population rates (notifications per 100,000 population) by local health district for the 2018-2019 financial year.

Local Health District	Barmah Forest virus		Ross River virus	
	Notifications	Population Rate*	Notifications	Population Rate*
Central Coast	0	0.00	36	10.46
Far West	0	0.00	14	46.69
Hunter New England	12	1.29	168	18.02
Illawarra Shoalhaven	2	0.48	18	4.36
Mid North Coast	14	6.33	53	23.98
Murrumbidgee	0	0.00	53	17.93
Nepean Blue Mountains	0	0.00	4	1.05
Northern NSW	35	11.53	69	22.74
Northern Sydney	1	0.11	26	2.78
South Eastern Sydney	0	0.00	8	0.85
South Western Sydney	0	0.00	6	0.60
Southern NSW	3	1.42	30	14.21
Sydney	0	0.00	6	0.89
Western NSW	3	1.06	81	28.70
Western Sydney	0	0.00	13	1.30
Total	**70**	**0.88**	**585**	**7.35**

*Notifications per 100,000 estimated resident population, based on ABS population estimates. Population projections by the Centre for Epidemiology and Evidence, NSW Ministry of Health, based on data from the NSW Department of Planning and Environment.

Notifications of BFV and RRV infection by geographic region (Coastal, Inland, and Sydney metropolitan) of residence are shown in Figures 8 and 9, respectively, by financial year of disease onset from 2009-2010 to 2018-2019.

The Coastal region again accounted for the vast majority of BFV notifications (n=65, 92.9%) followed by the Inland region (n=4, 5.7%) with only 1 notification reported in a resident of the Sydney region (Figure 8).

Notification maps of BFV and RRV infection by ABS statistical area level 2 (SA2) of residence for the 2018-2019 financial year are shown in Figures 10 and 11, together with maps of population notification rates.

The SA2 areas with the highest total number of BFV notifications were Maclean-Yamba-Iluka (n=8) and Brunswick Head – Ocean Shores (n=6) (Figure 10(a)). The two SA2 areas with the highest notification rates per 100,000 population were also Brunswick Head – Ocean Shores (70) and Maclean-Yamba-Iluka (49) (Figure 10(b)).

The SA2 areas with the highest total number of RRV notifications were Griffith (n=12) and Old Bar – Manning Point – Red Head (n=11) (Figure 11(a)). The SA2 areas with the highest notification rates per 100,000 population were Far West (179) and Forster-Tuncurry Region (151) (Figure 11(b)).

Figure 8: Barmah Forest virus infections in NSW residents: annual notifications by year of disease onset and geographical region for the past 10 years (2009-2010 to 2018-2019).

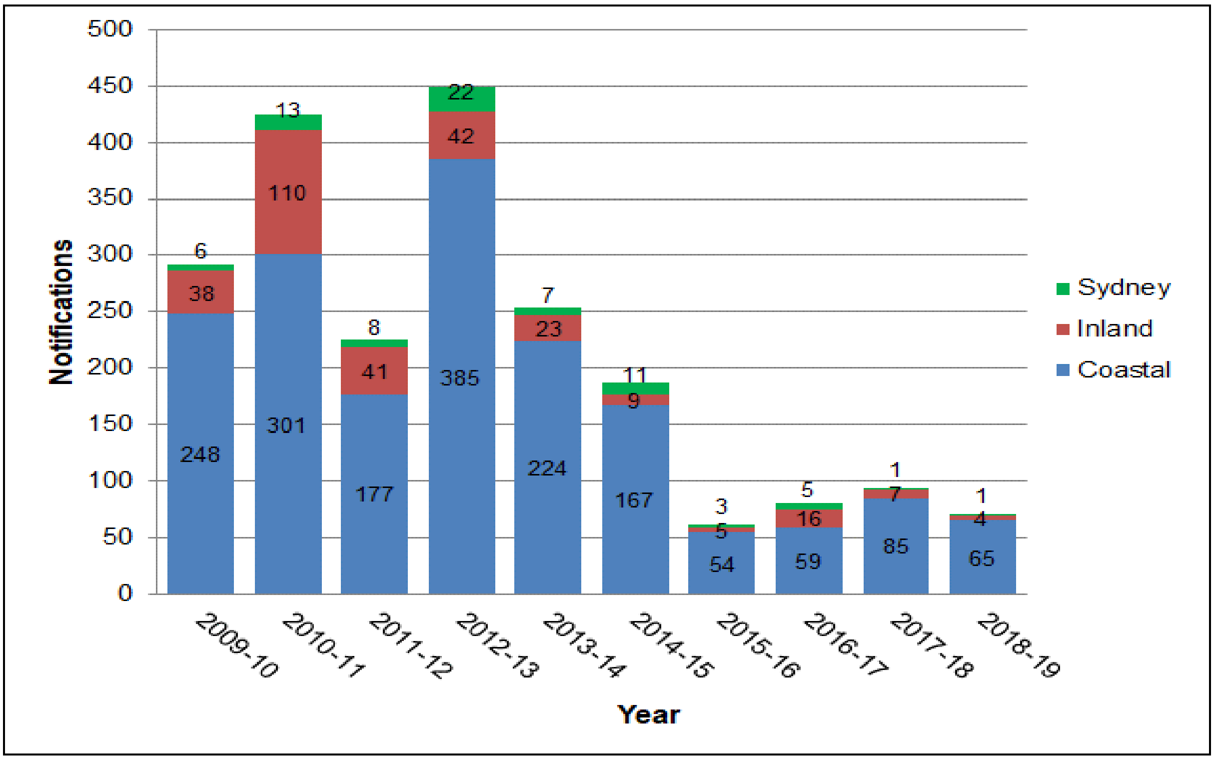


Figure 9: Ross River virus infections in NSW residents: annual notifications by year of disease onset and geographical region for the past 10 years (from 2009-2010 to 2018-2019).

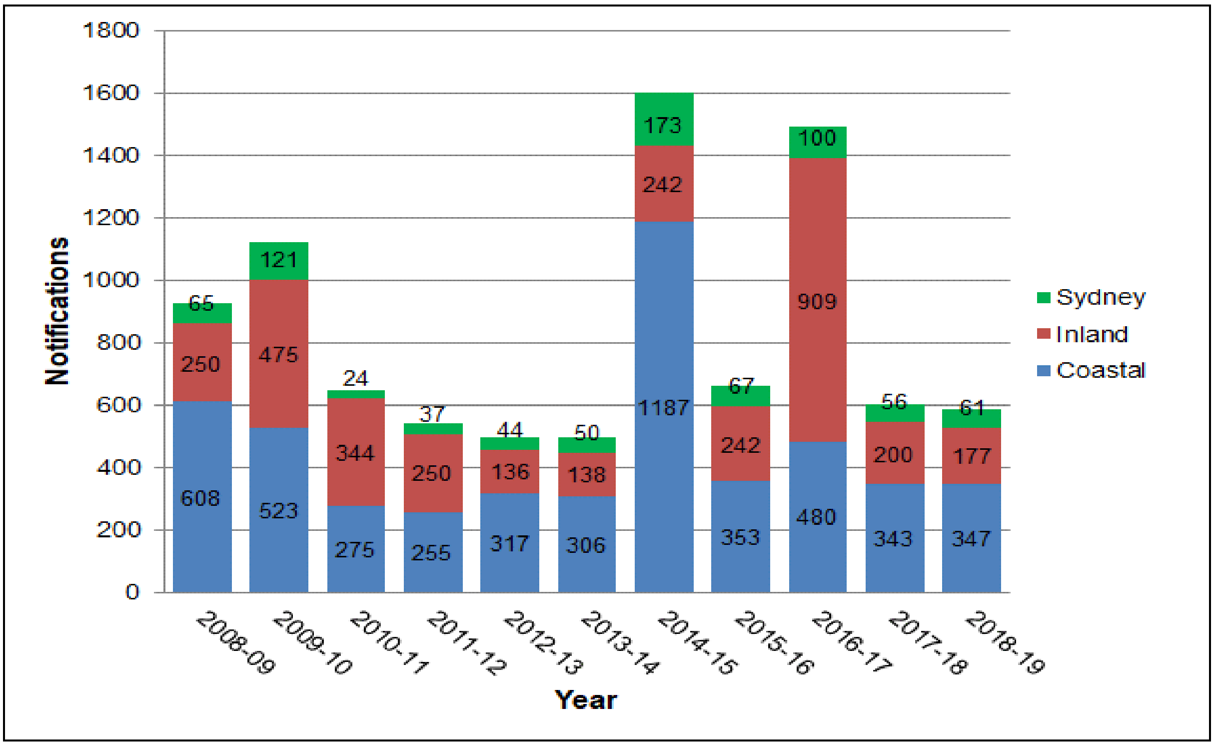
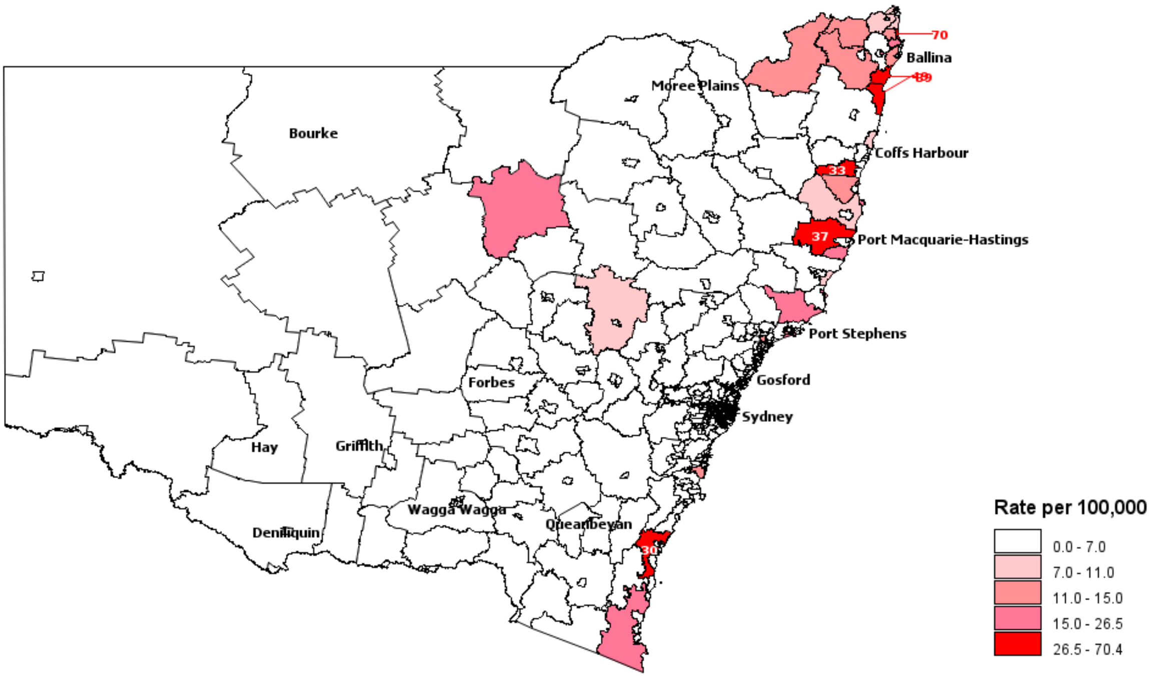


Figure 10: Barmah Forest virus infections in NSW residents.

(a) Notifications by statistical area level 2 (SA2), for 2018-2019.

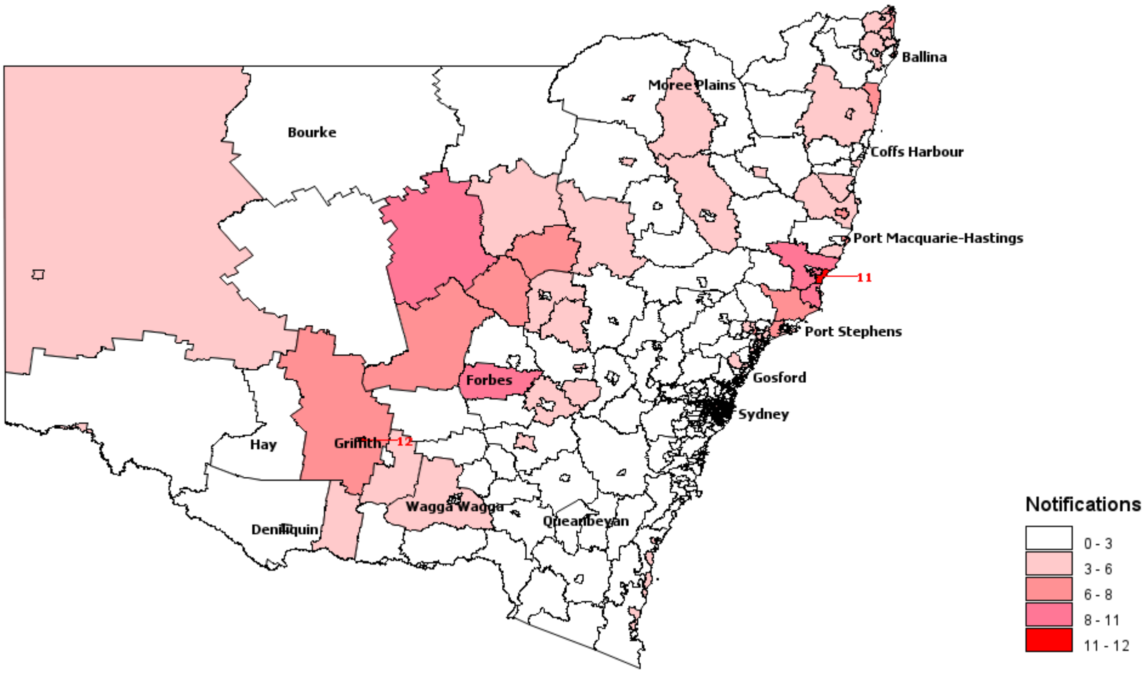
(b) Population notification rates* by statistical area level 2 (SA2), for 2018-2019.



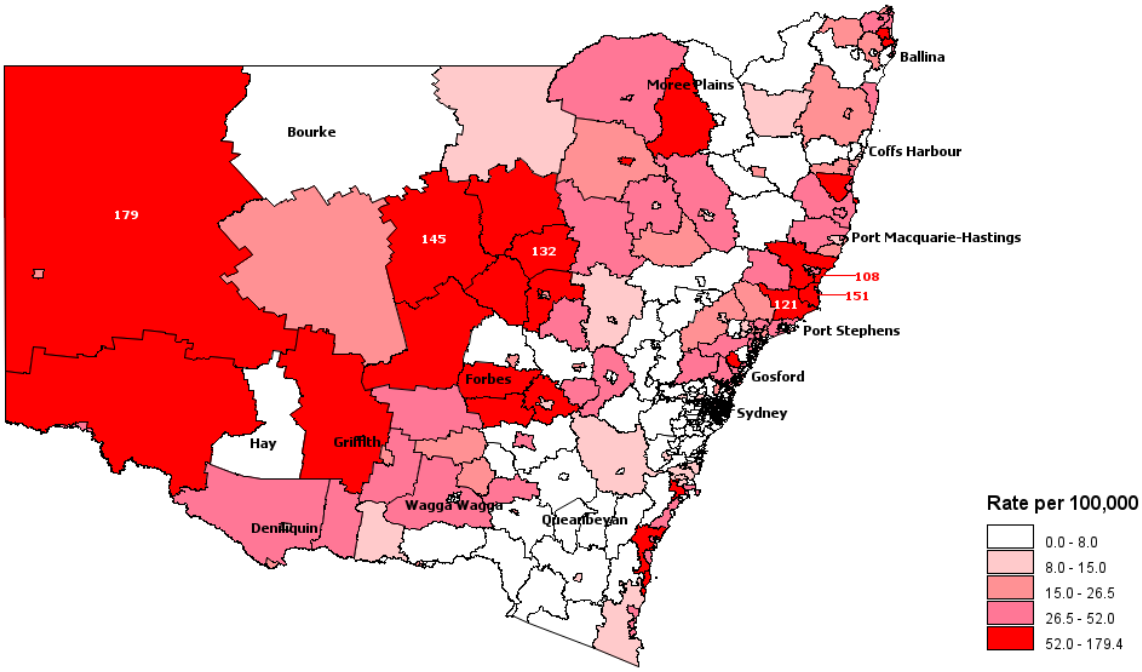
* Notifications per 100,000 estimated resident population based on ABS census data.

Figure 11: Ross River virus infections in NSW residents.

(a) Notifications by statistical area level 2 (SA2), for 2018-2019.



(b) Population notification rates* by statistical area level 2 (SA2), for 2018-2019.



*Notifications per 100,000 estimated resident population based on ABS census data.

For further information on surveillance for human infections with vector-borne diseases, including exotic arbovirus infections, see the following:

- NSW Health [Vector-borne diseases reports]
- NSW Health [Notifiable diseases data] (and select the relevant disease).

DISCUSSION

The 2018-2019 season, as per last year, was dominated by a protracted period of continually hot and dry weather patterns. The entire year of 2018 produced rainfall that was below average for the entire state, and most of NSW even had very much below average precipitations. In spite of the last three months of 2018 having average rainfall, the ongoing dry conditions and parched soils meant that any free standing water was quickly absorbed. Furthermore, the state experienced temperatures of up to 2.5^{o}C above average for the entire 2018 and 1-2^{o}C above average for the first six months of 2019.

As a consequence, mosquito numbers were at a record low from the inland, with only 7,998 collected from the entire season from all set traps. In a more typical year, this number is often collected on the one night, from one trap. The one arboviral isolate, no seroconversions in the sentinel flocks, and few human notifications, were in accordance with the low mosquito numbers. For the inland, in total there were 181 notifications, which included 177RRV & 4BFV, and this was almost half the previous nine season average of 343 (311RRV & 32BFV). The statistical local areas that produced the highest notifications for RRV from the inland was Griffith (12), while the Far West (179/100,000) had the highest notification rate.

With the hot and dry weather patterns continuing into August 2019, the SOI being neutral, and that current models are in the negative, an MVEV outbreak would seem unlikely for 2019-2020. However, not dissimilar conditions have existed in the past when MVEV activity has occurred following the southerly movement of low pressure cells formed from tropical cyclones. The hypothesis is that the low pressure cell pushed infected vectors south. Thus the residual weather patterns from tropical cyclones are now closely monitored by the Department of Medical Entomology. The forecast ahead is for temperatures that are higher than the norm and this may push forward the mosquito season, with activity occurring earlier than normal. If this does happen, then the start of the surveillance season may need to be brought forward.

While the coast experienced similar dry conditions, other environmental factors influence mosquito numbers, notably tides. In fact dry conditions can result in larger collections of the saltmarsh mosquito, *Aedes vigilax*. However for this season, mosquito collections from the coastal region were not extraordinary, the 48,214 trapped was similar to last year’s total (48,660). Arboviral notifications were also very similar to last year; for 2018-2019 there were 412 cases, including 347RRV and 65BFV, compared to 2017-2018 when there was a total of 428 notifications, including 343 RRV and 85BFV. Both of these years were around 30% lower than average (627 cases, including 438RRV & 189BFV).

One key difference this season for the coastal sites was the higher number of arboviral detections from the trapped mosquitoes (seven this year compared with three in 2017-2018). However, this is probably due to the enhancements made this season to improve the sensitivity of the arboviral detection procedures.

While mosquito activity for both the inland and coast were unremarkable, in strong contrast the sites around Sydney, notably those along the Georges and Parramatta River were extremely productive. In fact this season produced the highest collections to date from the Sydney region. From the Georges River, the site of Picnic Point alone trapped over 50,000 mosquitoes and five arboviral isolates were yielded (4EHV & 1KOKV, Table 6). *Aedes vigilax* comprised more than 90% of the sample, suggesting that the dry conditions coupled with the high tides contributed to this seasons dramatic increase (if the mudflats are continually dry, the mosquito eggs can maturate producing larger hatches in subsequent tidal flushings). From the other trapping locations along the Georges River, there were another seven arboviral isolates, including from Alfords Point, 1RRV, 3EHV & 1STR, and from Deepwater, 1RRV & 1EHV.

In spite of the high mosquito numbers and multiple arboviral detections, human notifications were below average with a total of 62 reports (61RRV and 1BFV). In comparison, the average from the previous nine seasons was 81 notifications, including 73RRV and 8BFV. In the past there have been large numbers of arboviral isolates from the Georges River without a concomintant increase in arboviral notifications. In fact notifications from Sydney tend to only increase when there is a large amount of activity elsewhere along the coast.

The Picnic Point trapping site is located along Henry Lawson Drive, east of Yeramba Lagoon. The Department of Medical Entomology has undertaken sampling from this water body and it is not the source for the large mosquito populations. Rather, the mosquitoes appear to be coming from the south side of the Georges River where there are two moderately sized mudflats, as can be observed on Google Maps. Fortunately there is little housing directly around these areas. Access to the site to survey the habitat is difficult, as are most of the mosquito breeding sites along the Georges River, due to the topography, lack of vehiclar access, and privately owned lands. However, plans are underway to better document the breeding sites along the river this coming season.

The other Sydney location that produced an extraordinary high number of mosquitoes this season, was the new trapping site at Duck River, which is a tributary of the Parramatta River, and west of Sydney Olympic Park. Duck River trapped over 45,000 mosquitoes, the majority being *Aedes vigilax*, plus there were four isolates.

Duck River runs through industrial estates; a petroleum plant is situated to the west and factories towards the east. The river is bordered by a narrow strip of vegetation along each bank (Figure 12), with some small areas of mudflats. Like the Georges River, access for mosquito sampling is very difficult due to the topography and that the river is fenced off for most of its margin. However, preliminary surveys undertaken with Unmanned Aerial Vehicles (UAVs or ‘drones’) by Medical Entomology has revealed that the size of the potential mosquito habitat is quite small, probably under 300 square metres. Yet this location is extremely productive and many factor workers in the area complained of the mosquito nuisance problem when staff were setting the traps.

As a comparison, the three nearby trapping sites of Sydney Olympic Park (SOP) trapped a total of 18,331 mosquitoes for the entire season (Figure 13). Active mosquito management through aerial application of larvicides is undertaken on a routine basis at SOP, successfully reducing the mosquito nuisance problem. Even a small scale management program with the use of UAVs at Duck River should be highly effective a reducing mosquito numbers. Further mapping of the mosquito breeding habitats will be undertaken this season.

Figure 12. Drone footage showing the nature of Duck River, with large industrial estates to the east and a petroleum plant to the west. Habitat for mosquito breeding is limited to small areas of mudflats.

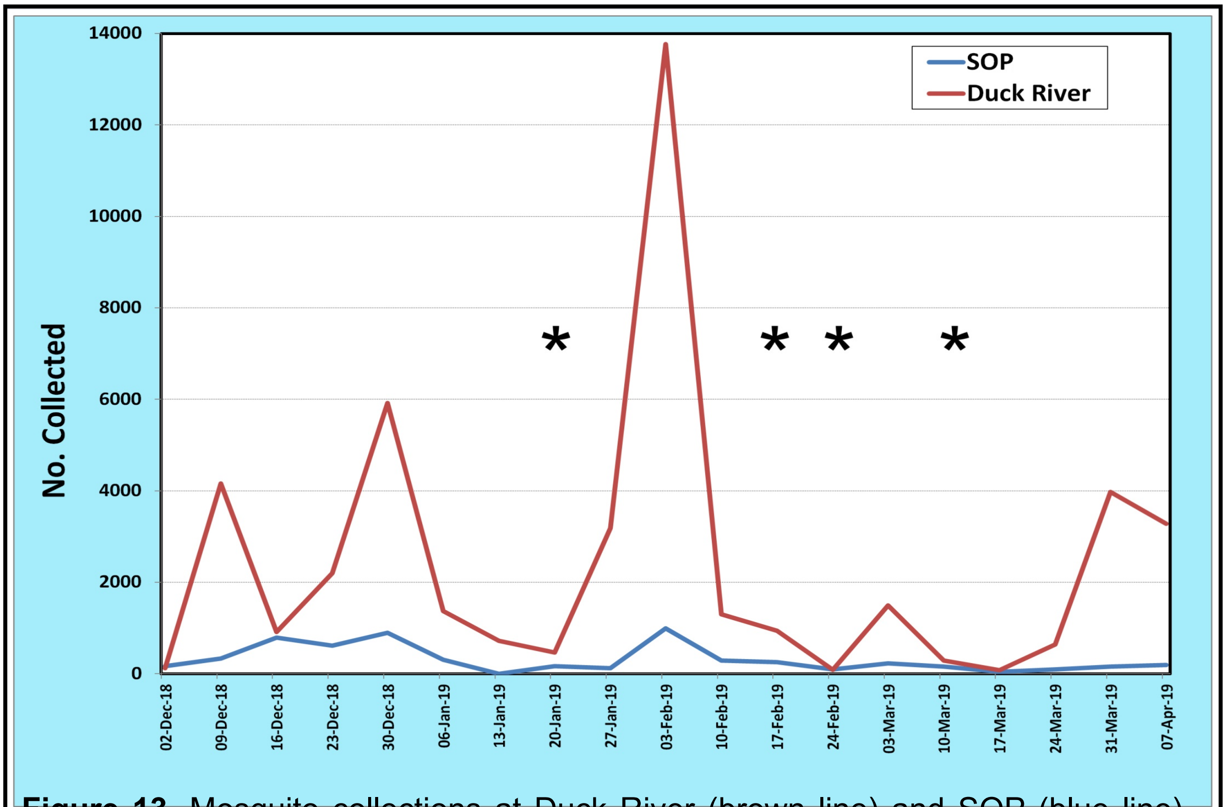


Figure 13. Mosquito collections at Duck River (brown line) and SOP (blue line). The asterisks represent viral detections at Duck River.

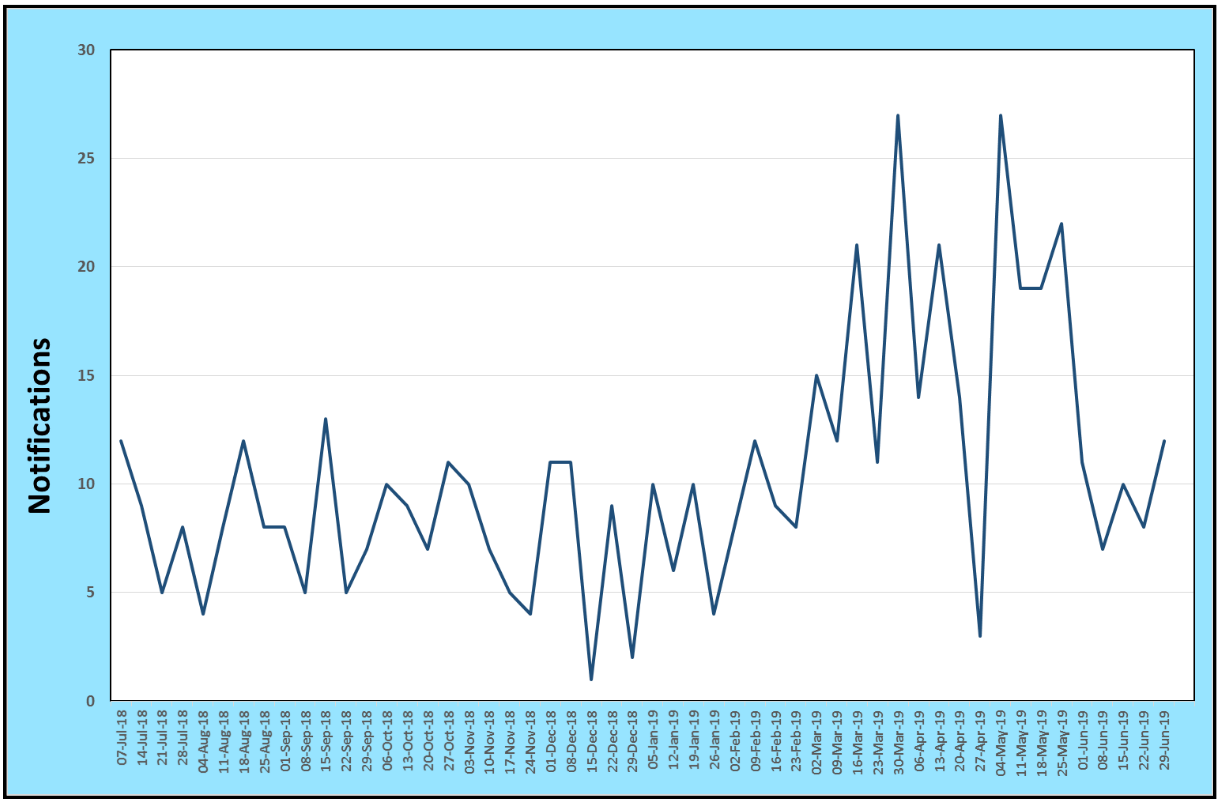


Figure 14. Weekly Ross River virus notifications in NSW residents, Jul 2018 to June 2019. (Source: NSW Heath Communicable Diseases Weekly Report, www.health.nsw.gov.au/Infectious/reports/Pages/CDWR.aspx).

Figure 14 represents reports of RRV by notification date, which is directly from the NSW Health Communicable Diseases Weekly Report, and is the data that is included in the weekly report of the NSW Arbovirus Surveillance Program. In comparison, Figure 7 shows notifications by date of onset.

In the case of the RRV reports by notification date (Figure 14), just under 40% of the reports during the fiscal year of 2018-19 occurred over the months of July to December 2018, and approximately 35% of the RRV positives by date of onset (Figure 7) originated from the same period. All of these reported cases occurred during the cooler months before mosquito numbers notably increased. This highlights the ongoing issues with the reliability of notification data as an indication of recent infection as patients reported during the cooler months were clearly not infected with the virus at that time of the year as mosquito numbers were low and there was little evidence of arbovirus activity. However, it should be noted that date of onset is the better indication of the two notification types in terms of an indication of possible viral acquisition date.

The data points to the key limitation of testing for RRV (and BFV) infection as it relies solely upon serological tests that detect the person's antibody response to an infection rather than the virus itself. Furthermore, most cases that are notified are suggestive (single specimen with both IgM and IgG RRV antibodies) and not definitive (where specimens are taken during the acute and convalescent stage and a four-fold rise in antibodies are recorded). As IgM antibody levels can remain elevated for months to even years (and IgG for years to decades), it is likely that most of the RRV notifications reported in these cooler months actually represent infections that were acquired during the previous season when RRV activity was high. The possibility of cross-reacting antibodies cannot be discounted with a single positive result. Issues with the serological tests must also be considered, as happened with the commercial BFV kit over 2012-2013 whereby almost 90% of those that tested positive were subsequently found to be false (Doggett, 2014; Knope *et al*., 2016; Kurucz *et al*., 2016). The limitations in the notification data also indicates why RRV models are unlikely to ever accurately predict activity and future outbreaks (Doggett, 2018).

FTA CARDS VS WHOLE TRAP GRINDS

Arboviral detection methodologies from the trapped mosquitoes continued to be investigated within the surveillance program, in order to produce the most sensitive assay system with results available in the shortest possible time. In recent years, the use of FTA cards have been evaluated, which enabled results to be quickly available. The power of this technique is that arboviral detections from mosquitoes are typically available in under 24hours of receipt of the sample into the laboratory. However, analysis from the last two seasons have shown that FTA cards were not the most sensitive system available. Instead, Medical Entomology has been testing a novel approach, grinding all the mosquitoes from the same catch in the one vial, and testing the supernatant via PCR as per described methods.

Last season, this updated methodology was found to be considerably more sensitive than FTA cards. For example, while there were 8 arboviral detections with the whole trap grind (WTG) protocol, the FTA cards yielded only two positive results. This season produced an even more compelling result for the whole trap grinds; of the 28 detections only two were picked up by the FTA cards.

Furthermore, not only are WTGs more sensitive, the system offers a number of advantages over FTA cards: no FTA card preparation by field operatives; no FTA card elution; and no FTA cards. Thus WTGs are cheaper, quicker, as well as being more sensitive.

For the 2019-2020 season, FTA cards will no longer be deployed in the mosquito traps.

EXOTIC MOSQUITO DETECTIONS AT SYDNEY INTERNATIONAL AIRPORT

Background. Over the decade there has been an increasing number of detections of exotic mosquitoes at major Australian ports. The main species have been the Dengue/Yellow Fever mosquito, *Aedes aegypti*, and the Asian Tiger Mosquito, *Aedes albopictus*. Both of these pose a serious biosecurity risk to Australia being major vectors of serious arboviral diseases including Dengue, Yellow Fever, Zika, and Chikungunya viruses.

Aedes aegypti, being a tropical species, mainly poses a threat to the more northern regions of the nation, whereas *Aedes albopictus* is more cold tolerant. This species has the potential to become established along the eastern coast of Australia including the major population centre of Sydney. As such, *Aedes albopictus* has the potential to cost the national economy hundreds of millions of dollars, through the transmission of diseases and vector control costs. Thus, it is imperative that these mosquitoes are kept out of regions of the country where they presently do not exist. Furthermore, the mosquitoes that have been collected have undergone genetic analysis for the presence of insecticide resistant genes and most of the mosquitoes have these. In Australia, there is no evidence for insecticide resistance in our local mosquitoes, including invading species such as *Ae. aegypti*. The presence of resistant insects will make the mosquito much more difficult to control and limit management options.

Sydney has seen numerous detections of exotic mosquitoes in recent years, the majority being *Ae. aegypti*, although specimens of *Ae. albopictus* and *Culex gelidus* have also been trapped. Most detections have occurred within the baggage handling area of Sydney International Airport. However, some detections have occurred at various freight handling facilities, both at Sydney Airport and in a facility close to the airport.

Table 5 details the detections for the period July 2018 to August 2019. The finding of *Aedes quasirubrithorax* was of initial concern, for it is very similar in appearance to the highly invasive species, *Aedes japonicus*, which has been detected on a number of occasions in Brisbane port facilities. *Aedes quasirubrithorax* is a native mosquito species but previously unknown from the south coast of NSW.

Table 5. Detection of Exotic Mosquitoes in NSW, July 2018 – August 2019.

Date	Mosquito Species & Sex	Location
3 Aug 2019	*Aedes aegypti* ♀	Sydney International Airport
31 Jul 2019	*Aedes aegypti* ♀	Sydney International Airport
14 May 2019	*Aedes albopictus* ♂	Sydney International Airport
2 May 2019	*Aedes aegypti* ♀	Approved Arrangement Facility, Sydney
23 Apr 2019	*Aedes aegypti* ♂	Sydney International Airport
6 Feb 2019	*Aedes aegypti* ♀	Approved Arrangement Facility, Sydney Airport
16 Jan 2019	*Aedes aegypti* ♂	Approved Arrangement Facility, Sydney Airport
2 Jan 2019	*Aedes aegypti* ♀	Approved Arrangement Facility, Sydney
7 Dec 2018	*Aedes aegypti* ♀	Approved Arrangement Facility, Sydney
23 Nov 2018	*Aedes aegypti* ♀	Approved Arrangement Facility, Sydney
9 Oct 2018	*Aedes quasirubrithorax* ♀*	Eden Sea Port

*see text for discussion.

In response to the *Aedes aegypti* detections a number of actions have been implemented. NSW Health established regular teleconferences, the Department of Agriculture and Water Resources (DAWR) undertook enhanced surveillance (both increasing the number of traps used and the frequency of trap inspections), insecticidal treatment of the detection areas were undertaken, and vector surveys were conducted both within and around the sites with previously unrecorded detections, which included staff from the Department of Medical Entomology at Westmead Hospital.

The reason for the recent increase in detections is not presently fully clear. In spite of this, there has been a co-ordinated effort across multiple agencies to ensure the exotic mosquitoes do not become established in NSW. Recently a new invasive mosquito species was detected in Europe, *Aedes flavopictus* (Ibáñez-Justicia *et al*. 2019), which is closely related to *Aedes albopictus* and shown to be an efficient vector of dengue virus. This is another species that will be closely monitored for.

Appendix 1. LOCATION-BY-LOCATION SUMMARY

Inland Locations

Albury: mosquito numbers were ‘low’ for the entire season. There were no arboviral detections from the trapped mosquitoes. Sentinel chicken flocks did not operate at Albury.

Bourke: mosquito collections were ‘low’ for the entire season. There were no arboviral isolates and sentinel chickens did not operate at this location.

Deniliquin: no mosquito collections were undertaken at this location. There were no seroconversions to MVEV or KUNV in the sentinel chickens.

Forbes: other than the one ‘high’ yield in mid-December, mosquito numbers were ‘low’ for most of the season. There were no arboviral isolations nor any seroconversions to MVEV or KUNV in the sentinel chickens.

Griffith: during January to early February, mosquito numbers were ‘high’, but never reached ‘very high’ as per most years. Outside this period, numbers were ‘low’ to ‘medium’. There was one arboviral isolate this season from Griffith, an EHV isolated from a collection made at Lake Wyangan trapped on 29/Jan/2019. There were no seroconversions to MVEV or KUNV in the sentinel chickens.

Hay: no mosquito collections were undertaken this season, and there were no seroconversions to MVEV or KUNV in the sentinel chickens.

Leeton: mosquito numbers were ‘low’ to ‘medium’ throughout the entire season. There were no arboviral isolates from the mosquitoes nor any seroconversions in the sentinel chickens.

Macquarie Marshes: five mosquito collections were made this season and numbers were mostly ‘low’, with one ‘high’ collection in mid-December. There were no arboviral detections or any seroconversions to MVEV or KUNV in the sentinel chickens.

Menindee: no mosquito collections were undertaken this season, and there were no seroconversions to MVEV or KUNV in the sentinel chickens.

Moree: no mosquito collections were undertaken this season, and there were no seroconversions to MVEV or KUNV in the sentinel chickens.

Wagga Wagga: mosquito numbers were ‘low’ for the entire the season and there were no arboviral detections. Sentinel chickens did not operate at Wagga Wagga.

Coastal Locations

Ballina: mosquito numbers were 'high' throughout the season with three 'very high' traps late in the season, in mid-March and April. For the most part, these elevated numbers coincided with larger collections of *Aedes vigilax*. However for most of the season, collection of *Aedes vigilax* were 'low' and only became 'high' in mid-March. In spite of the lower numbers of this species, collections were dominated by salt water breeding mosquitoes including *Culex sitiens* and *Verrallina funerea*, neither of which are notable vectors. There were three RRV isolates detected, including one from North Creek Rd from mosquitoes trapped on 23/Apr/2019, and two detections from Pacific Pines, one each on 17/Apr/2019 and 23/Apr/2019.

Coffs Harbour: trapping was undertaken at Moller Drive and Christmas Bells Road. Collections tended to be 'low' to 'medium', with the one 'high' yield late in early January. Numbers of *Aedes vigilax* remained 'low' for the entire season. No arboviral isolates were detected.

Gosford: as per usual, the Empire Bay site dominated the mosquito collections on the Central Coast, where numbers were 'high' for most of the season. However, *Aedes vigilax* collections were mostly 'low', with only the two 'high' yields. *Aedes notoscriptus* was the most common species, comprising more than 50% of the overall collections. No arboviral isolates were detected.

Lake Macquarie: collections were undertaken from three sites: Belmont Lagoon, Teralba, and Dora Creek. Mosquito numbers were 'low' for most of the season until late March with two 'high' collections. *Aedes vigilax* were 'low' for the majority of the season. No arboviral isolates were detected.

Nambucca: four traps were set towards the end of the season and mosquito numbers were mostly 'low' to 'medium'. No arboviral isolates were detected.

Port Macquarie: trapping was again undertaken at three sites; Wall Reserve, Fernbank Creek Road, and Steven Street. Mosquito collections were 'low' throughout most of the season with some 'medium' catches in March and April. *Aedes vigilax* collections remained 'low' throughout the entire season. There was one BFV detected at Stevens St from mosquitoes trapped on 2/Apr/2019.

Tweed Heads: trapping continued at the three sites of Koala Beach, Beltana Drive, and Piggabeen Road. Beltana Drive again yielded the largest collections for the season. Overall numbers were mostly 'medium', with a series of 'high' numbers over February and March. *Aedes vigilax* collections peaked during the same period but were mostly 'medium' in number. *Culex sitiens* close to half of the overall collections. There was one BFV detected at Piggabeen Rd from mosquitoes trapped on 2/Apr/2019.

Wyong: trapping was undertaken at three sites: Ourimbah, Halekulani, and North Avoca. Mosquito numbers were 'low' for the entire season, except for some 'medium' collections early in the season. There were two arboviral isolates detected from Ourimbah. This included one STRV from 4/Feb/2019 and one RRV from 13/Mar/2019

Sydney Locations

Bankstown: collections this season were undertaken at Deepwater and Picnic Point. These sites are known for intense local *Aedes vigilax* production, which again dominated the catches this year comprising over 93% of the collections. Picnic Point was especially productive, trapping over 50,000 mosquitoes from this trap alone. Mosquito numbers were consistently 'high' to 'very high' for the entire period from early December to mid-April. There were seven arboviral isolates and these are listed in the table below.

Table 6. Arboviral isolates from the Georges River sites, 2018-2019.

LOCATION – Site	Date Trapped	Detection Method	Virus
GEORGES RIVER – Alfords Point	14/Mar/2019	Whole trap grind	Stratford
GEORGES RIVER – Alfords Point	20/Feb/2019	Whole trap grind	Ross River
GEORGES RIVER – Deepwater	12/Feb/2019	Whole trap grind	Ross River
GEORGES RIVER – Deepwater	12/Feb/2019	Whole trap grind	Edge Hill
GEORGES RIVER – Picnic Point	12/Feb/2019	Whole trap grind	Edge Hill
GEORGES RIVER – Alfords Point	6/Feb/2019	Whole trap grind	Edge Hill
GEORGES RIVER – Alfords Point	24/Jan/2019	Whole trap grind	Edge Hill
GEORGES RIVER – Picnic Point	23/Jan/2019	Whole trap grind	Edge Hill
GEORGES RIVER – Picnic Point	16/Jan/2019	Whole trap grind	Edge Hill
GEORGES RIVER – Alfords Point	10/Jan/2019	Whole trap grind	Edge Hill
GEORGES RIVER – Picnic Point	9/Jan/2019	Whole trap grind	Edge Hill
GEORGES RIVER – Picnic Point	9/Jan/2019	FTA card	Kokobera

FTA Card = Sugar based surveillance. Whole trap grind = all the mosquitoes are ground (or a subsample of the larger collections) and tested for arboviral nucleic acid.

Blacktown: mosquito trapping was undertaken at Nurrangingy Reserve and Ropes Crossing. Numbers were 'low' for most of the season with the very occasional 'medium' collection. There was one EHV yielded from mosquitoes trapped on 22/Jan/2019 from Ropes Crossing.

Georges River: trapping continued to be undertaken at Alfords Point and Illawong, with the former site producing the vast majority of the catch. Collections were 'high' or greater for the majority of the season, with numbers peaking during December/January. *Aedes vigilax* comprised more than 90% of the collections. There were five arboviral detections this season, which are detailed in Table 6 above.

Hawkesbury: trapping was undertaken at four main sites, including at Wheeny Creek, McGraths Hill, Yarramundi, and Richmond. Mosquito numbers tended to be 'low' throughout most of the season with some 'medium' collections in late March. No arboviral isolates were detected.

Hills Shire: mosquito trapping was undertaken at Rouse Hill, Glenorie, and Baulkham Hills. Numbers were 'low' for most of the season. There was one EHV yielded from mosquitoes trapped on 23/Jan/2019 from Glenorie.

Parramatta: collections were undertaken at the three sites of George Kendall Reserve, Eric Primrose Reserve and Duck River. The latter site yielded the largest collections, trapping over 45,000 mosquitoes during the season. Here, *Aedes vigilax* comprised over 90% of the collections. For the season, overall numbers were consistently ‘high’ and ‘very high’ during late March and early April. There were four STRV isolated from mosquito collections at Duck River, one each for 23/Jan/2019, 18/Feb/2019, 25/Feb/2019 and 12/Mar/2019.

Penrith: trapping was undertaken at the sites of Emu Plains, Muru Mittiger, Glenmore Park, and Werrington. Mosquito numbers were ‘low’ from most of the season, with a series of ‘high’ collections during December and January. No arboviral isolates were detected.

Sydney Olympic Park (SOP): mosquito monitoring at this location included the long-term locations of Narawang and Haslams Creek, as well as Newington. Mosquito numbers were consistently ‘high; for almost the entire season, with *Aedes vigilax* being the predominant species. There were two arboviral detections this season, one STRV from Newington (12/Feb/2019) and one RRV from Haslams Creek (18/Feb/2019).

Appendix 2. THE MOSQUITOES

The following briefly details the main mosquito species collected in NSW.

	**The Common Domestic Mosquito**, ***Aedes notoscriptus***. A common species that breed in a variety of natural and artificial containers around the home. It is the main vector of dog heartworm and laboratory studies shows it be an excellent transmitter of both RRV and BFV.
	**The Bushland Mosquito**, ***Aedes procax***. Common throughout coastal NSW and breeds in bushland freshwater groundwater. Numerous isolates of BFV have been recovered from this species and it is probably involved in the transmission of the virus.
	**The Northern Saltmarsh Mosquito**, ***Aedes vigilax***. An important species along coastal NSW. This species breeds on the mud flats behind saltmarshes and can be extremely abundant and a series nuisance biter. It is a major vector for RRV and BFV along the coast.
	**The Common Australian Anopheline,** ***Anopheles annulipes***. A mosquito from throughout NSW, but is most common in the irrigated region of the Murrumbidgee where it can be collected in the 1,000's. Despite its abundance, it is not thought to be a serious disease vector.
	**The Common Marsh Mosquito**, ***Coquillettidia linealis***. Found throughout NSW but especially in areas with freshwater marshes such as the Port Stephens area. Both BFV & RRV have been isolated from this species and is probably involved in some transmission.
	**The Common Banded Mosquito**, ***Culex annulirostris***. The species is common in the NSW inland regions that have intense irrigation. This species is highly efficient at transmitting most viruses and is responsible for the spreading of most of the arboviruses to humans inland. It is also involved in coastal RRV transmission.

Appendix 3. THE VIRUSES

Alphaviruses

Barmah Forest virus (BFV): disease from this virus is clinically similar to that of RRV disease, although BFV disease tends to be associated with a more florid rash and a shorter duration of clinical severity. Serological over diagnosis of this condition through the non-specificity of the commercial kit was a major issue, and the kit was withdrawn from the market. This has resulted in a dramatic reduction in BFV notifications and the disease may now be under reported. Despite being first isolated from an inland region, cases of BFV disease tend to occur mostly in coastal regions in NSW. The main vector in NSW is *Aedes vigilax* although other species are involved, notably *Aedes procax*. In 2010-2011 for the inland, there was a small epidemic of BFV, but this was the largest outbreak to date for the region.

Ross River virus (RRV): this virus causes RRV disease and is the most common cause of human arboviral disease in Australia. In NSW, approximately 700 cases per season are reported. A wide variety of symptoms may occur from rashes with mild fever, to arthritis that can last from months to years. The virus occurs in both inland and coastal rural regions. The main vectors are *Culex annulirostris* (inland) and *Aedes vigilax* (coast), although other mosquitoes are undoubtedly involved in the transmission of the virus as isolates have been made from many species.

Sindbis virus (SINV): this is an extremely widespread virus throughout the world and occurs in all mainland states of Australia. In contrast with Africa and Europe where outbreaks have been reported, disease from SINV is relatively uncommon in Australia; only 24 infections were notified in NSW from Jul/1995-Jun/2003 (Doggett 2004), with few cases reported since then. Symptoms of disease include fever and rash. Birds are the main host, although other animals can be infected, including macropods, cattle, dogs and humans. The virus has been isolated from many mosquito species, but most notably *Culex annulirostris* in south-eastern Australia. It is also not routinely tested for any longer and it is possible that this would cross react with RRV in the commercial tests.

Flaviruses*

Alfuy virus (ALFV): no clinical disease has been associated with this virus and it has not been isolated from south-eastern Australia.

Edge Hill virus (EHV): a single case of presumptive infection with EHV has been described, with symptoms including myalgia, arthralgia, and muscle fatigue. *Aedes vigilax* has yielded most of the EHV isolates in southeast Australia, although it has been recovered from several other mosquito species. The virus is quite common, with isolates from most years. The vertebrate hosts may be wallabies and bandicoots, but studies are limited.

Kokobera virus (KOKV): only three cases of illness associated with KOKV infection have been reported and all were from southeast Australia. Symptoms included mild fever, aches and pains in the joints, and severe headaches, and lethargy. Symptoms were still being reported by the patients five months after onset. This virus historically was only known from inland regions of NSW until it was detected in a mosquito trapped from the coastal region in 2009-2010. *Culex annulirostris* appears to be the principal vector.

Kunjin virus (KUNV): disease from this virus is uncommon, with only two cases being notified from 1995-2003 (Doggett 2004), and one case in 2011 (Doggett *et al*. 2012). Historically, activity has been confined to the inland region of NSW where it is detected every few years. However, in the summer of 2010-2011, the virus was detected on the coast, which resulted in an outbreak amongst horses with a number of animal deaths resulting. *Culex annulirostris* appears to be the main vector.

Murray Valley Encephalitis (MVEV): major activity of this virus is rare in south-eastern Australia and the last epidemic occurred in 1974. However, since the year 2000 there has been six seasons when MVEV activity has been detected within the state: 2000-2001, 2003-2004, 2007-2008, 2010-2011, 2011-2012, and most recently, 2013-2014. There have been four human cases reported over 2008-2012. The virus occurs only in inland regions of the state and symptoms are variable, from mild to severe with permanent impaired neurological functions, to sometimes fatal. *Culex annulirostris* is the main vector.

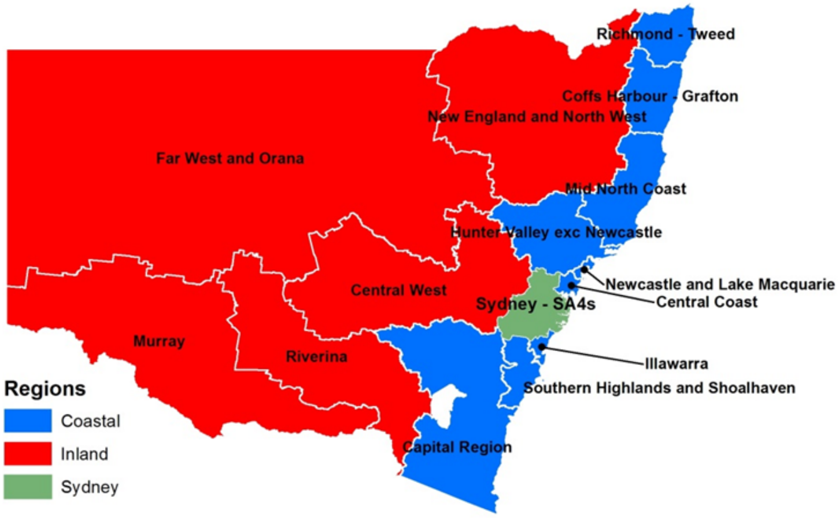
Stratford virus (STRV): there have been very few documented symptomatic patients, only three described to date and symptoms included fever, arthritis, and lethargy. The virus has mostly been isolated from coastal NSW, particularly from the saltmarsh mosquito, *Aedes vigilax*, although recent isolates from the Sydney metropolitan area have been from *Aedes notoscriptus* and *Aedes procax*. This is a common virus, being isolated most years.

*__Note that not all the flaviviruses above (excluding MVEV and KUNV) are tested for, and so it is not possible to determine the disease burden associated with these arboviruses. In light of some of these viruses being extremely common, it may be that disease is unrecognised (as symptoms are non-specific) and without supportive testing, is likely to remain undetected.__**

Appendix 4. ABBREVIATIONS

AHS	Area Health Service
BFV	Barmah Forest virus
BOM	Bureau of Meteorology
CC	Central Coast Public Health Unit
CS	Central Sydney Public Health Unit
EHV	Edge Hill virus
FW	Far West Public Health Unit
GM	Greater Murray Public Health Unit
HUN	Hunter Public Health Unit
IgG	Immunoglobin G (a type of antibody)
IgM	Immunoglobin M (a type of antibody)
ILL	Illawarra Public Health Unit
ICPMR	Institute for Clinical Microbiology and Medical Research
MAC	Macquarie Public Health Unit
MNC	Mid North Coast Public Health Unit
MVEV	Murray Valley Encephalitis virus
MW	Mid West Public Health Unit
NE	New England Public Health Unit
NR	Northern Rivers Public Health Unit
NS	Northern Sydney Public Health Unit
KOKV	Kokobera virus
KUNV	Kunjin virus
PHU	Public Health Unit
RRV	Ross River virus
SA	Southern Area Public Health Unit
SA2	Statistical area level 2
SES	South Eastern Sydney Public Health Unit
SINV	Sindbis virus
SLA	Statistical Local Area
SO	Southern Oscillation
STRV	Stratford virus
SWS	Public Health Unit
TC	Tropical Cyclone
WEN	Public Health Unit
WS	Western Sydney Public Health Unit
VADCP	Victorian Arbovirus Disease Control Program
Virus?	Virus unknown (not BFV, RRV, SINV, EHV, KOKV, KUNV, MVEV, STRV)

Appendix 5. NSW GEOGRAPHIC REGIONS - COASTAL, INLAND, AND SYDNEY METROPOLITAN – USING ABS STATISTICAL AREA LEVEL 4 (SA4) GROUPINGS.



SA4 Name	Region
Capital Region	Coastal
Coffs Harbour - Grafton	Coastal
Newcastle and Lake Macquarie	Coastal
Southern Highlands and Shoalhaven	Coastal
Illawarra	Coastal
Hunter Valley excluding Newcastle	Coastal
Central Coast	Coastal
Richmond - Tweed	Coastal
Mid North Coast	Coastal
Central West	Inland
Far West and Orana	Inland
New England and North West	Inland
Riverina	Inland
Murray	Inland
Sydney - all 14 Sydney SA4s	Sydney

ACKNOWLEDGMENTS

This project is funded and supported by the Environmental Health Branch of the NSW Ministry of Health. The following are acknowledged for their efforts in the Arbovirus Program:

Kishen Lachireddy, Anna Bethmont, Neil Hime, Aditya Vyas & Richard Broome (**Environmental Health Branch, NSW Health**); Tracey Oakman, James Allwood, Tony Burns, Ian Hardinge & Kev Prior (**Murrumbidgee & Southern LHDs**); David Ferrall, Gerard van Yzendoorn & Jason Harwood (**Far West & Western LHDs**); Dr David Durrheim, Philippe Porigneaux, Glenn Pearce (**Hunter New England LHD**); Paul Corben, Kerryn Lawrence, David Basso, Greg McAvoy, Matthew Rand, Greg Bell, Tony Kohlenberg & Geoff Sullivan (**Mid North Coast & Northern NSW LHDs**), Dr Peter Lewis, Sam Curtis, Adam McEwen, Wayne McCallum & Kerry Spratt (**Northern Sydney & Central Coast LHDs**); Prof. Mark Ferson, Toni Cains & Brian Huang (**South Eastern Sydney LHD**); Helen Noonan, Haylee Sneesby & Annie Truong (**Western Sydney & Nepean Blue Mountains LHD**); Peter Cavagnino, John Birkett, Angela Daly & Mike Cassidy (**South Western Sydney & Sydney LHDs**); Philip Palenkas & Lauriston Muirhead (**Albury City Council, Albury**); Kristy Bell & Tom McAully (**Ballina Shire Council, Ballina**); Jackie Davis (**Nursery on Mertin, Bourke**); Krystle Knowles, Erin Downes, Calee Bancroft & James Moss (**Coffs Harbour City Council, Coffs Harbour**); Mathew Teale (**Forbes Council**), Laura Turner & Fiona De Wit (**Griffith Shire Council, Griffith**); Anthony Gleeson (**Hawkesbury City Council, Windsor**); Bill Larkin (**Kempsey**), Derek Poulton & Keith Lainson (**Lake Macquarie City Council**); Peter Skarlis & Craig McVittie (**Leeton Shire Council, Leeton**); Linda McLellan (**Macquarie Marshes**), Emma Shaw & Daniel Walsh (**Nambucca**), David Durie, Stevie McCormack & Belinda Comer (**Penrith City Council, Penrith**); Brian Falkner (**Tweed Shire Council, Murwillumbah**).

The chicken handlers included: Maggie McCalman (**Deniliquin**), Denyell Woodhouse (**Dubbo**), Mathew Teale (**Forbes**), Renae Foggiato & Fiona de Wit (**Griffith**), Kevin Rosser (**Hay**), David Lang (**Leeton**), Linda McLellan (**Macquarie Marshes**), Barbara Turner (**Menindee**), Lester Rodgers (**Moree**). The laboratory staff within CIDMLS are acknowledged, particularly Heang Lim, Laurence McIntyre & James Goodwin.

The section on 'Notifications of Locally-Acquired Arbovirus Infections' was produced by the Communicable Diseases Branch, Health Protection NSW, NSW Ministry of Health.

The input of Dr Ross Matthews, Director of Animal Care, Westmead Hospital in the continuation of the chicken surveillance program is greatly appreciated. The Sydney Olympic Park Authority funds the Department to undertake mosquito surveillance in the Homebush area.

The cooperation of the Department of Agriculture and Water Resources (especially Laura Marsh) and the Sydney Airport Corporation were integral in the surveillance of mosquitoes at ports and approved arrangement facilities (freight). Our apologies to anyone inadvertently omitted.

REFERENCES

Bureau of Meteorology, Australia. (2019). **Rainfall Maps**. http://www.bom.gov.au/cgi-bin/climate/rainmaps.cgi, *accessed 15/Aug/2019.*

Dobrotworsky N.V. (1965). **The Mosquitoes of Victoria**. *Melbourne University Press, Carlton*.

Doggett S. (2004). **Population health aspects of mosquito-borne disease in New South Wales**. *NSW Public Health Bulletin*, 15: 193-199.

Doggett S.L. (2014). **The Forum: It's Barmah…or is it?** *Mosquito Bites*, 8(2): 40-42.

Doggett S.L. (2018). **Your number's up!** *Mosquito Bites*, *13*(1): 24-27.

Doggett S., Clancy J., Haniotis J., Russell R.C., Hueston L., Marchetti M. and Dwyer D. (2001). **The New South Wales Arbovirus Surveillance & Mosquito Monitoring Program. 2000 –2001 Annual Report**. *Department of Medical Entomology, Westmead*. 27pp.

Doggett S., Clancy J., Haniotis J., Russell R.C., Hueston L., Marchetti M. and Dwyer D. (2004). **The New South Wales Arbovirus Surveillance & Mosquito Monitoring Program. 2003 – 2004 Annual Report**. *Department of Medical Entomology, Westmead*. 23pp.

Doggett S., Clancy J., Haniotis J., Russell R.C., Hueston L., and Dwyer D. (2011). **The New South Wales Arbovirus Surveillance & Mosquito Monitoring Program. 2010 – 2011 Annual Report**. *Department of Medical Entomology, Westmead*. 37pp.

Doggett S.L. and Russell R.C. (2005). **The epidemiology of Ross River and Barmah Forest viruses in New South Wales.** *Arbovirus Research in Australia*, 9: 86-100.

Doggett S.L., Russell R.C., Clancy J., Haniotis J. and Cloonan M.J. (1999). **Barmah Forest virus epidemic on the south coast of New South Wales, Australia, 1994-1995: Viruses, Vectors, Human Cases, and Environmental Factors**. *Journal of Medical Entomology*, 36: 861-868.

Doggett S.L., Webb C., Clancy J. and Haniotis J. (2017). **Mosquito survey of the QANTAS freight terminal, Sydney International Airport, 2017**. *Report for the NSW Ministry of Health*.

Finlaison D.S., Read A. J. and Kirkland P.D. (2008). **An epizootic of bovine ephemeral fever in New South Wales in 2008 associated with long-distance dispersal of vectors**. *Australian Veterinary Journal*, 88(8): 301-306.

Forbes J.A. (1978). **Murray Valley encephalitis 1974 - also the epidemic variance since 1914 and predisposing rainfall patterns**. *Australasian Medical Publishing Co., Glebe*. 20pp.

Ibáñez-Justicia A., de Vossenberg B., van den Biggelaar R., Voogd J, Metx E, Jacobs F., Dik M. and Stroo A. (2019). **Detection of *Aedes flavopictus* (Yamada, 1921), Netherlands, June 2019**. *EuroSurveillance*, 24(30): pii=1900433.

Lee D.J., Hicks M.M., Griffiths M., Russell R.C., Geary M. and Marks E.N. (1980 – 1989). **The Culicidae of the Australian Region. Vols. 1 - 12**. *Australian Government Publishing*

Service, Canberra.

Knope K.E., Kurucz N., Doggett S.L. *et al*. (2016). **Arboviral diseases and malaria in Australia, 2012-23: annual report of the National Arbovirus and Malaria Advisory Committee**. *Communicable Diseases Intelligence*, 40(1): E17-E47.

Kurucz N., Markey P., Draper A. *et al.* (2016). **Investigation into High Barmah Forest Virus Disease Case Numbers Reported in the Northern Territory, Australia in 2012-2013**. *Vector-Borne and Zoonotic Diseases*, 16(2): 110-116.

Moureau G*, et al.* (2007). **A real-time RT-PCR method for the universal detection and identification of flaviviruses**. *Vector Borne Zoonotic Diseases,* 7(4):467-477.

Nicholls N. (1986). **A method for predicting Murray Valley encephalitis in southeast Australia using the Southern Oscillation**. *Australian Journal of Experimental Biology and Medical Science,* 64: 587-94

Pyke, A. T., I. L. Smith, A. F. van den Hurk, J. A. Northill, T. F. Chuan, A. J. Westacott, and G. A. Smith. 2004. **Detection of Australasian Flavivirus encephalitic viruses using rapid fluorogenic TaqMan RT-PCR assays**. *Journal of Virological Methods*, 117: 161-167.

Russell R.C. (1993). **Mosquitoes and mosquito-borne disease in southeastern Australia**. *Department of Medical Entomology, Westmead, NSW*, 310pp.

Russell R.C. (1996). **A Colour Photo Atlas of Mosquitoes of Southeastern Australia**. *Department of Medical Entomology, Westmead, NSW*, 193pp.

van den Hurk, A. F., S. Hall-Mendelin, M. Townsend, N. Kurucz, J. Edwards, G. Ehlers, C. Rodwell, F. A. Moore, J. L. McMahon, J. A. Northill, R. J. Simmons, G. Cortis, L. Melville, P. I. Whelan, and S. A. Ritchie. 2014. **Applications of a sugar-based surveillance system to track arboviruses in wild mosquito populations.** *Vector Borne Zoonotic Diseases*, 14: 66-73.