THE NEW SOUTH WALES ARBOVIRUS SURVEILLANCE & MOSQUITO MONITORING PROGRAM

2017-2018 Annual Report

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NSW GOVERNMENT | Health Pathology

EXECUTIVE OVERVIEW

- **For the 2017-2018 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 metropolitan Sydney, (ii) monitored flavivirus transmission through the testing of sentinel chickens across inland NSW. Surveillance operated over mid-October to April.
- **The climatic conditions** leading up to 2017-2018 was a spell of extremely dry weather over July to September, 2017. A weak La Niña formed late in 2017 producing rainfall that was above average over much of the state for the last three months of the year. However, conditions soon became dry again with well below average rainfall for the first six months of 2018 and temperatures were above average for the entire season. Neither the Forbes nor the Nicholls hypotheses were suggestive of a potential MVEV epidemic for the 2017-2018 season.
- **For the inland**, 33,527 mosquitoes were trapped and this was around one sixth that of the previous season collection of 180,992, but similar to that yielded in 2015-2016. There were three RRV detections, all from Griffith. There were no seroconversions in the sentinel chickens.
- **Human notifications from the inland** of RRV and BFV totalled 207 (200RRV & 7BFV), which was less than one quarter that of the previous season and below average. The statistical local areas that produced the highest notifications for RRV from the inland was Griffith (12), while Moree (191.8/100,000) had the highest notification rate. There were no human cases of flavivirus infection.
- **As of August 2018**, the Forbes hypothesis is not suggestive of a possible MVEV epidemic for 2018-2019 and such an outbreak seems unlikely with the ongoing drought. The El Niño-Southern Oscillation is currently neutral, although there is a 50% chance of an El Niño forming, suggesting drier conditions later this year.
- **For the coast**, almost 49,000 mosquitoes were trapped, which was slightly below the previous season. There were three isolates from the coast; 2BFV & 1RRV all from the Central Coast region.
- **Human notifications from the coast** totalled 428 cases, including 343 RRV and 85 BFV, and this was below average.
- **Around 40% of the RRV notifications for the season** occurred over Jul-Dec 2017, when mosquito and arbovirus activity was minimal. This highlights that notification data is not always a good indication of when infection occurred.
- **Sydney** experienced a notable decrease in mosquito numbers upon the previous season with almost 31,000 mosquitoes trapped, compared with the 57,000 from the previous season. There were five arboviral isolates from the Georges River area including 2BFV and 3STRV.
- **Detections of exotic mosquitoes at Sydney Airport continue**. This included *Aedes aegypti* trapped at the Sydney International Airport on 12/Jul/2017, 9/Aug/2017, 24/Oct/2017, 22/Nov/2017, 21/Mar/2018, and 18/Apr/2018. *Aedes aegypti* were also collected at a freight facility near the domestic airport on 7/Feb/2018. *Culex gelidus* was trapped at the international airport on 2/May/2018. Responses included regular teleconferences initiated by the NSW Ministry of Health, enhanced surveillance at the airport, vector surveys, insecticidal applications, and ongoing larval treatments.

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NSW ARBOVIRUS SURVEILLANCE AND MOSQUITO MONITORING PROGRAM 2017-2018

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 2017-2018 season follow.

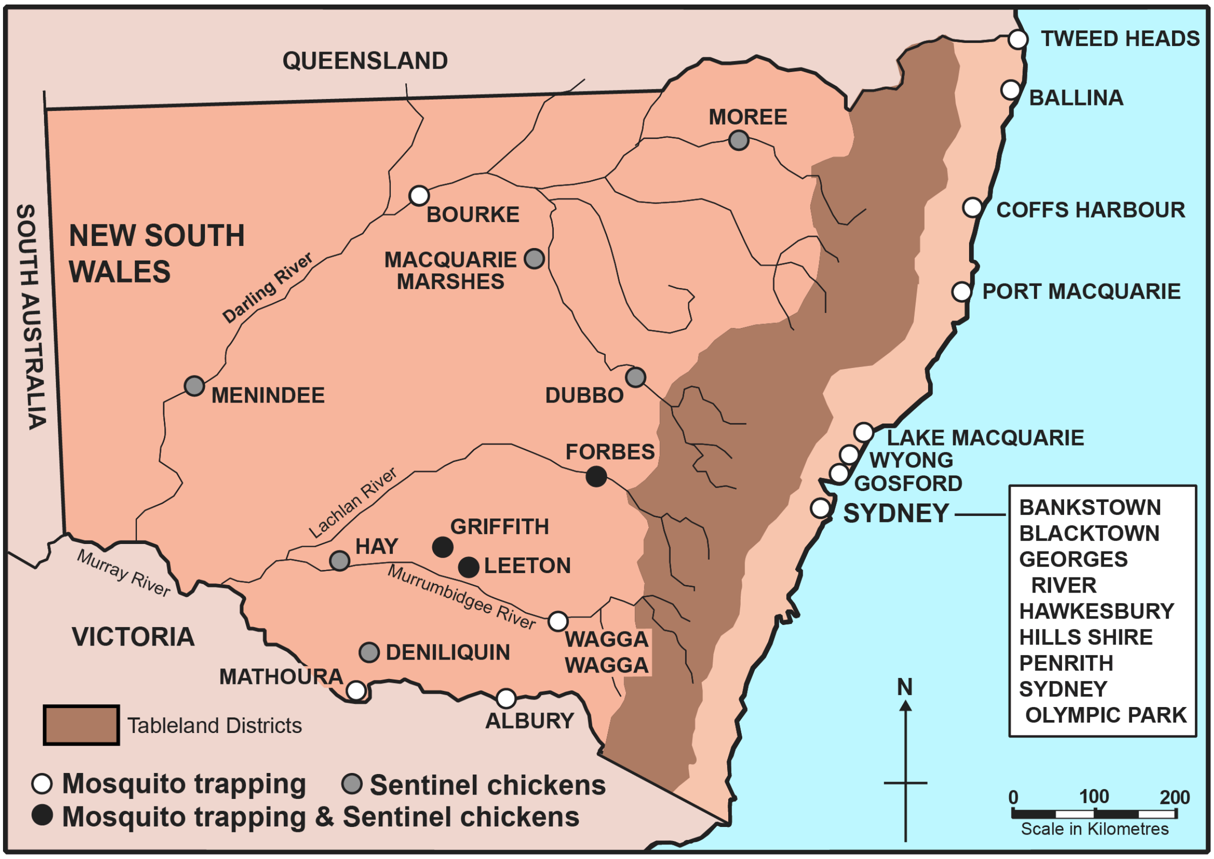


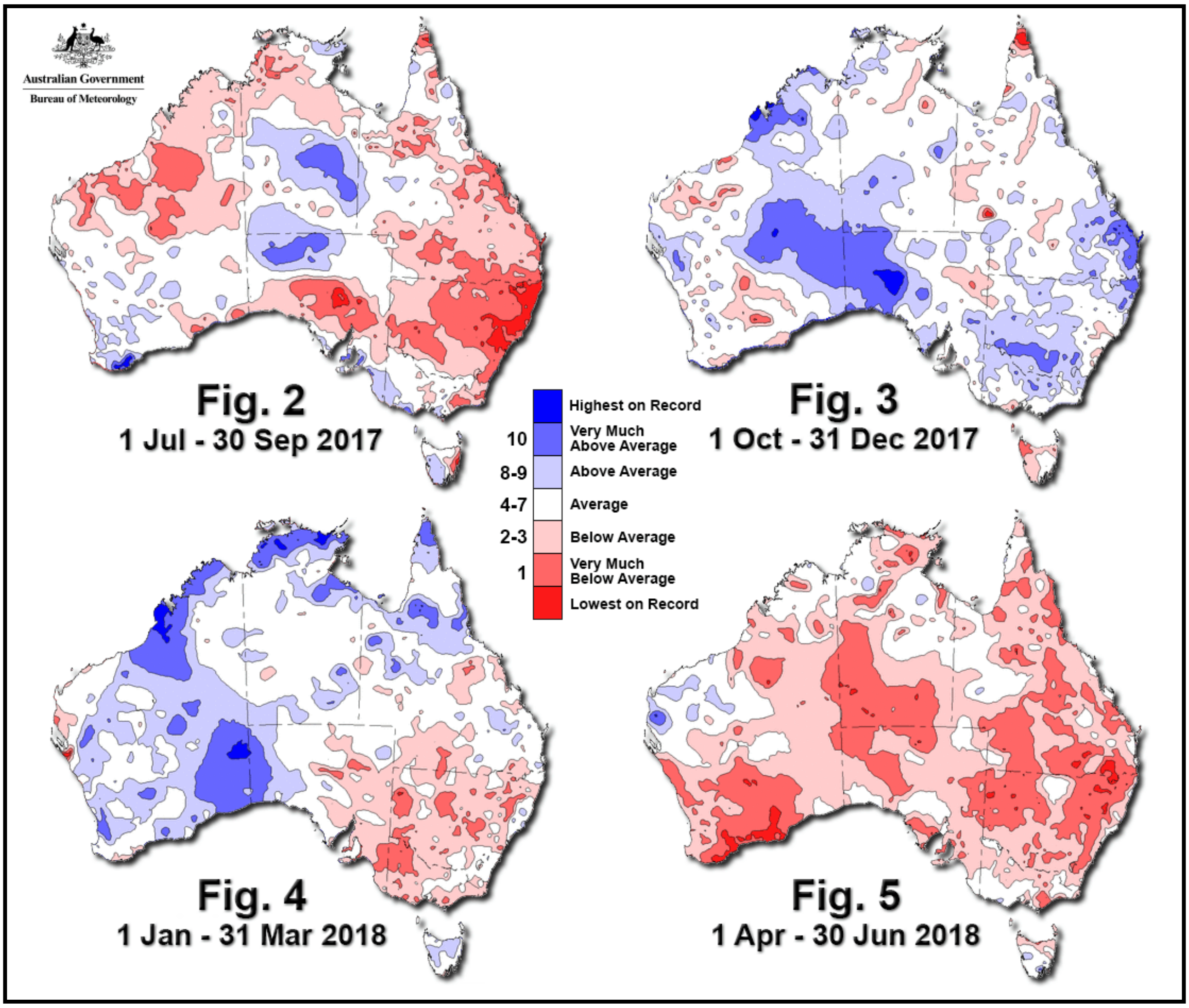
Fig 1. Mosquito trapping locations and Sentinel Chicken sites, 2017-2018.

MONITORING LOCATIONS

For 2017-2018, mosquito-trapping sites were operated at 7 inland, 7 coastal and 7 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 2017, Oct-Dec 2017, Jan-Mar 2018 & Apr-Jun 2018. The stronger the red, the drier the conditions. Conversely, the stronger the blue, the wetter the conditions. *Modifed from the Australian Bureau of Meterology, 2018*.

The first quarter of 2017 (January to March, not shown) produced normal rainfall patterns for most of the state with above average rainfall along the north coast. For the second quarter (also not shown), rainfall was below to very much below average for much of the state, but especially so for the slope region. The entire state experienced extremely low precipitation during the third quarter of 2016 (Figure 2), with some areas of the coast having record low rainfall levels. A weak La Niña formed late in 2017 producing rainfall that was above average across the inland for the final quarter of the year (Figure 3). However, conditions soon became dry again with well below average rainfall for the first six months of 2018 (Figures 4 & 5).

Currently (as of August, 2018), there is a 50% chance of a spring El Niño, which means that last part of the year may remain dry.

Maximum temperatures for the last half of 2017 were slightly (1-2º) above average for both inland and coastal regions. The first three months of 2018 continued to be above average with maximum temperatures of 2-3 degrees above normal for 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.

MVEV Predictive Models

Two main models have been developed for the prediction of MVEV epidemic activity in southeastern 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 2016) or the first quarter of the current year (January-March 2017) and the last quarter of the current year (October-December 2017), 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 two of the catchment basins for the last quarter of 2016, was above decile 7 in one catchment basin in the first quarter of 2017, and above decile 7 in only one of the catchment basins for the last quarter of 2017, thus the Forbes hypothesis was not fulfilled for 2017-2018 (Table 1). Additionally, decile 7 or above rainfall did not occur across all the catchment basins during the first quarter of 2018. Therefore according to Forbes', there should be a lower risk of an MVEV epidemic for the upcoming 2018-2019 season.

Table 1. Rainfall indices for the main catchment basins of eastern Australia as per Forbes hypothesis, relevant to the 2016-2017 and 2017-2018 seasons.

Catchment Basin	Oct-Dec 2016	Jan-Mar 2017	Oct-Dec 2017	Jan-Mar 2018
Darling River	0.72	0.67	0.58	0.81
Lachlan/Murrumbidgee/ Murray Rivers	0.70	1.14	0.92	1.01
Northern Rivers	1.35	0.57	0.98	1.03
North Lake Eyre system	1.35	0.63	1.09	0.73

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 2017, the SO values were respectively: 1009.60mm, 1013.23mm and 1009.70mm (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 season were not included as there was only one detection of MVEV, which may have resulted from over-wintering mosquitoes.

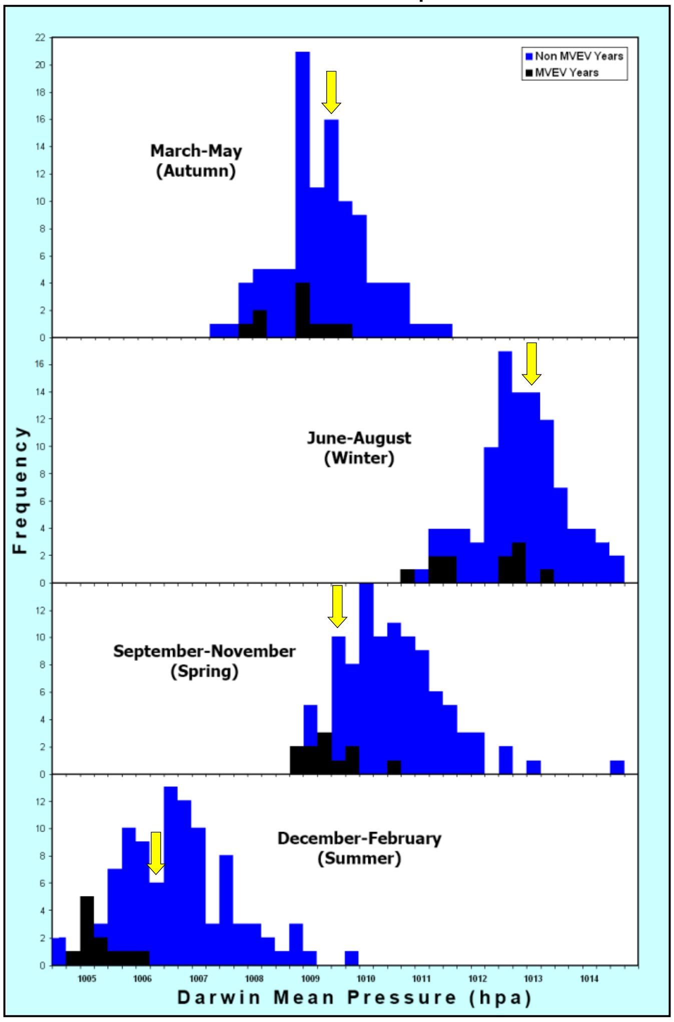


Figure 6. The SO by seasons prior to MVEV active years, according to Nicholls (1986), updated up to Spring 2017. The black bars represent the pre-MVEV active seasons. The yellow arrows indicate the respective SO values relevant to the 2017-2018 season.

As of August 2018, the autumn Nicholls' value is 1009.30mm and the winter value is 1013.30. While both values are within the range of values for past MVEV outbreak years (albeit just for the winter period), the ongoing drought suggests that an MVEV outbreak would be unlikely for 2018-2019. The El Niño-Southern Oscillation is currently neutral but (as noted above) there is a 50% chance of an El Niño forming, suggesting below average rainfall patterns ahead. Conditions are expected to be warmer than average, which may bring the mosquito season forward.

Table 2. The seasonal atmospheric pressures (in mm) according to Nicholls' hypothesis, relevant to the 2017-2018 season.

	Autumn 2017	Winter 2017	Spring 2017
2017 Values	1010.30	1012.57	1010.07
Pre past MVEV seasons	<1009.74	<1013.50	<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, 113,132 mosquitoes representing 49 species were collected in NSW during 2017-2018, with the total being around one third of the previous season (with a total of 299,239 mosquitoes), but similar to the number trapped in 2015-2016 (108,663). *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 33,527 mosquitoes comprising 19 species was around one sixth that of the previous season collection of 180,992, but similar to that yielded in 2015-2016, when inland mosquito numbers totalled 37,615. *Culex annulirostris* was the dominant species trapped at most sites and comprised 65.3% of the total inland collections. *Anopheles annulipes* (28.3%) was the next most common species followed by *Culex quinquefasciatus* (4.2%).

Coastal

In total, 48,660 mosquitoes comprising 36 species were collected from coastal NSW and was below the previous season's collection (56,935). The most common species collected were *Culex sitiens* (26.8%), *Aedes vigilax* (22.8%), *Culex annulirostris* (11.5%), *Aedes notoscriptus* (9.5%), *Verrallina funerea* (9.1%), *Aedes multiplex* (9.0%), and *Aedes procax* (2.7%).

Metropolitan Sydney

A total of 30,945 mosquitoes, comprising 29 species, was collected from metropolitan Sydney and this was around half the previous season's total collection (56,935). *Aedes vigilax* (66.1% of the total Sydney mosquitoes trapped) was the most common species, followed by *Culex annulirostris* (9.8%), *Culex quinquefasciatus* (7.4%), *Anopheles annulipes* (4.3%), *Aedes notoscriptus* (4.1%), and *Coquillettidia linealis* (2.2%).

ARBOVIRUS ISOLATIONS FROM MOSQUITOES

Methods

Viral detection now incorporates both traditional cell culture methodology and modern molecular techniques for identifying viral nucleic acid. Cell culture isolation methods were as per earlier annual reports (Doggett *et al*., 1999, 2001). ELISA assays were used to identify any suspected viral isolate and can identify the alphaviruses - BFV, RRV, and Sindbis (SINV), and the flaviviruses - MVEV, KUNV, Alfuy (ALFV), Edge Hill (EHV), Kokobera (KOKV), and Stratford (STRV). Any isolate that was not identified by the assays was labelled as 'unknown'.

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. (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), 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 11 detections, including 4 RRV, 4 BFV, and 3 STRV (Table 3). From the inland, three RRV were made from Griffith. From the coast, Georges River produced five isolates (2BFV & 3STRV), while another three isolates (2BFV & 1 RRV) were made from the Central Coast.

Table 3. Arboviral isolates from NSW, 2017-2018.

LOCATION - Site	Date Trapped	Mosquito Species	Virus
GRIFFITH – Lake Wyangan	3/Jan/2018	*Culex annulirostris*	Ross River
GEORGES RIVER - Deepwater	30/Jan/2018	*	Stratford
GRIFFITH – Lake Wyangan	31/Jan/2018	*Culex annulirostris*	Ross River
GRIFFITH – Hanwood	5/Feb/2018	*Culex annulirostris*	Ross River
GEORGES RIVER – Alfords Point	7/Feb/2018	*	Stratford
GEORGES RIVER - Deepwater	12/Feb/2018	*Aedes vigilax*	Stratford
CENTRAL COAST – Empire Bay	27/Feb/2018	*	Barmah Forest
CENTRAL COAST – Halekulani	14/Mar/2018	*	Barmah Forest
GEORGES RIVER – Alfords Point	21/Mar/2018	*	Barmah Forest
GEORGES RIVER – Deepwater	21/Mar/2018	*	Barmah Forest
CENTRAL COAST – Empire Bay	26/Apr/2018	*	Ross River

* Detection via PCR on pooled samples, the mosquito species cannot be determined. BFV = Barmah Forest virus, RRV = Ross River virus, STRV = Stratford virus.

SENTINEL CHICKEN PROGRAM

Location of flocks

The 2017-2018 season began on 2nd November 2017 with the first bleed and ended on 9th April 2018 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

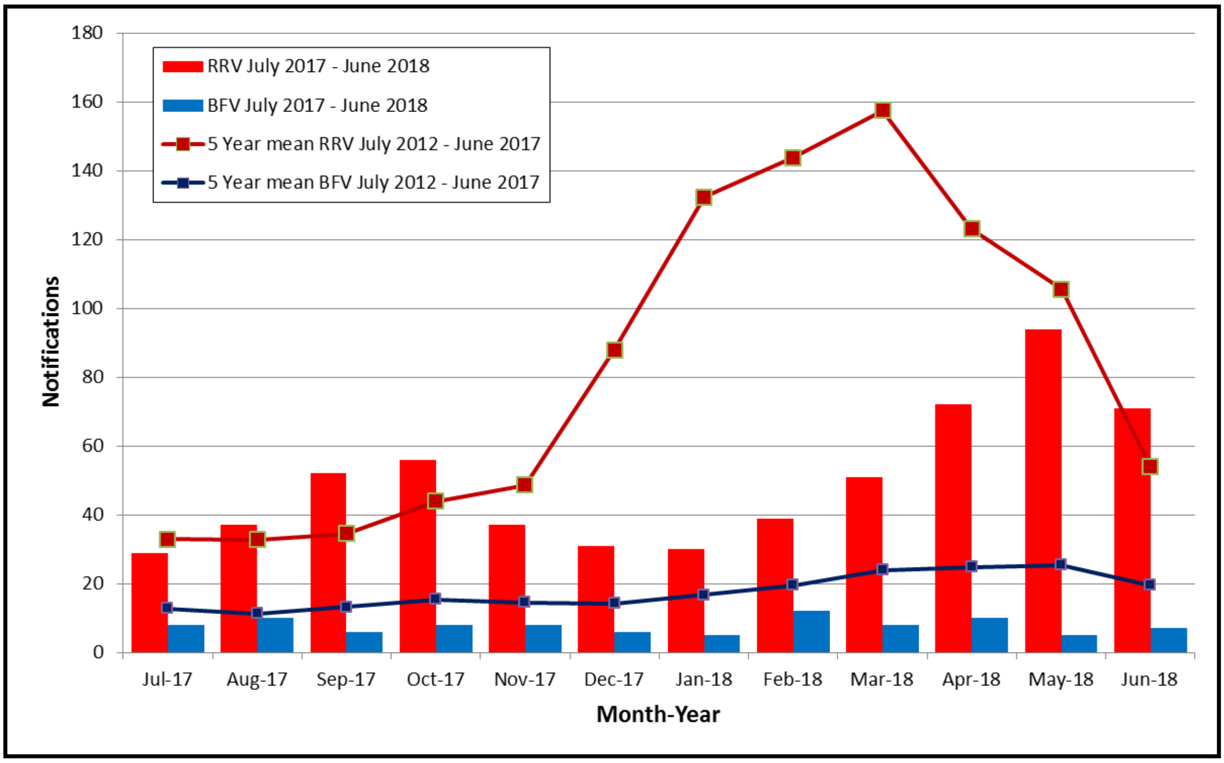
The season began with 135 pullets. A total of 2,500 samples was received from the ten flocks in NSW over the five-month period in 2017-2018. This represented 5,000 ELISA tests (excluding controls and quality assurance samples), with each specimen being tested for MVEV and KUNV antibodies. There no seroconversions in the sentinel chickens.

NOTIFICATIONS OF LOCALLY-ACQUIRED ARBOVIRUS INFECTIONS

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 the 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.

Figure 7. Barmah Forest virus and Ross River virus infections in NSW residents: notifications by month and year of onset for 2017-2018 financial year, compared to the 5 year monthly means for the period July 2012 to June 2017.



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 2017-2018 financial year, there were 599 notifications of RRV in NSW residents (Table 4), a 61% decrease compared to the previous year (1,522 notifications). There were 93 notifications of BFV (Table 4), which is 16% higher than the previous year (80 notifications). There were no notifications of other locally-acquired arbovirus infections in NSW during 2017-2018.

Monthly BFV notifications were generally low throughout the year, with a peak of 12 notifications in February 2017 (Figure 7).

RRV notifications from August to October 2017 were above the historical average for this period (Figure 7). Notifications were highest in the autumn months of 2018 with a peak in May 2018 (n=94). This marked a return to the typical seasonal pattern of RRV activity.

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 2017-2018 financial year.

Local Health District	Barmah Forest virus		Ross River virus	
	Notifications	Population Rate*	Notifications	Population Rate*
Central Coast	3	0.86	23	6.58
Far West	0	0	18	58.97
Hunter New England	18	1.92	175	18.65
Illawarra Shoalhaven	1	0.24	17	4.13
Mid North Coast	12	5.34	67	29.81
Murrumbidgee	0	0	71	24.00
Nepean Blue Mountains	0	0	5	1.28
Northern NSW	51	16.48	81	26.18
Northern Sydney	0	0	26	2.81
South Eastern Sydney	0	0	10	1.06
South Western Sydney	0	0	6	0.60
Southern NSW	3	1.38	26	11.98
Sydney	1	0.15	6	0.90
Western NSW	4	1.43	62	22.10
Western Sydney	0	0	4	0.40
Total	**93**	**1.16**	**599**	**7.50**

*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.

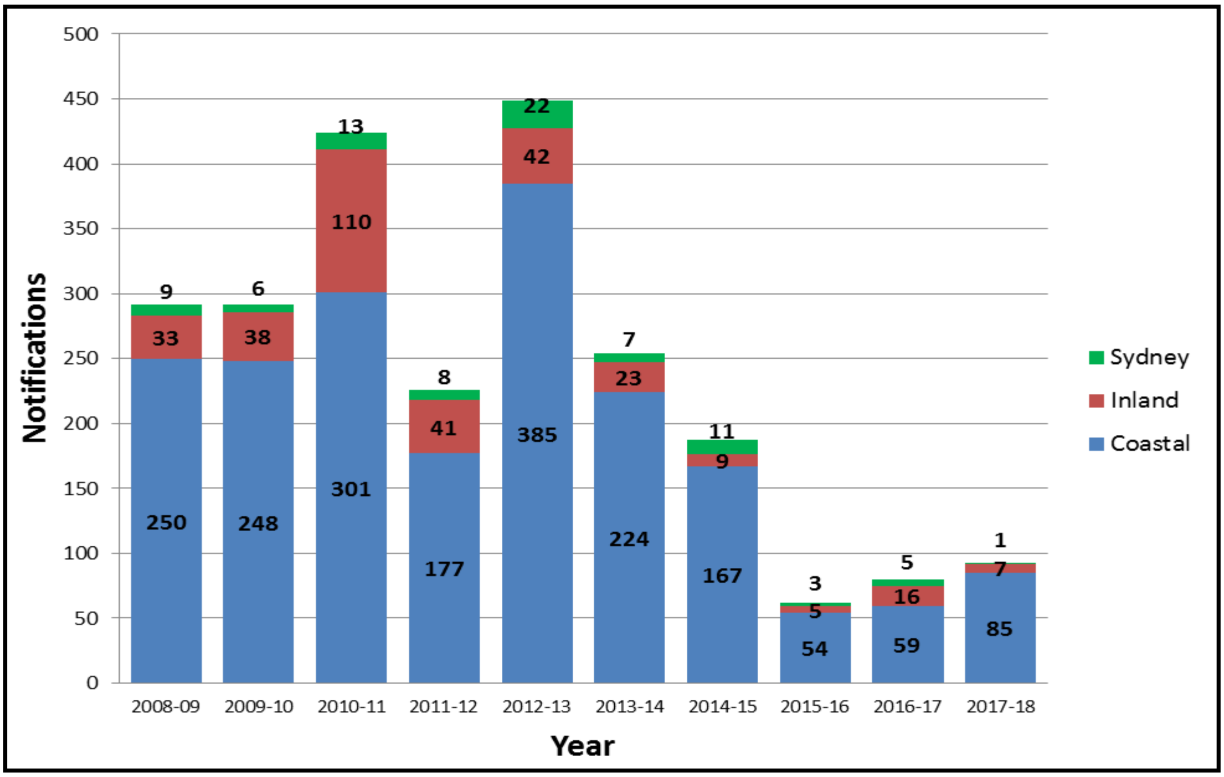
BFV and RRV notifications by place of residence of the case are presented by NSW local health district (LHD, Table 4), by geographic region (Coastal, Inland, and Sydney metropolitan, Figure 8) and by Australian Bureau of Statistics (ABS) statistical area level 2 (SA2, Figures 10&11). Population rates are based on ABS estimated resident population data (using the 2017-2018 financial year estimates for LHDs and the 2015 estimates for SA2s). It should be noted that the place of residence of a case may not be where the infection was acquired.

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

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

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 2007-2008 to 2017-2018. The Coastal region again accounted for the majority of BFV notifications (n=85, 91.4%) followed by the Inland region (n=7, 7.5%) with only 1 notification reported in a resident of the Sydney metropolitan region (Figure 8).

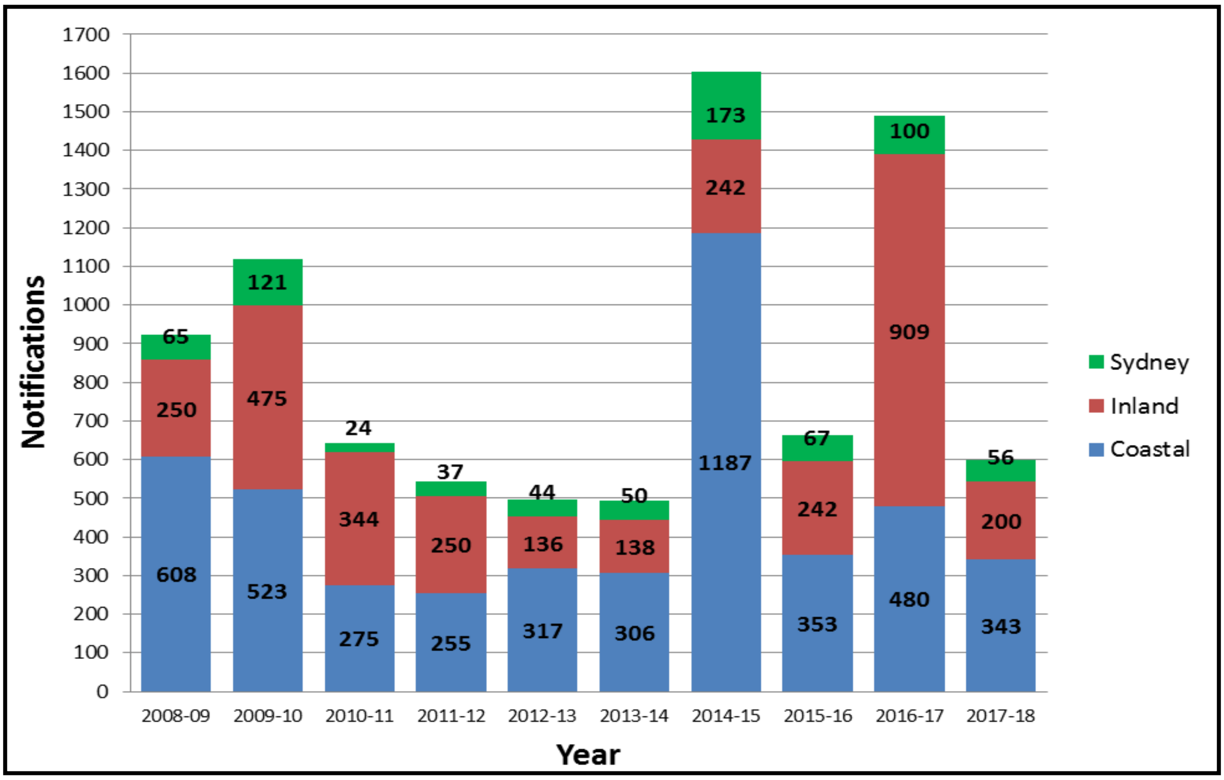
Figure 8: Barmah Forest virus infections in NSW residents: annual notifications by year of disease onset and geographical region for the past 10 years (2008-2009 to 2017-2018).



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.

The Coastal region also accounted for the majority of RFV notifications (n=343, 57.3%), in contrast to the previous season when there were more notifications from residents of the Inland region. In 2017-2018, the Inland region accounted for a third of all RRV notifications, with RRV infections in Sydney residents accounting for just under 10% of notifications (Figure 9).

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 2008-2009 to 2017-2018).



See Appendix 4 for definitions of the Coastal, Inland, and Sydney metropolitan regions. Due to incomplete address information, a handful of cases (about one a year) could not be allocated to a region.

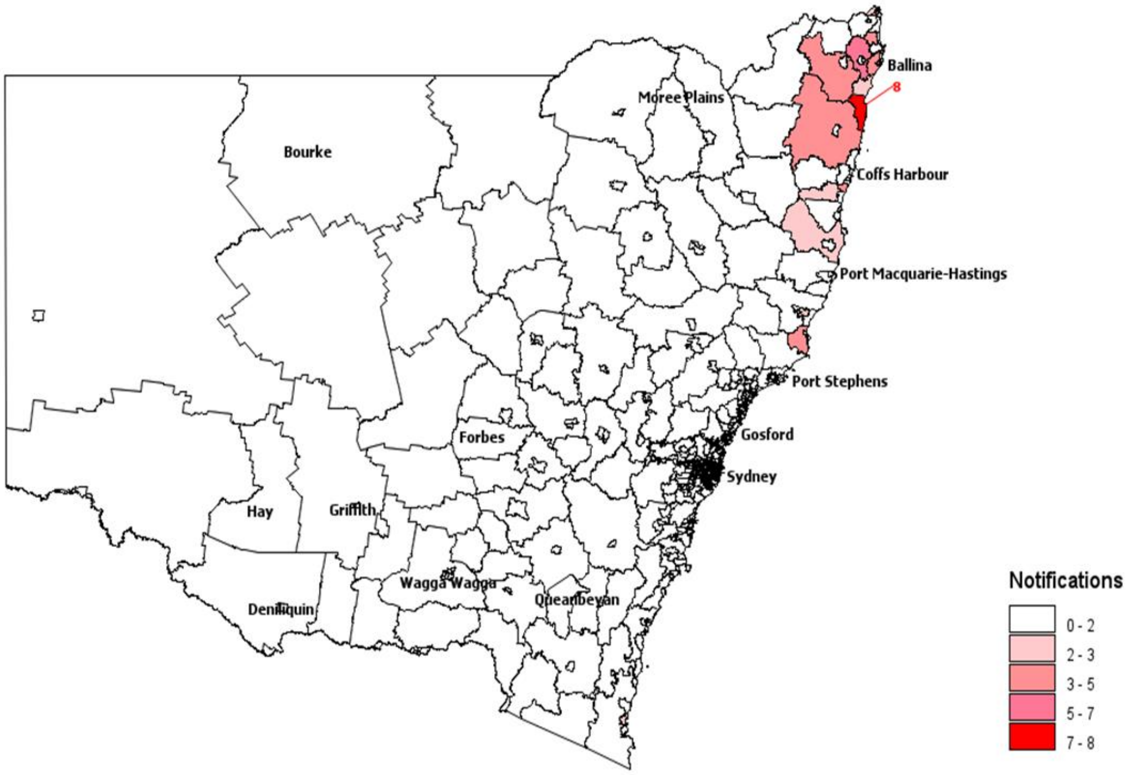
Notification maps of BFV and RRV infection by ABS statistical area level 2 (SA2) of residence for the 2017-2018 financial year are shown in Figures 10 and 11, together with maps of population notification rates.

The SA2 areas with the highest total number of BFV notifications were Maclean-Yamba-Iluka (n=8) and Lismore Region (n=6) (Figure 10a). The three SA2 areas with the highest notification rates per 100,000 population were the Casino Region (56.4), Forster-Tuncurry Region (50.5), and Maclean-Yamba-Iluka (48.6) (Figure 10b).

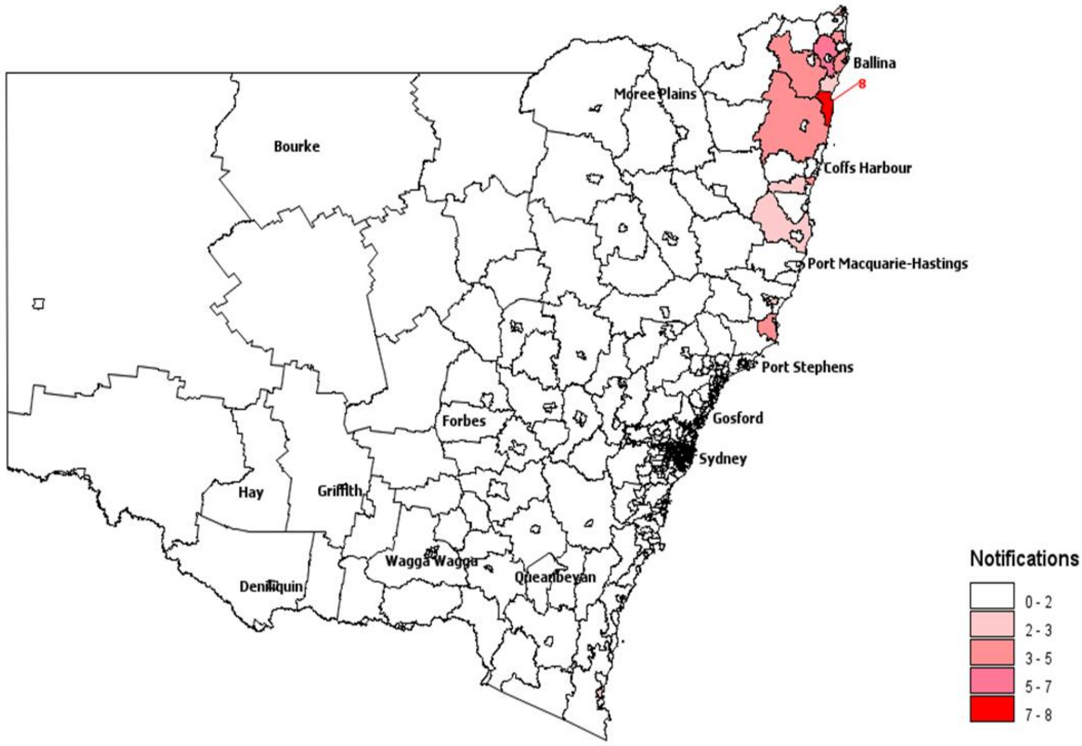
The SA2 areas with the highest total number of RRV notifications were the Taree Region (n=14) and the Griffith Region (n=12) (Figure 11a). The SA2 areas with the highest notification rates per 100,000 population were Moree Region (191.8), Far West (143.5), and Narrabri (136.5) (Figure 11b).

Figure 10: Barmah Forest virus infections in NSW residents.

(a) Notifications by statistical area level 2 (SA2), for 2017-2018.



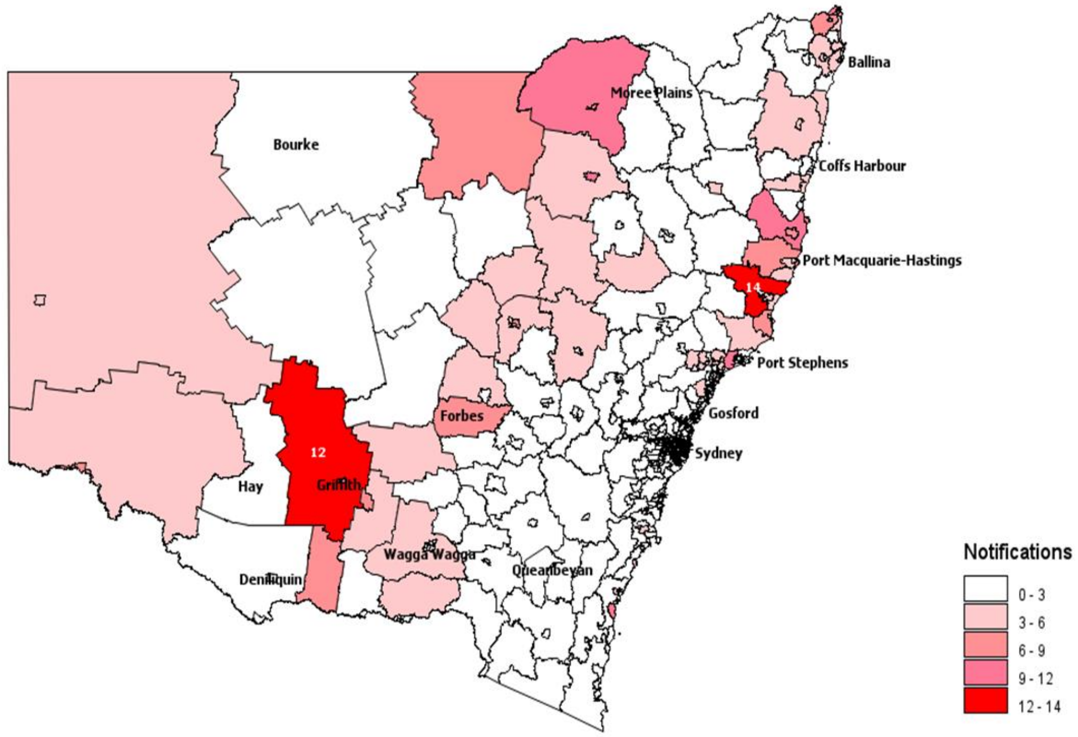
(b) Population notification rates* by statistical area level 2 (SA2), for 2017-2018.



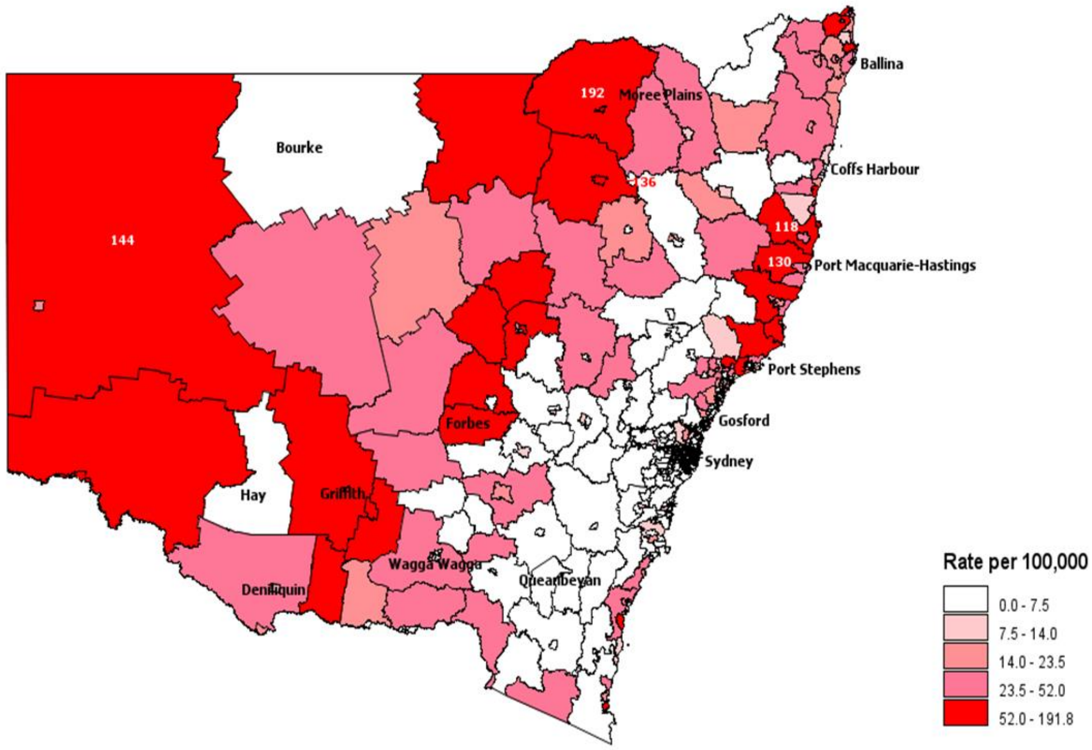
*Notifications per 100,000 estimated resident population for 2015, based on ABS census data.

Figure 11: Ross River virus infections in NSW residents.

(a) Notifications by statistical area level 2 (SA2), for 2017-2018.



(b) Population notification rates* by statistical area level 2 (SA2), for 2017-2018.



*Notifications per 100,000 estimated resident population for 2015, 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 2017-2018 season was very much dominated by a year of exceptionally dry and hot weather. This was in spite of a weak La Niña forming late in the year that produced some above average rainfall in the last quarter. However, the entire state during the second and third quarter of 2017, experienced well below average precipitation, as occurred again during the first and second quarters of 2018.

With the hot and dry conditions predominating, mosquito numbers were well below normal for both the inland and coastal regions. There were few arboviral isolations from the mosquitoes and no seroconversions to MVEV or KUNV in the sentinel chickens from the inland.

Thus it is not surprising that human notifications for local arboviruses were well below previous years, with a combined total of only 692 (599 RRV, 93 BFV) notifications for the season. This included 207 (200 RRV, 7 BFV) from the inland and 428 (343 RRV, 85 BFV) from the coast. However, the accumulated data for 2017-2018 hides the fact that many RRV notifications were reported during the cooler months (Figure 7 & 12).

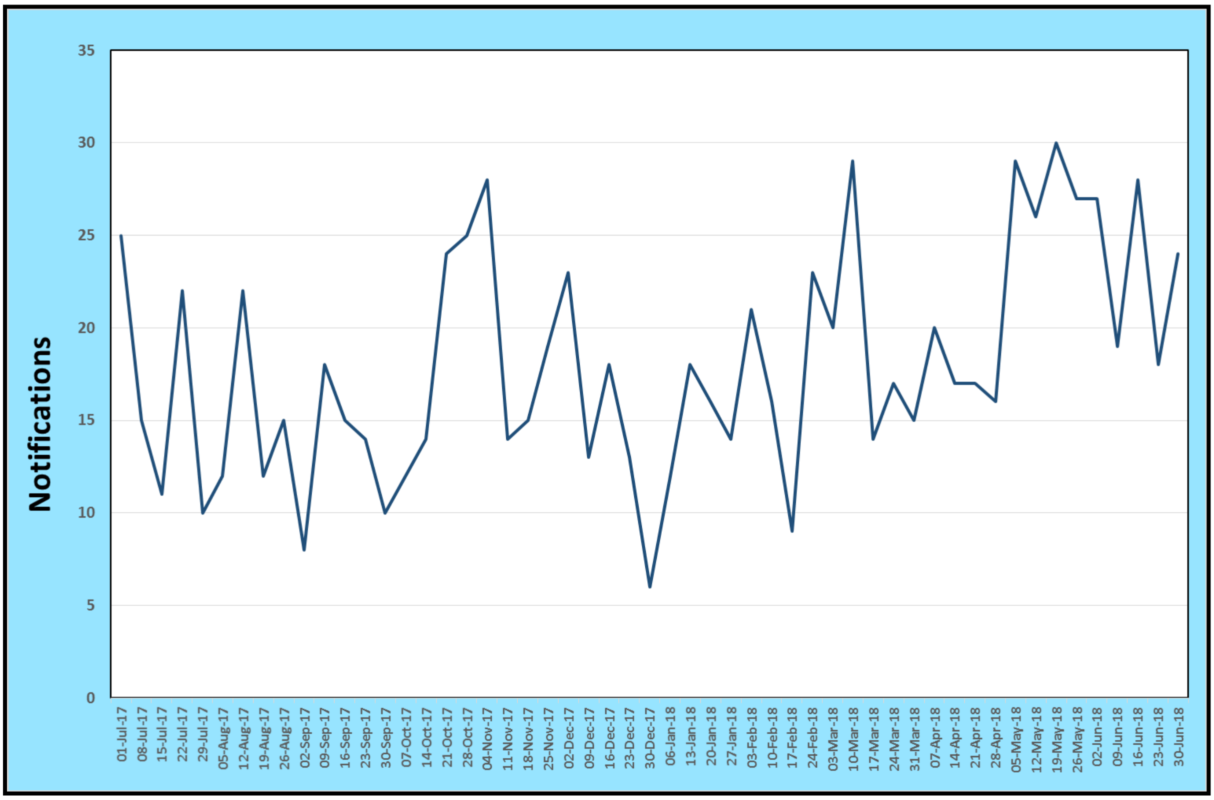


Figure 12. Weekly Ross River virus notifications in NSW residents, Jul 2017 to June 2018. (Source: NSW Heath Communicable Diseases Weekly Report, www.health.nsw.gov.au/Infectious/reports/Pages/CDWR.aspx).

Figure 12 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 12), almost half (45%) of the reports during the fiscal year of 2017-18 occurred over the months of July to December 2017, and approximately 40% 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 the 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).

With the ongoing dry conditions, the possibility of a La Nina forming, and that the predictive models are currently not indicative, the probability of a MVEV outbreak for 2018-2019, at present, seems remote. In spite of this, above average temperatures are forecast ahead, which may bring the onset of the mosquito season on earlier.

FTA CARDS VS CELL CULTURE

Arboviral detection methodologies from the trapped mosquitoes continue to be validated within the surveillance program. As noted in the methods above, the FTA cards produced few arboviral detections and, thus late in the season, an alternative method was implemented. This involved grinding all the mosquitoes from the same catch in the one vial, and testing the supernatant via PCR as per described methods.

There was a total of two arboviral detections via FTA cards out of 767 cards tested, while for the whole trap grinds (WTGs) there were 8 positives out of 45 traps tested. Perhaps this result should not be unexpected as typically an infected mosquito only expectorates 1-2 logs of virus, while there may be 3-7 logs of virus within the body. Thus WTGs offers a number of advantages: no FTA card preparation by field operatives; no FTA card elution; and no FTA cards. Thus WTGs are cheaper, quicker, and initially appears more sensitive. There are however questions about the upper limit of how many mosquitoes can be ground in one batch before inhibitors affect the PCR. Plus live mosquitoes are required and grinding whole mosquitoes does not demonstrate potential transmission as does a mosquito expectorating virus.

This method will undergo further validation next season.

EXOTIC MOSQUITO DETECTIONS AT SYDNEY INTERNATIONAL AIRPORT

Background. Over the last eight years 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.

In the 2015-2016 arbovirus report, it was noted that there were 11 separate detections of *Aedes aegypti* at Sydney International Airport, the first occurring on 14/Jan/2016, and another nine through the weeks until early March. A further detection occurred in September, while a live male *Aedes albopictus* was found in a flower consignment during Apr/2016. The *Aedes aegypti* at Sydney International Airport were mostly detected in the basement areas of the terminal, though the use of surveillance traps. The basement area is where passenger bagging is unloaded from the air cans (these are essentially crates for the passenger bags, which are then loaded into aircraft holds).

On 3 February 2017, DAWR during their routine surveillance for exotic mosquitoes detected a male *Aedes aegypti* in one of the BG GAT traps at a different part of the Sydney International Airport, the Qantas Freight Terminal (QFT). Subsequently a larval survey of the QFT was undertaken by staff of Medical Entomology in conjunction with DAWR and recommendations made (Doggett *et al*., 2017). Fortunately, no larval mosquitoes were detected during this survey and the site was deemed low risk for potential vector breeding.

There were further detections of *Aedes aegypti* from the basement areas of the Sydney International Airport in 2017. This included a female trapped on 24/May, a male collected on 29/May, and a male trapped on 14/June. In response to these a new larval survey undertaken on 20/June (Clancy *et al*., 2017). Again no larvae were found, although adult *Culex quinquefasciatus* were observed flying in one drain, suggesting that larval breeding was occurring nearby prompting the need for follow up actions. Subsequently there were other *Aedes aegypti* detections in the basement area; one female trapped on 12/July/2017, and another female *Aedes aegypti* trapped on 9/August/2017.

Further detections at the airport occurred during the recent season of 2017-2018 at Sydney International Airport. This included one adult female *Aedes aegypti* on 24/Oct/2017 and another adult female *Aedes aegypti* on 22/Nov/2017. Subsequently two female *Aedes aegypti* were trapped at a freight handling facility near the domestic airport on 7/Feb/2018, which was soon followed by detections of the same species on 10 & 11/Feb/2018 at the same locations. Another *Aedes aegypti* was collected in a BG trap on 21/Mar/2018 at the baggage handling area of Sydney International Airport. Two further *Aedes aegypti* were trapped in a similar area on 18/Apr/2018. The most recent exotic detection occurred on 2/May/2018, also at Sydney Airport and this was one female *Culex gelidus*. This particular species was introduced into Australia during the 1990's and is widespread in many tropical areas. It is possible this could have been the origin of the mosquito.

In response to the *Aedes aegypti* detections a number of actions have been implemented. The NSW Ministry of 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 airport, 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.

The salient piece of legislation pertaining to exotic mosquitoes is the Biosecurity Act 2015 (the Act), which came into effect on 1/July/2017. It replaces, wholly or in part, 14 separate pieces of biosecurity related legislation and is administered by the NSW Department of Primary Industries. The Act provides provisions to manage negative impacts of pests, diseases, weeds, and contaminants, which could cause a significant biosecurity impact on the economy, environment, or community of NSW.

Tools beneath the Act for the management of a response to an exotic mosquito incursion include:

- A high-risk category known as "prohibited matter" within Schedule 2 of the Act. This category acknowledges the severe consequences of some pests and diseases (note: Currently *Aedes albopictus* is included as "prohibited matter" within the Act, but not *Aedes aegypti*).
- Emergency powers that allow swift action to be taken to respond to significant biosecurity risks to the economy, environment and community;

- A general biosecurity duty that provides that people who deal with biosecurity matter or a carrier, and who have knowledge of the biosecurity risks posed are to take reasonable steps to manage those risks; and
- Numerous other management tools such as biosecurity zone control orders, registration, biosecurity certificates, biosecurity directions, and permits.

Furthermore, the Australian Government Department of Health released in August 2017, a '*Response Guide for Exotic Mosquito Detections at Australian First Points of Entry*'. This guide aims to provide a "nationally consistent approach to the management and control of exotic mosquitoes at first points of entry into Australia" and includes "the roles and responsibilities of stakeholders in order to strengthen responses to exotic mosquito detections at Australia's borders". The guide was developed in conjunction with the National Arbovirus and Malaria Advisory Committee and follows 'best practice' in terms of mosquito management.

Appendix 1. LOCATION-BY-LOCATION SUMMARY

http://medent.usyd.edu.au/arbovirus/results/results.htm

Inland Locations

Albury: mosquito numbers were mostly ‘low’ for the season, with the one ‘high’ collection in early January. 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: no mosquito collections were undertaken this season. There were no seroconversions to MVEV or KUNV in the sentinel chickens.

Griffith: the trapping at Lake Wyangan continued this season, replacing Barren Box. Mosquito collections were ‘low’ at the start of the season in early November and quickly rose to ‘high’ levels by mid-November. Collections were ‘very high’ over mid-December to mid-January, and thereafter remained ‘high’ until March. There were three arboviral isolates this season from Griffith. This included two RRV from *Culex annulirostris* trapped at Lake Wyangan (one each from mosquitoes trapped on 3/Jan and 31/Jan/2018), and one RRV from *Culex annulirostris* trapped at Hanwood on 5/Feb/2018. 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 trapping began in early November with ‘low’ catch yields early in the season. Collections became ‘high’ in number by mid-December and remained at this level until mid-February. Thereafter, mosquito numbers became ‘low’. There were no arboviral isolates from the mosquitoes nor any seroconversions in the sentinel chickens.

Macquarie Marshes: only two mosquito collections were made this season and numbers were ‘low’ to ‘medium’, with no arboviral detections. There were no seroconversions to MVEV or KUNV in the sentinel chickens.

Mathoura: only four mosquito collections were made this season and numbers were mainly ‘low’. There were no arboviral isolates from the mosquitoes. No sentinel chickens operated at this location for the season.

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: trapping continued at the two sites of North Creek Road and Pacific Pines, with the latter site trapping almost two thirds of the total number of mosquitoes yielded. Numbers were 'high' throughout the season with one 'very high' total in mid-March. Overall few *Aedes vigilax* were trapped (typically numbers were 'low'), although collections were predominated by saltwater mosquitoes, notably *Culex sitiens* and *Verrallina funerea*, neither of which are notable vectors. No arboviral isolates were detected.

Coffs Harbour: trapping was undertaken at Moller Drive and Christmas Bells Road. Collections tended to be 'low' to 'medium', with some 'high' yields late in the season. Overall numbers of *Aedes vigilax* remained 'low'. 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, and dominated by *Aedes vigilax*. There were two arboviral isolates detected; one BFV from mosquitoes trapped at Empire Bay on 27/Feb/2018 and one RRV from 26/Apr/2018.

Lake Macquarie: collections were undertaken from three sites: Belmont Lagoon, Teralba, and Dora Creek. Mosquito numbers were 'low' until mid-February with some 'high' collections in the ensuing weeks, with 'medium' yields of *Aedes vigilax*. No arboviral isolates were detected.

Port Macquarie: trapping was undertaken at three sites; Wall Reserve, Fernbank Creek Road, and Steven Street. *Aedes vigilax* collections remained 'low' throughout the season but overall mosquito numbers were 'high' from early January until late in the trapping program. *Culex annulirostris* was the most common species collected. No arboviral isolates were detected.

Tweed Heads: trapping was undertaken at three sites; Koala Beach, Beltana Drive, and Piggabeen Road. Beltana Drive yielded the largest collections trapping over 10,000 mosquitoes for the season. Overall numbers were 'high' for most of the trapping period, while *Aedes vigilax* collections peaked during the months of January and February. Large numbers of *Culex sitiens* were also trapped throughout the season. No arboviral isolates were detected.

Wyong: trapping was undertaken at three sites: Ourimbah, Halekulani, and North Avoca. Mosquito numbers were 'low' for the entire season, except for one week in mid-March. There was one detection of Barmah Forest virus from mosquitoes trapped at Halekulani on 14/Mar/2018.

Sydney Locations

Bankstown: collections this season were exclusively undertaken at Deepwater, a site known for intense local *Aedes vigilax* production, which again dominated the catches this year. Trapping was conducted over late January to mid-March, with 'high' numbers being yielded most weeks. There were three arboviral detections this season. These included; one STRV from mosquitoes trapped on 30/Jan/2018, one STRV isolated from *Aedes vigilax* trapped on 12/Feb/2018, and one BFV from 21/Mar/2018.

Blacktown: mosquito trapping was undertaken at Nurrangingy Reserve and Kings Langley. Numbers were mostly 'low' with the occasional 'medium' collection during summer. No arboviral isolates were detected.

Georges River: trapping was undertaken at Alfords Point and Illawong. As per usual, the mosquito collections were much greater at Alfords Point and 'high' for most weeks, dominated by *Aedes vigilax* (which comprised 97.4% of all mosquitoes trapped). There was one 'very high' collection in mid-February with almost 4,000 mosquitoes trapped. There were two arboviral detections this season, both from Alfords Point. These included one STRV from mosquitoes trapped on 7/Feb/2018 and one BFV from 21/Mar/2018.

Hawkesbury: trapping was undertaken at four main sites, including at Wheeney Creek, McGraths Hill, Yarramundi, and Richmond. Mosquito numbers tended to be 'low' through until March, when there were some 'high' collections. The biggest of these was from McGraths Hill in mid-March, with a collection strongly dominated by *Coquillettidia linealis*. No arboviral isolates were detected.

Hills Shire: mosquito trapping was undertaken at Rouse Hill, Glenorie, and Baulkham Hills. Numbers were 'low' for the entire season. No arboviral isolates were detected.

Penrith: trapping was undertaken at the sites of Emu Plains, Woodford, Glenmore Park, and Werrington. Mosquito numbers were 'low' from most of the season. 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. Every week yielded 'high' mosquito numbers or greater, with the biggest collection occurring mid-January with a 'very high' collection of over 3,000 mosquitoes trapped. As per usual, these large yields were associated with enhanced *Aedes vigilax* activity. No arboviral isolates were detected.

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.

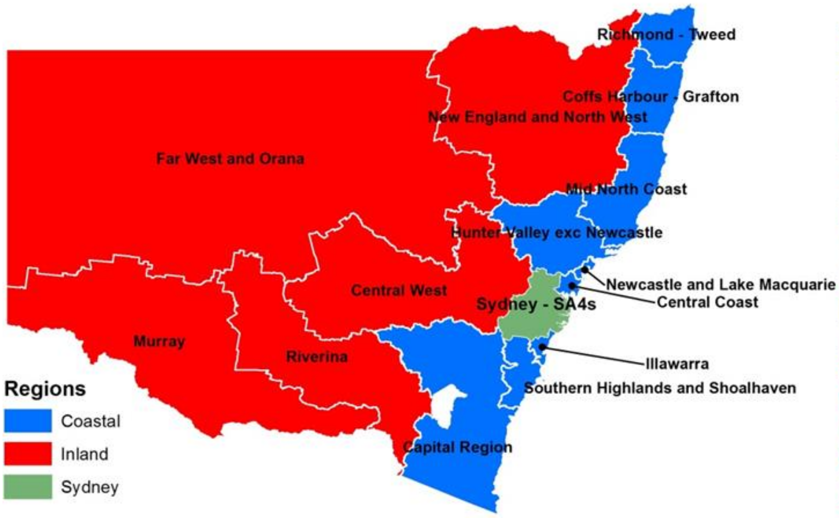
Stratford virus (STRV): there have been very few documented symptomatic patients, only three described to date and symptoms included fever, arthritis, and lethargy. The virus has mostly been isolated from coastal NSW, particularly from the saltmarsh mosquito, *Aedes vigilax*, although recent isolates 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

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