THE NEW SOUTH WALES ARBOVIRUS SURVEILLANCE & MOSQUITO MONITORING PROGRAM

2016-2017 Annual Report

Aedes vigilax

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NSW GOVERNMENT Health Pathology

EXECUTIVE OVERVIEW

- **For the 2016-2017 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. Most sites operated between mid-October and April.
- **The climatic conditions** leading up to the 2016-2017 season for the inland was one of extraordinary rainfall during the period July to September, 2016. This resulted in widespread flooding and resulted in massive mosquito problems which prompted an early start to the season. For the last quarter of 2016 and first three months of 2017, precipitation levels were normal, however the climate was considerably hotter than normal. Despite the intense rainfall activity, neither the Forbes nor the Nicholls hypotheses were suggestive of a potential MVEV epidemic for the season. For the coast, the last quarter of the year was exceptionally dry. Intense rainfall (with local flooding) did occur in the north coast in the wake of the low pressure cell formed from Tropical Cyclone Debbie.
- **For the inland**, 180,992 mosquitoes were trapped, around five times the previous seasons total and more than double that yielded in 2014-2015. There were 105 arboviral detections, including 6BFV, 47RRV, 46SINV, 2KOKV, 1KUNV, and 3TRUV. The majority were from Griffith. There were six KUNV seroconversions during mid-January in the sentinel chickens located at Macquarie Marshes.
- **Human notifications from the inland** of RRV and BFV totalled 925 (909RRV & 16BFV), which was over three times long term average. The RRV outbreak was the largest epidemic since the disease became notifiable. The statistical local areas that produced the highest notifications for RRV where in the south of the state and included Tocumwal-Finley-Jerilderie (n=51), Deniliquin region (n=38), and Albury (n=37). There were no human cases of flavivirus infection reported.
- **As of September 2017**, the Forbes hypothesis is not suggestive of a possible MVEV epidemic for 2017-2018, although the Nicholls hypothesis is not excluding such activity. The El Niño-Southern Oscillation remains neutral, which is indicative of average rainfall patterns ahead. However, conditions are expected to be warmer than average, which may bring the mosquito season forward.
- **For the coast**, almost 57,000 mosquitoes were trapped, which was around double the previous season. There was one SINV isolate from Port Macquarie.
- **Human notifications from the coast** totalled 539 cases, including 480 RRV and 59 BFV, and this was below average. Many of the RRV notifications occurred late in the season following the rainfall/flooding event associated with TC Debbie.
- **Sydney** experienced a moderate increase in mosquito numbers upon the previous season and there were 33 arboviral isolates from the Georges River including 23RRV, 5EHV and 5STRV.
- **The detections of *Aedes aegypti* at Sydney International Airport continue**. This included the first detection at the Qantas Freight Terminal (male mosquito) and four other detections at the passenger terminal from late May to mid-July. Responses included regular teleconferences initiated by the NSW Ministry of Health, enhanced surveillance at the airport, vector surveys of the freight and passenger terminals, insecticidal applications, and ongoing larval treatments.

TABLE OF CONTENTS

NSW ARBOVIRUS SURVEILLANCE AND MOSQUITO MONITORING PROGRAM 2016-2017

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 2016-2017 season follow.

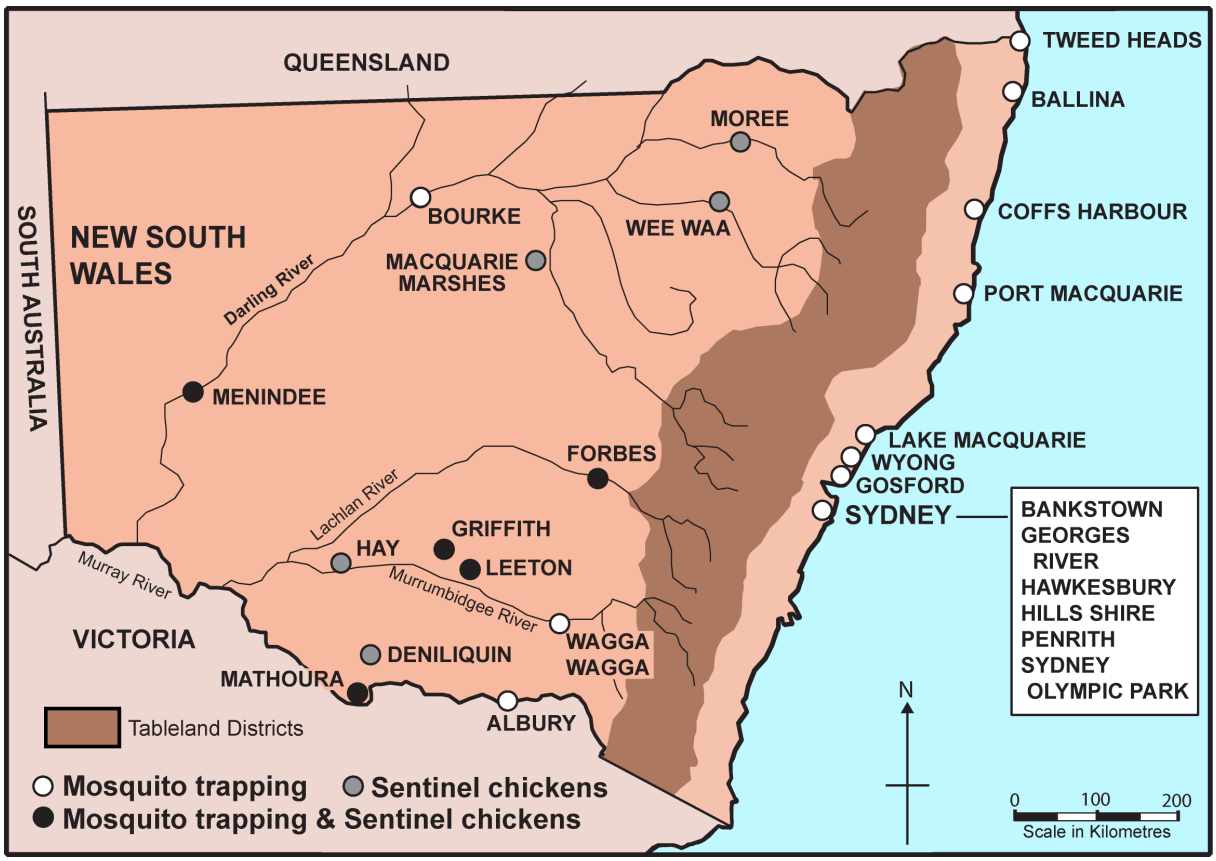


Fig 1. Mosquito trapping locations and Sentinel Chicken sites, 2016-2017.

MONITORING LOCATIONS

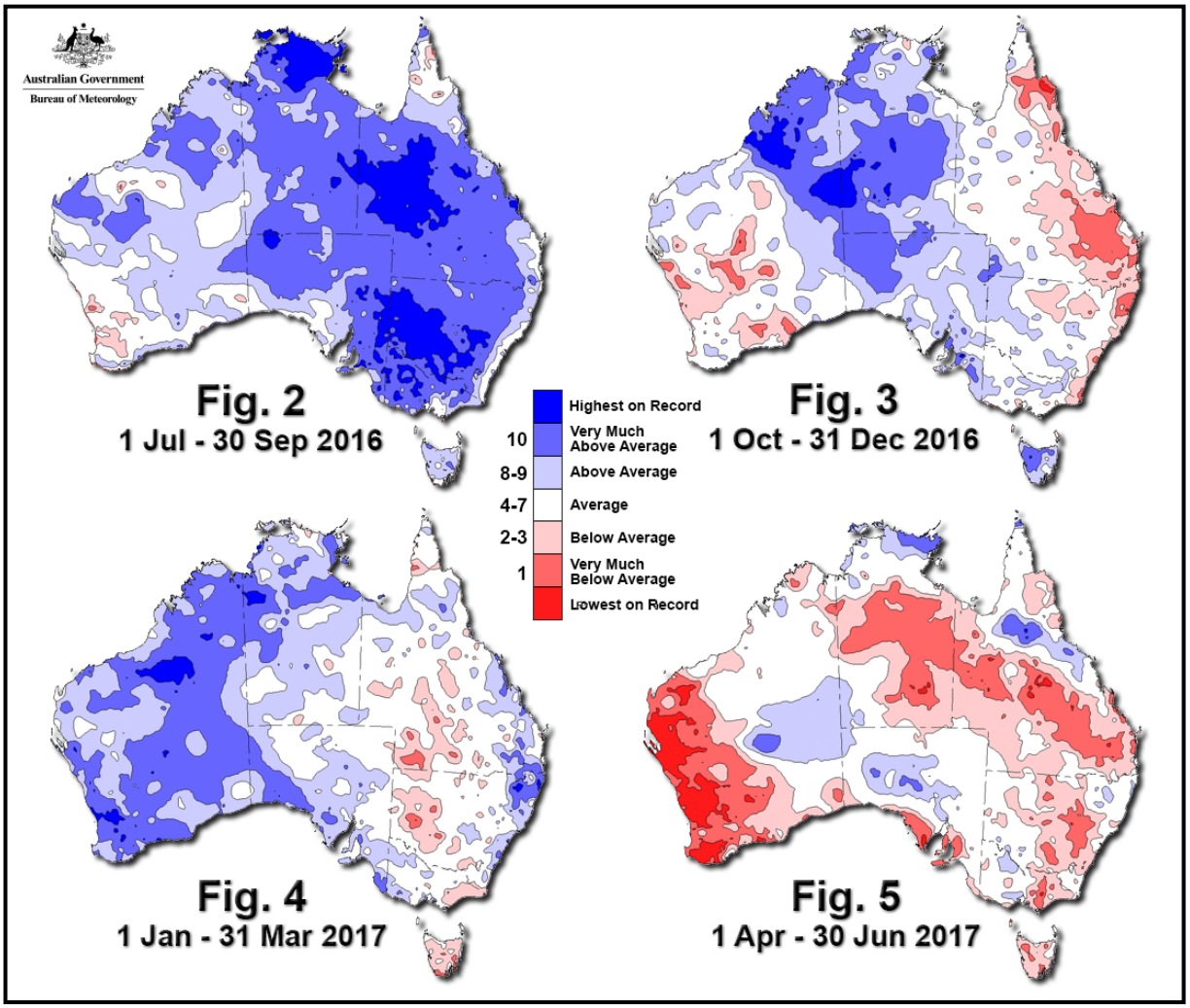
http://medent.usyd.edu.au/arbovirus/location/locations.htm

For 2016-2017, mosquito-trapping sites were operated at 5 inland, 7 coastal and 6 Sydney locations. Chicken sentinel flocks were located at 10 locations (Fig 1).

WEATHER DATA

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

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 2016, Oct-Dec 2016, Jan-Mar 2017 & Apr-Jun 2017. The stronger the red, the drier the conditions. Conversely, the stronger the blue, the wetter the conditions. *Modifed from the Australian Bureau of Meterology, 2017*.

The first quarter of 2016 (January to March, not shown) produced normal rainfall patterns for most of the state with dry conditions along the north coast. For the second quarter (not shown), rainfall was above to very much above average for the majority of the state. The inland experienced extremely precipitation across the state during the third quarter of 2016 (Figure 2), with some areas having record rainfall level. This did result in widespread flooding across many areas. The final quarter for 2016 (Figure 3), rainfall amounts returned to more normal levels for the inland, however conditions along the coast were extremely dry. Conversely, conditions were wetter than normal for the coast in the first quarter of 2017 (Figure 4), while the inland experienced mostly average rainfall. Conditions were much drier during the second quarter of 2017 (Figure 5), with most of the inland having below average rainfall.

For the north coast, intense rainfall occurred with flooding over 30-31/March/2017. This was in the wake of Tropical Cyclone Debbie, which developed into a low pressure cell that subsequently moved through southeast Queensland and into northern NSW, causing the flooding.

Maximum temperatures for the last half of 2016 were close to average for both inland and coastal regions. In contrast the first three months of 2017 were exceptionally hot with maximum temperatures up to 4 degrees above average for the inland, and 3 degrees above for the coast. By the second quarter of 2017, temperatures were again normal.

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 2015) or the first quarter of the current year (January-March 2016) and the last quarter of the current year (October-December 2016), 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 2015, was

above decile 7 in only one catchment basin in the first quarter of 2016, and above decile 7 in only one of the catchment basins for the last quarter of 2016, thus the Forbes hypothesis was not fulfilled for 2016-2017 (Table 1). Additionally, decile 7 or above rainfall did not occur across all the catchment basins during the first quarter of 2017, therefore according to Forbes', there should be a lower risk of an MVEV epidemic for the upcoming 2017-2018 season.

Table 1. Rainfall indices for the main catchment basins of eastern Australia as per Forbes hypothesis, relevant to the 2015-2016 and 2016-2017 seasons.

Catchment Basin	Oct-Dec 2015	Jan-Mar 2016	Oct-Dec 2016	Jan-Mar 2017
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 2016, the SO values were respectively: 1010.30mm, 1012.57mm and 1010.07mm (indicated on Figure 6 by the yellow arrows and Table 2). The graph on the right has been modified (i.e. updated) 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

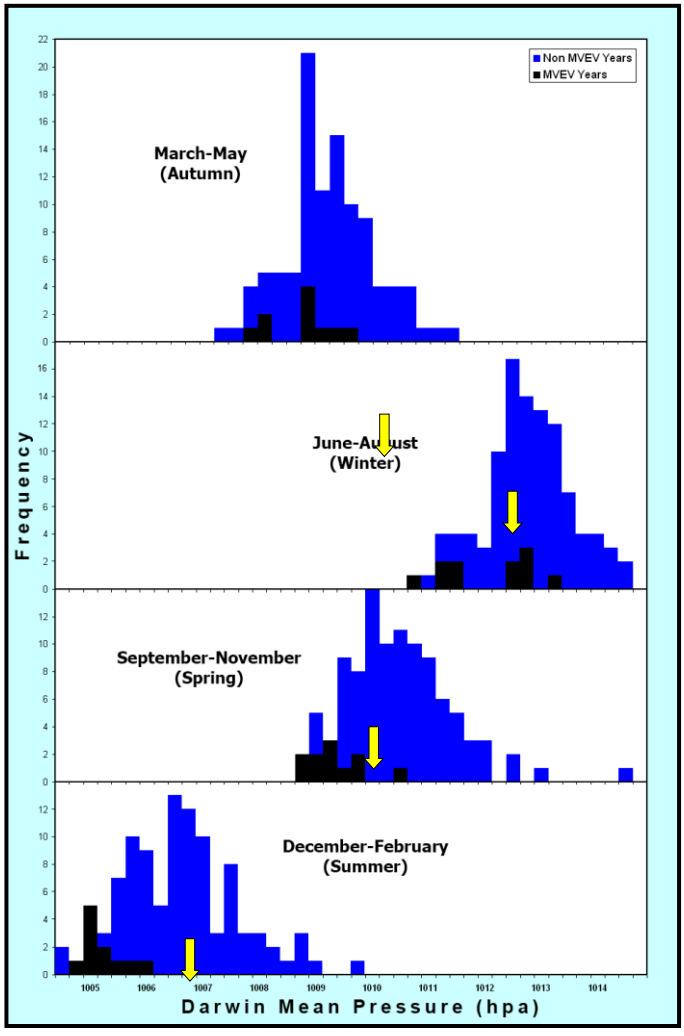


Figure 6. The SO by seasons prior to MVEV active years, according to Nicholls (1986), updated up to Spring 2016. The black bars represent the pre-MVEV active seasons. The yellow arrows indicate the respective SO values relevant to the 2016-2017 season.

have resulted from over-wintering mosquitoes.

As of September 2017, the autumn Nicholls' value is 1009.60mm and the winter value is 1013.23. Both values are within the range of values for past MVEV outbreak years, suggesting a possible increased risk for 2017-2018. The El Niño-Southern Oscillation remains neutral, indicating average rainfall patterns ahead. However, 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 2016-2017 season.

	Autumn 2016	Winter 2016*	Spring 2016*
2016 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 in a weekly report, which was disseminated to stakeholders.

Results

Overall, 299,239 mosquitoes representing 52 species were collected in NSW during 2016-2017, which was around three times the previous season (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 180,992 mosquitoes comprising 19 species was around five times that of the previous season collection of 37,615 and more than double that yielded in 2014-2015, when inland mosquito numbers totalled 88,111. *Culex annulirostris* was the dominant species yielded at most sites and comprised 48.4% of the total inland collections. *Anopheles annulipes* (30.3%) was the next most common species followed by *Culex australicus* (16.0%).

Coastal

In total, 56,935 mosquitoes comprising 43 species were collected from coastal NSW and this almost double the previous season's collection (30,101). The most common species collected were *Culex sitiens* (29.9%), *Aedes vigilax* (23.4%), *Aedes multiplex* (13.7%), *Aedes notoscriptus* (8.8%), *Verrallina funerea* (7.8%), *Aedes procax* (5.8%) and *Culex annulirostris* (3.7%).

Metropolitan Sydney

A total of 61,312 mosquitoes, comprising 31 species, was collected from metropolitan Sydney and this was around 50% higher the previous season's total collection (40,947). *Aedes vigilax* (64.6% of the total Sydney mosquitoes trapped) was the most common species, followed by *Culex annulirostris* (6.8%), *Aedes notoscriptus* (5.2%), and *Anopheles annulipes* (4.6%) and *Coquillettidia linealis* (3.9%).

ARBOVIRUS ISOLATIONS FROM MOSQUITOES

http://medent.usyd.edu.au/arbovirus/about/methods.htm

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).

Alignment of an 894 bp region of the RdRp gene on the L-segment of the three PCR positive TRUV 2017 isolates showed a 97.7% identity with the Trubanaman virus (TRUV) strain MRM3630, KP792684.1. Bayesian analysis of the isolates indicated a posterior probability of 1 confirming that the isolates were TRUV.

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.

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

Results

From the mosquitoes processed, there were 139 arboviral detections; 34 from the coast (Table 3) and 105 from the inland (Table 4). Some 25 RRV isolates from FTA cards and cell culture were Sanger sequenced by the Australian Genome Research Facility (AGRF). A Pairwise comparison of the E2 region (680 bp) showed 99.2% identity with 94.5% identical sites shown between isolates from different locations and the Genbank reference strains. The most number of differences (N=19-25) were evident between isolates and the RRV T48 GQ433359.1, prototype of 1959.

Table 3. Arboviral isolates from Coastal NSW, 2016-2017.

LOCATION	Date Trapped	Mosquito Species	Virus				
			RRV	SINV	EHV	STRV	Total
GEORGES RIVER	20-Apr-17	*Aedes notoscriptus*			1		**1**
GEORGES RIVER	20-Apr-17	*Aedes procax*			1		**1**
PORT MACQUARIE	10-Apr-17	*		1			**1**
GEORGES RIVER	26-Mar-17	*	1				**1**
GEORGES RIVER	26-Mar-17	*Aedes vigilax*	2				**2**
GEORGES RIVER	26-Mar-17	*Coquillettidia linealis*	4				**4**
GEORGES RIVER	26-Mar-17	*			1		**1**
GEORGES RIVER	19-Mar-17	*Aedes vigilax*	1			1	**2**
GEORGES RIVER	19-Mar-17	*	1				**1**
GEORGES RIVER	13-Mar-17	*Aedes vigilax*	7			4	**11**
GEORGES RIVER	13-Mar-17	*	1				**1**
GEORGES RIVER	07-Mar-17	*			1		**1**
GEORGES RIVER	02-Mar-17	*Aedes vigilax*	2		1		**3**
GEORGES RIVER	02-Mar-17	*	1				**1**
GEORGES RIVER	29-Dec-16	*Aedes alboannulatus*	1				**1**
GEORGES RIVER	29-Dec-16	*	1				**1**
GEORGES RIVER	08-Dec-16	*	1				**1**
		TOTAL	**23**	**1**	**5**	**5**	**34**

*Detection via Honey-Baited Cards, the mosquito species cannot be determined. RRV = Ross River virus, SINV = Sindbis virus, EHV = Edge Hill virus, KOKV = Kokobera virus, STRV = Stratford virus.

Table 4. Arboviral isolates from Inland NSW, 2016-2017.

LOCATION	Date Trapped	Mosquito Species	Virus						
			BFV	RRV	SINV	KOKV	KUNV	TRUV	Total
GRIFFITH	06-Feb-17	*Culex annulirostris*			1				**1**
GRIFFITH	06-Feb-17	*				1			**1**
GRIFFITH	31-Jan-17	*Culex annulirostris*					1		**1**
GRIFFITH	31-Jan-17	*Culex annulirostris*			3				**3**
GRIFFITH	31-Jan-17	*Anopheles annulipes*			1				**1**
ALBURY	23-Jan-17	*				1			**1**
GRIFFITH	22-Jan-17	*Culex annulirostris*			2				**2**
GRIFFITH	22-Jan-17	*			2				**2**
LEETON	17-Jan-17	*Culex annulirostris*			2				**2**
LEETON	17-Jan-17	*			1				**1**
ALBURY	16-Jan-17	*Culex annulirostris*		1					**1**
ALBURY	16-Jan-17	*		1					**1**
GRIFFITH	16-Jan-17	*	1						**1**
GRIFFITH	16-Jan-17	*Culex annulirostris*	1						**1**
GRIFFITH	10-Jan-17	*Culex annulirostris*		1					**1**
GRIFFITH	10-Jan-17	*Culex annulirostris*			7				**7**
LEETON	09-Jan-17	*Culex annulirostris*		1					**1**
LEETON	09-Jan-17	*		1					**1**

GRIFFITH	03-Jan-17	*Culex annulirostris*			1				**1**
ALBURY	19-Dec-16	*		1					**1**
ALBURY	19-Dec-16	*Culex annulirostris*		1					**1**
GRIFFITH	19-Dec-16	*Culex annulirostris*			9				**9**
GRIFFITH	19-Dec-16	*			1				**1**
LEETON	13-Dec-16	*Culex annulirostris*		2	2				**4**
GRIFFITH	12-Dec-16	*Culex annulirostris*		2					**2**
GRIFFITH	12-Dec-16	*		1					**1**
GRIFFITH	12-Dec-16	*Anopheles annulipes*			1				**1**
GRIFFITH	12-Dec-16	*Culex annulirostris*			6				**6**
LEETON	07-Dec-16	*		1					**1**
LEETON	07-Dec-16	*Culex annulirostris*			1				**1**
MURRAY	06-Dec-16	*		1					**1**
ALBURY	05-Dec-16	*		1					**1**
ALBURY	05-Dec-16	*Culex annulirostris*		1					**1**
ALBURY	05-Dec-16	*Aedes bancroftianus*		1					**1**
FORBES	05-Dec-16	*		1					**1**
FORBES	05-Dec-16	*Culex annulirostris*		3					**3**
FORBES	05-Dec-16	*Culex australicus*		1					**1**
FORBES	05-Dec-16	*Anopheles annulipes*						1	**1**
GRIFFITH	05-Dec-16	*Culex annulirostris*		2	1				**3**
GRIFFITH	31-Nov-16	*Culex annulirostris*		1	3				**4**
GRIFFITH	31-Nov-16	*Anopheles annulipes*		2				2	**4**
GRIFFITH	31-Nov-16	*		1					**1**
FORBES	29-Nov-16	*Culex annulirostris*		2					**2**
FORBES	29-Nov-16	*Culex australicus*		1					**1**
LEETON	29-Nov-16	*Culex annulirostris*		1					**1**
GRIFFITH	21-Nov-16	*Culex annulirostris*		5	1				**6**
GRIFFITH	21-Nov-16	*Anopheles annulipes*		1					**1**
GRIFFITH	21-Nov-16	*		1					**1**
LEETON	16-Nov-16	*Culex annulirostris*		1					**1**
LEETON	16-Nov-16	*Anopheles annulipes*		1					**1**
LEETON	16-Nov-16	*		1					**1**
FORBES	15-Nov-16	*Culex annulirostris*	1	1					**2**
FORBES	15-Nov-16	*	1						**1**
GRIFFITH	14-Nov-16	*Aedes sagax*	1						**1**
GRIFFITH	14-Nov-16	*	1						**1**
MURRAY	08-Nov-16	*		1					**1**
MURRAY	08-Nov-16	*Aedes sagax*		1					**1**
FORBES	07-Nov-16	*Aedes sagax*			1				**1**
GRIFFITH	01-Nov-16	*Aedes theobaldi*		1					**1**
GRIFFITH	01-Nov-16	*Anopheles annulipes*		1					**1**
		Total	**6**	**47**	**46**	**2**	**1**	**3**	**105**

*Detection via Honey-Baited Cards, the mosquito species cannot be determined. BFV = Barmah Forest virus, RRV = Ross River virus, SINV = Sindbis virus, KOKV = Kokobera virus, KUNV = Kunjin virus, TRUV = Trubamanan virus.

SENTINEL CHICKEN PROGRAM

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

Location of flocks

The 2016-2017 season began on 20th October 2016 with the first bleed and ended on 4th April 2017 with the last. A total of ten flocks each containing up to 15 Isa Brown pullets was deployed, with one flock each at Deniliquin, Forbes, Griffith, Hay, Leeton, Macquarie Marshes, Menindee, Moama, Moree, and Wee Waa (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 2,580 samples was received from the ten flocks in NSW over the six-month period in 2016-2017. This represented 5,160 ELISA tests (excluding controls and quality assurance samples), with each specimen being tested for MVEV and KUNV antibodies. There were six seroconversions to KUNV in the sentinel chickens at Macquarie Marsh, with one positive from the bleed taken on 15/Jan/2017 and five positives from the bleed taken on 26/Jan/2017.

NOTIFICATIONS OF LOCALLY-ACQUIRED ARBOVIRUS INFECTIONS

All arboviral infections 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.

The two most common locally-acquired arbovirus infections notified in NSW are infections with Ross River virus (RRV) and Barmah Forest virus (BFV). It is important to note that current surveillance case definitions are more specific for both diseases since national surveillance case definitions were first introduced in 2004. There have also been changes in commercial testing products over time. Please refer to the 2015-2016 annual report for further information on these changes.

In the 2016-2017 financial year there were 1,522 notifications of RRV infection and 80 notifications of BFV infection in NSW residents. These represent increases on the 2015-2016 financial year for both RRV (679 notifications) and BFV (59 notifications). There were no notifications of other arbovirus infections acquired in NSW during 2016-2017.

Monthly BFV notifications were highest at the end of the season, with 21 notifications in May 2017 and in June 2017 (Figure 7).

There was a marked early increase in RRV notifications from December 2016, well above the five-year average for this period; notifications peaked in January 2017 (n=422) before a slow decline through February and March (Figure 7).

Figure 7. Barmah Forest virus and Ross River virus infections in NSW residents: notifications by month and year of onset for 2016-2017 financial year, compared to the five year monthly means for the period July 2011 to June 2016.

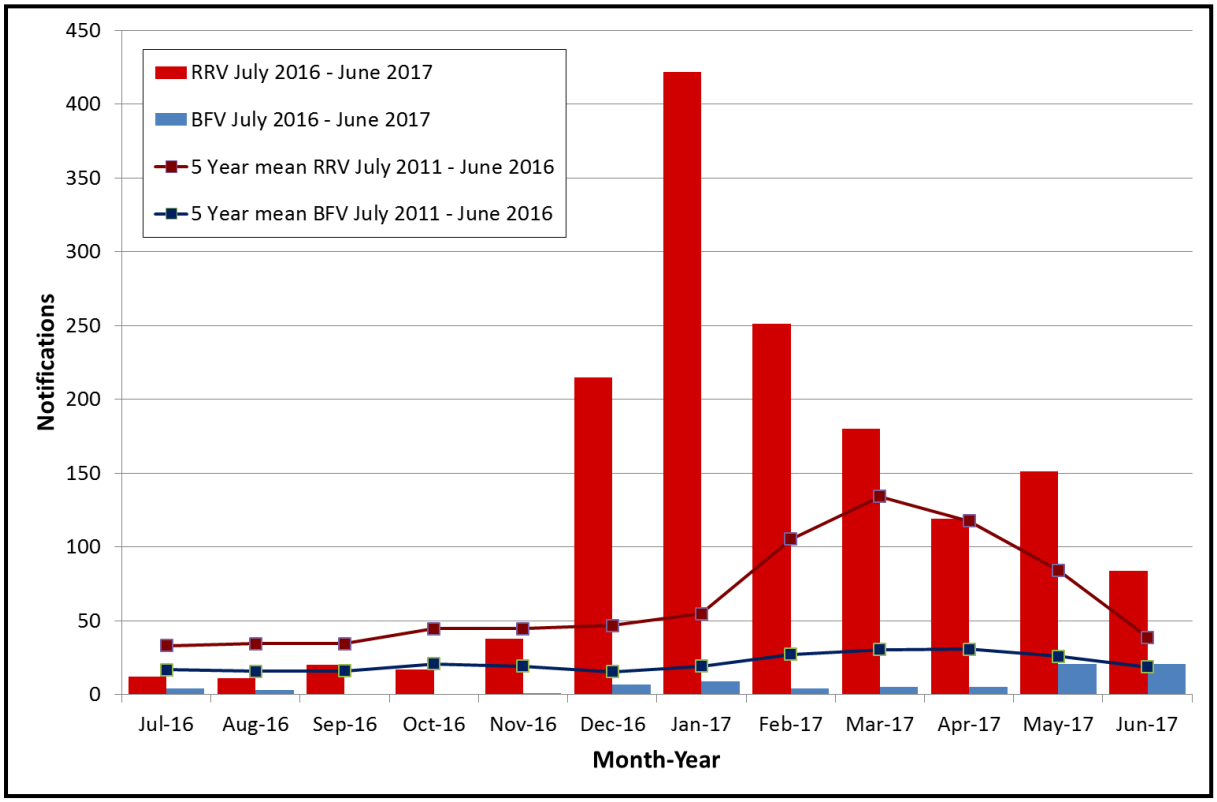


Table 5. Barmah Forest virus and Ross River virus infections in NSW residents: notifications and population notification rates* by local health district for the 2016-2017 financial year.

Local Health District	Barmah Forest virus		Ross River virus	
	Notifications	Population Rate*	Notifications	Population Rate*
Central Coast	2	0.58	44	12.84
Far West	0	0	44	143.42
Hunter New England	6	0.65	330	35.67
Illawarra Shoalhaven	3	0.74	40	9.84
Mid North Coast	17	7.71	41	18.59
Murrumbidgee	10	3.4	523	177.7
Nepean Blue Mountains	0	0	11	2.89
Northern NSW	30	9.86	113	37.13
Northern Sydney	2	0.22	31	3.4
South Eastern Sydney	2	0.22	25	2.72
South Western Sydney	0	0	17	1.74
Southern NSW	1	0.47	33	15.58
Sydney	1	0.15	10	1.54
Western NSW	6	2.15	247	88.46
Western Sydney	0	0	13	1.36
Total	**80**	**1.0**	**1,522**	**19.7**

*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.

The peak of RRV notifications in January 2017 was similar in magnitude to the peak in notifications seen in March 2015. However, the two outbreaks differed markedly in geographic distribution. The 2016-2017 RRV outbreak predominantly affected southern and inland regions of NSW (as described below), in contrast to the 2014-2015 RRV outbreak when NSW coastal regions were most affected, particularly along the north coast.

Arboviral 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). Population rates are based on ABS estimated resident population data (using the 2016-2017 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.

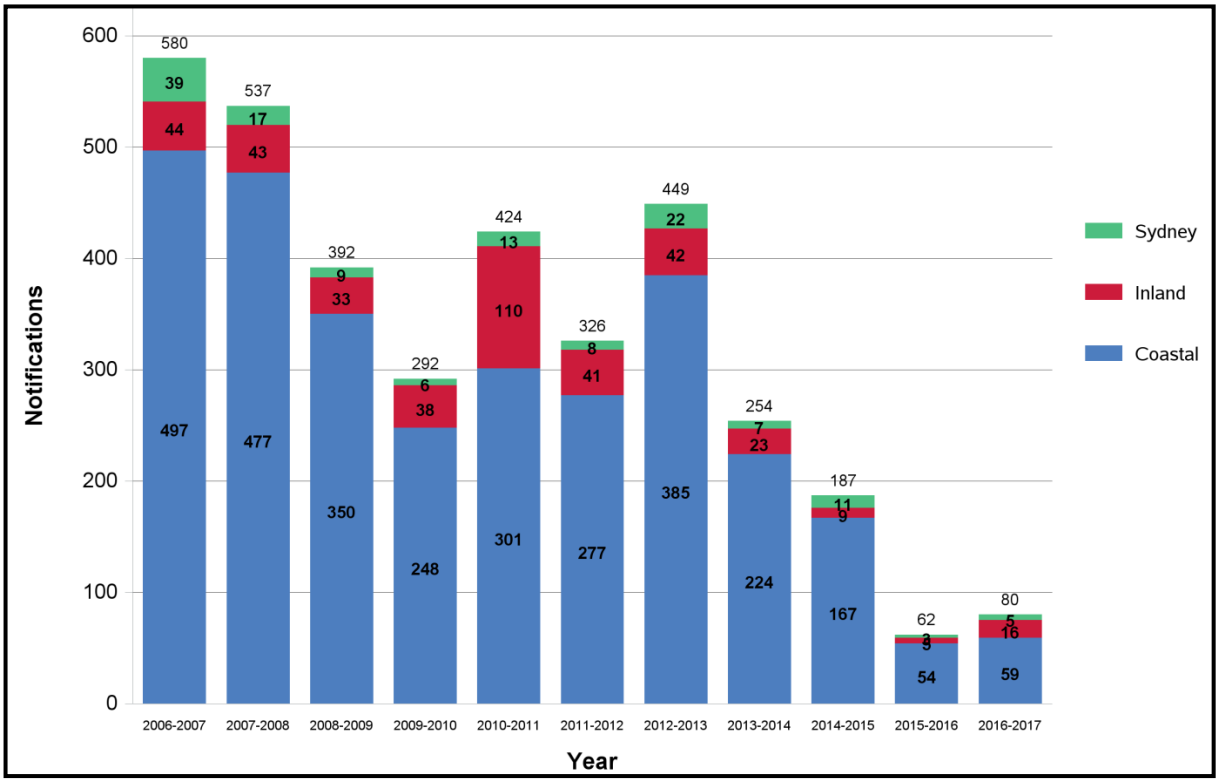
Notifications of BFV and RRV infection by LHD are shown in Table 5. The highest number of notifications and highest population notification rates for BFV infection were in the Northern NSW, Mid North Coast and Murrumbidgee LHDs, with few

notifications in other LHDs.

RRV notifications were highest in the Murrumbidgee, Hunter New England and Western NSW LHDs, while RRV population notification rates were highest in the Murrumbidgee, Far Western and Western 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 2006-2007 to 2016-2017. The Coastal region again accounted for the majority of BFV notifications (n=59, 73.8%) followed by the Inland region (n=16, 20.0%) with only 5 notifications reported in residents of Sydney region (Figure 8). The continuing decline in BFV notifications relates to the withdrawal of the commercial serological kit from the market.

Figure 8: Barmah Forest virus infections in NSW residents: annual notifications by year of disease onset and geographical region for the past 10 years (2007-2008 to 2016-2017).



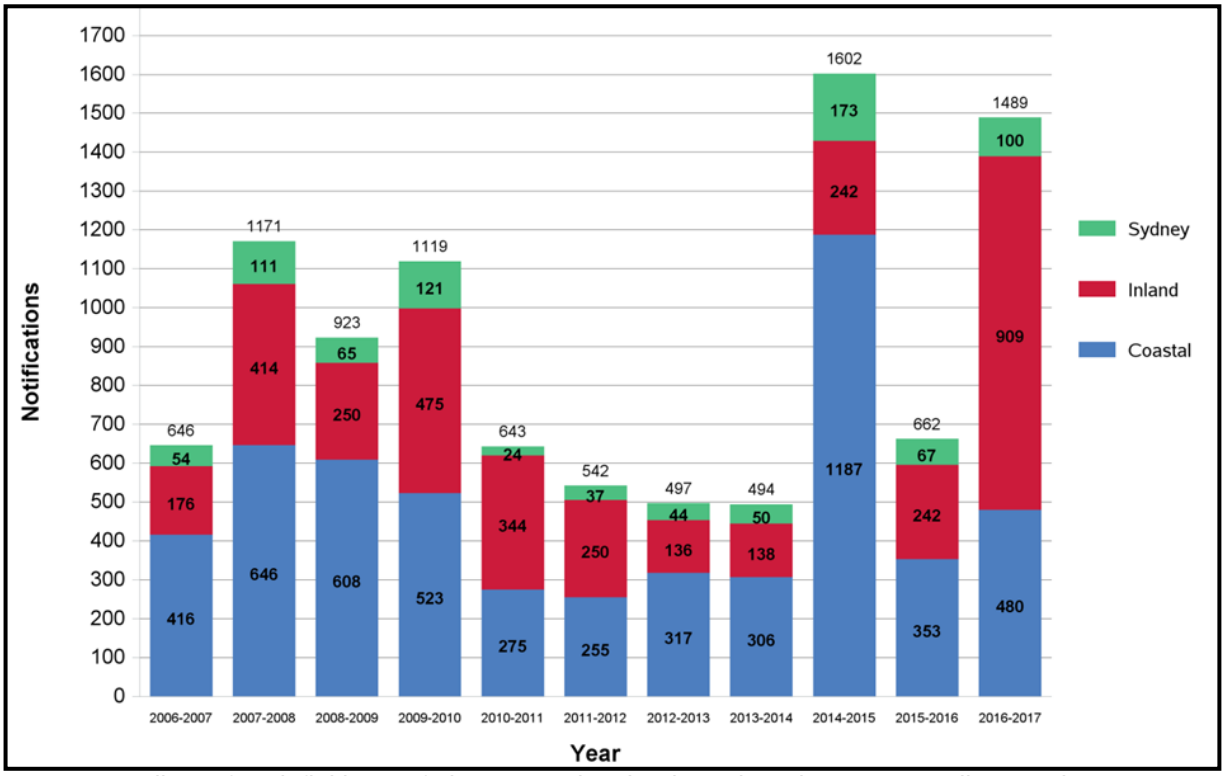
See Appendix 5 for definitions of the Coastal, Inland, and Sydney metropolitan regions. Due to incomplete address information, a handful of cases (approximately one per year) could not be allocated to a region.

Notification maps of BFV and RRV infection by ABS statistical area level 2 (SA2) of residence for the 2016-2017 financial year are shown in Figures 9 and 10, together with maps of population notification rates.

The SA2 areas with the highest total number of BFV notifications were Brunswick Heads-Ocean Shores (n=5) and Maclean-Yamba-Iluka (n=4); no other area had more than 3 notifications (Figure 10(a)). The three SA2 areas with the highest notification rates per 100,000 population were Brunswick Heads-Ocean Shores

(58.1), Deniliquin (40.4), and Evans Head (38.1) (Figure 10(b)).

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 2007-2008 to 2016-2017).



See Appendix X 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.

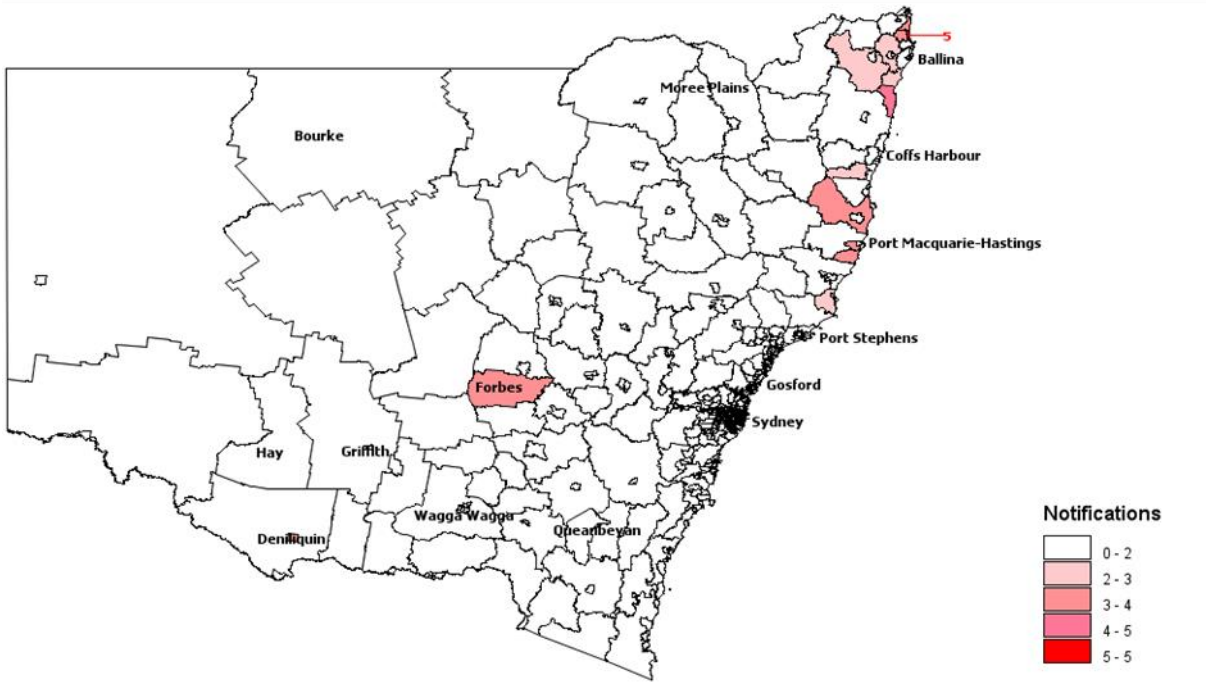
The three SA2 areas with the highest total number of RRV notifications were all in inland parts of the south of the state: Tocumwal-Finley-Jerilderie (n=51), Deniliquin region (n=38), and Albury region (n=37), and neighbouring areas in the south of the state (Figure 11(a)). The three SA2 areas with the highest notification rates per 100,000 population were Deniliquin region (550.6), Tocumwal-Finley–Jerilderie (526.8) and Wentworth-Balranald Region (424.2) (Figure 11(b)).

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

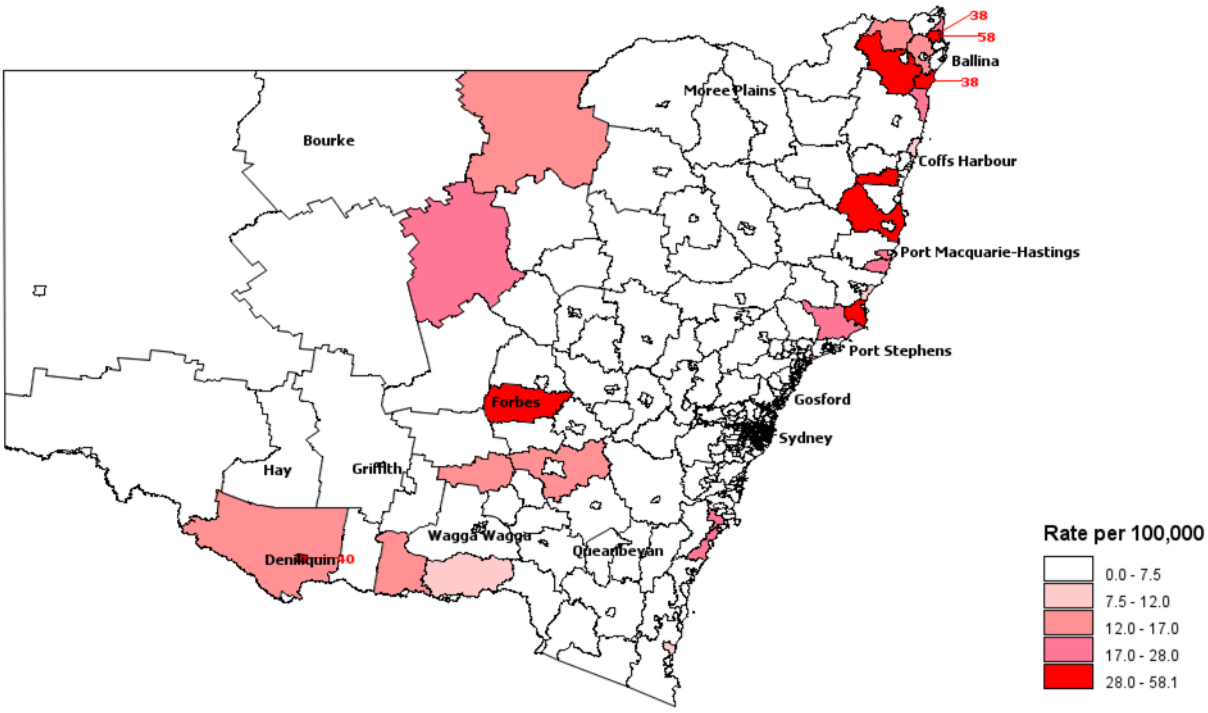
- NSW Health Vector-borne diseases reports
- NSW Health Notifiable diseases data (and select the relevant disease).

Figure 10: Barmah Forest virus infections in NSW residents.

(a) Notifications by statistical area level 2 (SA2), for 2016-2017.



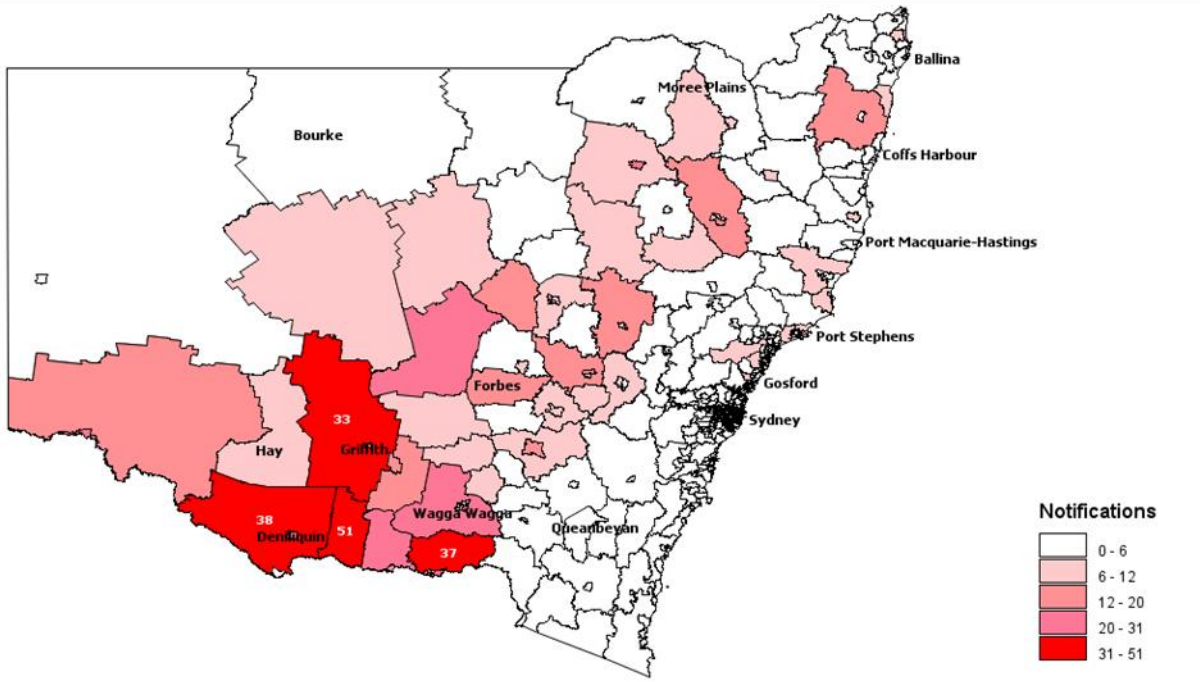
(b) Population notification rates* by statistical area level 2 (SA2), for 2016-2017.



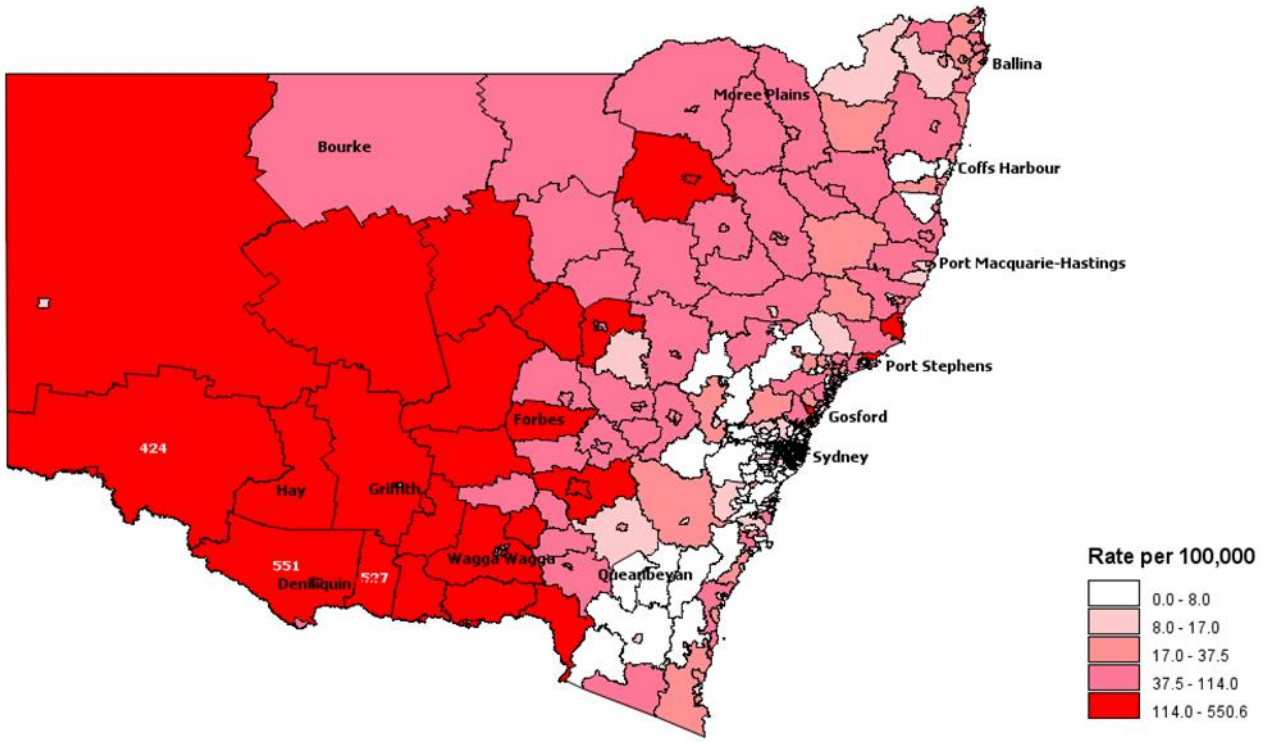
*Notifications per 100,000 estimated resident population for 2015, based on ABS census data.

Figure 11: Ross River virus infections in NSW residents.

(a) Notifications by statistical area level 2 (SA2), for 2016-2017.



(b) Population notification rates* by statistical area level 2 (SA2), for 2016-2017.



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

DISCUSSION

The Inland. For the 2016-2017 season, surveillance activities for the inland region was initiated earlier than normal due to the intense rainfall that resulted in widespread flooding, which in turn lead to an unprecedented rise in mosquito numbers. For example, during the last week of October, one trap from Griffith yielded over 10,000 mosquitoes when numbers at that time of the year are typically in the order of 100/trap. The mosquito collections from the first three weeks of the program totalled more than the entire 2015-2016 season! These extreme mosquito numbers from the inland continued well into December and thereafter reduced to be around average for the remainder of the season, as environmental conditions became drier and hotter.

For the inland, there were a total of 105 arboviral isolates including 6BFV, 47RRV, 46SINV, 2KOKV, 1KUNV and 3TRUV. For an average season, the number of isolates is around 30. November and December experienced a record number of isolates, which included 45RRV. It is worth noting for the history of the entire program (which has operated since 1985), a collective total of only 20RRV isolates have been made during the months of November and December (although it should be noted that since 2014, two forms of virus detection from mosquitoes; isolation through cell culture and sugar based surveillance using FTA cards). On 16/Jan/2017, the last RRV isolate was made, with the peak (with 12 RRV isolates) from the week beginning 4/Dec/2016. There were six seroconversions to KUNV, which occurred in mid-January, in the sentinel chicken flocks at Macquarie Marshes (no mosquito trapping was conducted at this site). The one KUNV isolated from mosquitoes occurred in the last week of January from Griffith.

With the rise in mosquito numbers and the high amount of arboviral activity, there was a major RRV outbreak in the region; the 909RRV notifications was over three times long term average. In fact this was the largest outbreak since the disease became nationally notifiable in 1985. Most of the activity occurred around the Riverina and Murray regions, with the statistical local areas that produced the highest cases being Tocumwal-Finley-Jerilderie (n=51), Deniliquin region (n=38), and Albury (n=37).

The genetic sequencing of the RRV isolates demonstrated that this season's viruses closely align with that of past isolates. Thus the outbreak related to environmental factors rather than changes to the viral genome.

It is worth noting that the peak in notifications occurred during the week ending 21/Jan/2017, some six weeks after the peak in the number of isolates, and a week after the last detection from the mosquitoes. The overall RRV notifications for the season were well above average (Figure 12). Despite the KUNV detections, there were no human cases reported, nor where there any reports of local flavivirus infection.

In response to the high mosquito numbers, record number of arboviral isolates and increased human notifications, NSW Health developed and distributed additional resources aimed at mitigating the risk of human arbovirus infections, particularly for

flood-affected communities. These resources can also be found at http://www.health.nsw.gov.au/environment/pests/vector/Pages/resources.aspx. State wide alerts warning of the RRV outbreak began in November. NSW Health Pathology staff (from Medical Entomology) travelled to flooded regions and provided advice on mosquito borne disease, disease avoidance and mosquito management directly to affected communities, local councils and event organisers.

Currently the two main climatic models for predicting an MVEV epidemic for 2017-2018 are at odds. Forbes is indicating that an outbreak is unlikely, while the Nicholl's theory is not excluding the possibility. However, as has been observed in recent years, MVEV can occur during seasons when the models have not been suggestive of an outbreak. Currently the El Niño-Southern Oscillation (ENSO) remains neutral and the Bureau of Meteorology is suggesting that the ENSO is unlikely to deviate from this neutrality for the remainder of the year. This would suggest that rainfall patterns will be average, however temperatures to be warmer than average, which is likely to bring the mosquito season forward.

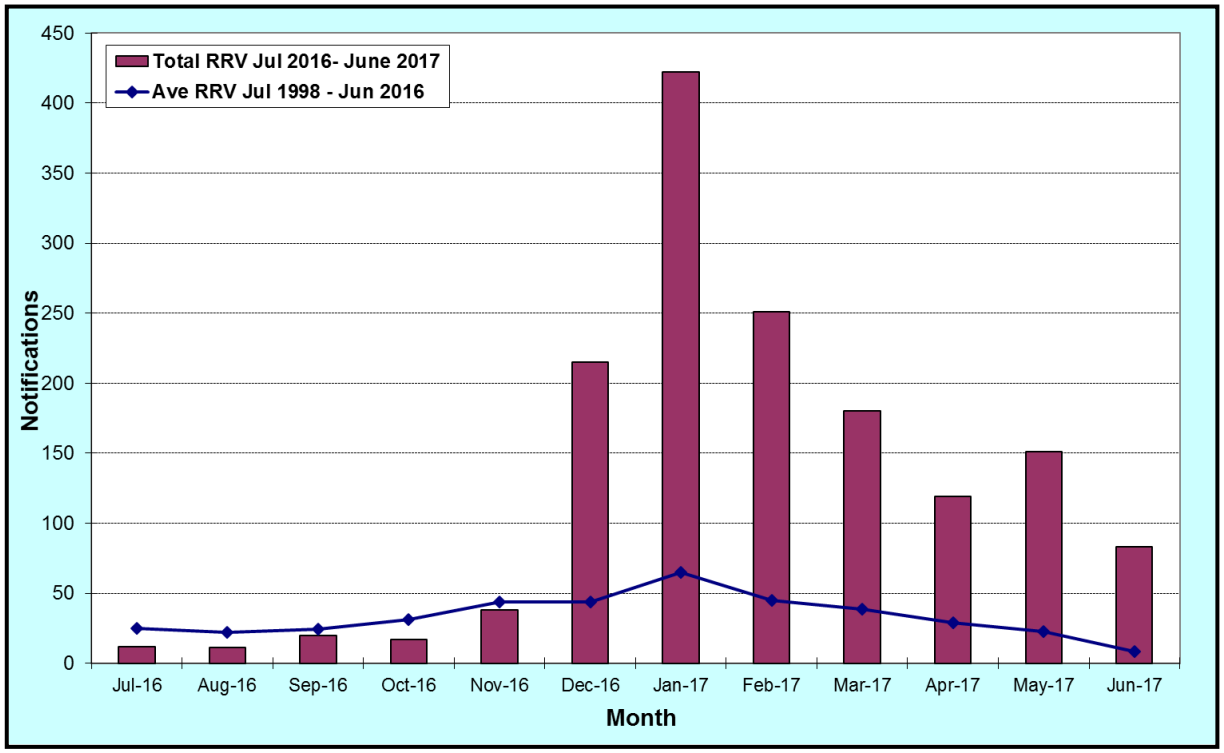


Figure 12. Notifications of RRV for NSW by month for the fiscal year 2016-2017, compared with the long term average over 1998-2016. Data from the NSW Ministry of Health, Communicable Diseases Weekly Report: http://www0.health.nsw.gov.au/data/diseases/rossriver.asp

The Coast. For the coast, the dry last quarter of 2016 meant that mosquito numbers were initially well below average. However, this did change following the intense rainfall associated with the low pressure cell that formed from TC Debbie and mosquito numbers dramatically rose and remained high in the far north coastal sites well into late April. As a result, mosquito numbers were almost twice that of the previous season.

For the coast, RRV and BFV notifications totalled 539 cases, including 480 RRV and 59 BFV, which was slightly below average. However the late season rains brought about an increase in notifications, which is represented by the rising numbers after April in Figure 2. The decline in the BFV notifications is artificial and relates to false positives with the commercial BFV serological testing kit, which led to its withdrawal from the market.

Sydney. Like elsewhere along the coast, Sydney experienced below average precipitation towards the end of 2016. However, March was very wet along the entire coast, which resulted in a late peak in mosquito numbers. As a consequence, total mosquito collections were considerably higher than the previous season. There were 100 RRV and 5BFV notifications within the Sydney region, being slightly above average, although how many were locally acquired is not known.

The trapping sites at Georges River produced 33 arboviral detections including 23RRV, 5EHV and 5 STRV. This location typically produces the greatest number of arboviral isolates for any Sydney trapping locality, due to its closeness to mosquito habitat along the river, and abundant wildlife that act as virus reservoirs. Interestingly, there were only two confirmed RRV cases that were believed to be locally acquired (Prof. Mark Ferson, SESLHD, *pers. comm*.). Following the arboviral isolations, the local public health unit undertook considerable local public prevention messaging with the local press and social media, and this may have contributed to the low cases numbers.

FTA CARDS VS CELL CULTURE

Arboviral detection methodologies from the trapped mosquitoes continue to be validated within the surveillance program. Table 6 details the number of isolates detected via honey-baited FTA cards and cell culture. For this season, cell culture produced more than three times the number of arboviral detections compared with FTA cards. However, several traps produced multiple isolates via cell culture and thus the table also includes the number of traps that produced a positive result.

Table 7 is a more direct comparison of the two technologies for arboviral detection. Thus cell culture detected 55 (85%) of the 65 viruses, FTA cards detected 30 (46%) of the viruses. Both technologies detected the same virus on only 20 (31%) occasions.

Table 8 depicts the average time taken to identify arboviral isolates via the two technologies, from the point of the specimen being received into the laboratory. As can be observed, the FTA cards are much quicker and being able to detect viruses compared with cell culture. Conversely however, cell culture was the more sensitive method this year at detecting arboviruses (especially the Flaviviruses). In light of the results depicted in the three tables, the current protocol of using both technologies for inland mosquitoes and the FTA cards only for coastal mosquitoes, will continue.

Table 6. Arboviral detections via FTA cards and Cell Culture, 2016-2017.

Detection Method	Virus‡							
	RRV	BFV	SINV	EHV	KOKV	KUNV	STRV	TOTAL
FTA Cards[1]	18	3	5†	2	2	0	0	**30**
Cell Culture[2] Trap +ve	29	3	18	2	0	1	2	**55**
Cell Culture Total Isolates	52	3	42	3	0	1	5	**106**

[1]1-4 cards added, mosquito density dependent.
[2]The number of traps that yielded an arboviral isolate via cell culture.
†Published SINV primers failed to detect the virus, once the primer was redesigned, detections were successful from mid-December.
‡TRUV was tested retrospectively on untyped cell culture isolates and hence the data is not included in this table.

Table 7. Comparison of the arboviral detection technologies.

Detection Method	Virus‡							
	RRV	BFV	SINV	EHV	KOKV	KUNV	STRV	TOTAL
Both	14	3	3	0	0	0	0	**20**
Cell Culture[1] Only	15	0	15	2	0	1	2	**35**
FTA Cards only	4	0	2	2	2	0	0	**10**

[1]In several cases, there were multiple cell culture isolations from the mosquitoes in the same trap. For this table, such multiple detections were counted as 1 detection.

Table 8. Comparison of the time in days taken to identify arboviral isolates via the two technologies.

Detection Method	Virus‡							
	RRV	BFV	SINV	EHV	KOKV	KUNV	STRV	TOTAL
FTA Cards	1.3	1.3	1.6	1.5	1	ND	ND	**1.3**
Cell Culture	6.5	6.7	6	16	ND	14	15	**10.7**

ND: no virus was detected via that methodology.

EXOTIC MOSQUITO DETECTIONS AT SYDNEY INTERNATIONAL AIRPORT

Background. Over the last seven 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 last year's 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).

In response to the *Aedes aegypti* detections a number of actions were initiated. 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.

On Friday, 3 February 2017, DAWR during their routine surveillance for exotic mosquitoes detected a male *Aedes aegypti* in one of the BG GAT traps at the Qantas Freight Terminal (QFT), Sydney International Airport Terminal. 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 was another *Aedes aegypti* detection in the

basement area; one female trapped on 12/July/2017. The most recent detection was a female *Aedes aegypti* trapped on 9/August/2017, again in the basement area.

The reason for the recent increase in detections is not presently clear. Despite this, there has been a co-ordinated effort across multiple agencies to ensure the exotic mosquitoes do not become established in NSW. With any detection, there is a period of enhanced surveillance, which is conducted by DAWR. Insecticide treatments (thermal fogging and residual application) is undertaken with the detection of female mosquitoes. The larval surveys has led to several recommendations, which have been acted upon. This has included the removal of some sites of known larval breeding, regular treatment of water bodies deemed a potential risk for larval breeding, and improvements in hygiene to minimize larval habitats.

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 that 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 will release later this year, 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: the first week of trapping in mid-October produced 'very high' numbers, with a combined total of almost 5,000 mosquitoes. Thereafter, trap yields were lower but remained 'high' until early February. After this time, mosquito numbers dropped to 'low' and remained down for the remainder of these season. The early collections were dominated by *Culex australicus*, with *Culex annulirostris* becoming more abundant from early December. There were eight arboviral isolates from the trapped mosquitoes and these are listed in Table 9 below. Sentinel chicken flocks did not operate at Albury.

Table 9. Arboviral isolations from Albury, 2016-2017.

Site	Date Trapped	Mosquito Species	Virus		
			RRV	KOKV	Total
Kremur St	23-Jan-17	*		1	**1**
Waterworks Rd	16-Jan-17	*Culex annulirostris*	1		**1**
Waterworks Rd	16-Jan-17	*	1		**1**
Kremur St	19-Dec-16	*	1		**1**
Kremur St	19-Dec-16	*Culex annulirostris*	1		**1**
Kremur St	05-Dec-16	*	1		**1**
Kremur St	05-Dec-16	*Culex annulirostris*	1		**1**
Kremur St	05-Dec-16	*Aedes bancroftianus*	1		**1**
		Total	**7**	**1**	**8**

*Detection via Honey-Baited Cards, the mosquito species cannot be determined. RRV = Ross River virus, KOKV = Kokobera virus, STRV = Stratford virus.

Table 10. Arboviral isolations from Forbes, 2016-2017.

Site	Date Trapped	Mosquito Species	Virus				
			BFV	RRV	SINV	TRUV	Total
Toms Lagoon	05-Dec-16	*Anopheles annulipes*				1	**1**
STP	05-Dec-16	*		1			**1**
STP	05-Dec-16	*Culex annulirostris*		3			**3**
STP	05-Dec-16	*Culex australicus*		1			**1**
STP	29-Nov-16	*Culex annulirostris*		1			**1**
STP	29-Nov-16	*Culex australicus*		1			**1**
Toms Lagoon	29-Nov-16	*Culex annulirostris*		1			**1**
Toms Lagoon	15-Nov-16	*Culex annulirostris*		1			**1**
STP	15-Nov-16	*Culex annulirostris*	1				**1**
STP	15-Nov-16	*	1				**1**
Toms Lagoon	07-Nov-16	*Aedes sagax*			1		**1**
		Total	**2**	**9**	**1**	**1**	**13**

*Detection via Honey-Baited Cards, the mosquito species cannot be determined. BFV = Barmah Forest virus, RRV = Ross River virus, SINV = Sindbis virus, TRUV = Trubanman virus.

Table 11. Arboviral isolations from Griffith, 2016-2017.

Site	Date Trapped	Mosquito Species	Virus						
			BFV	RRV	SINV	KOKV	KUNV	TRUV	Total
Lake Wyangan	06-Feb-17	*Culex annulirostris*			1				**1**
Lake Wyangan	06-Feb-17	*				1			**1**
Hanwood	31-Jan-17	*Culex annulirostris*					1		**1**
Hanwood	31-Jan-17	*Culex annulirostris*			2				**2**
Hanwood	31-Jan-17	*Anopheles annulipes*			1				**1**
Lake Wyangan	31-Jan-17	*Culex annulirostris*			1				**1**
Hanwood	22-Jan-17	*Culex annulirostris*			2				**2**
Hanwood	22-Jan-17	*			1				**1**
Lake Wyangan	22-Jan-17	*			1				**1**
Hanwood	16-Jan-17	*	1						**1**
Hanwood	16-Jan-17	*Culex annulirostris*	1						**1**
Hanwood	10-Jan-17	*Culex annulirostris*		1					**1**
Hanwood	10-Jan-17	*Culex annulirostris*			4				**4**
Lake Wyangan	10-Jan-17	*Culex annulirostris*			3				**3**
Lake Wyangan	03-Jan-17	*Culex annulirostris*			1				**1**
Barren Box	19-Dec-16	*Culex annulirostris*			4				**4**
Barren Box	19-Dec-16	*			1				**1**
Lake Wyangan	19-Dec-16	*Culex annulirostris*			5				**5**
Barren Box	12-Dec-16	*Culex annulirostris*		2					**2**
Barren Box	12-Dec-16	*		1					**1**
Barren Box	12-Dec-16	*Anopheles annulipes*			1				**1**
Barren Box	12-Dec-16	*Culex annulirostris*			3				**3**
Lake Wyangan	12-Dec-16	*Culex annulirostris*			3				**3**
Barren Box	05-Dec-16	*Culex annulirostris*			1				**1**
Lake Wyangan	05-Dec-16	*Culex annulirostris*		2					**2**
Hanwood	31-Nov-16	*Culex annulirostris*		1					**1**
Hanwood	31-Nov-16	*Culex annulirostris*			3				**3**
Lake Wyangan	31-Nov-16	*Anopheles annulipes*		2				2	**4**
Lake Wyangan	31-Nov-16	*		1					**1**
Barren Box	21-Nov-16	*Culex annulirostris*		2					**2**
Barren Box	21-Nov-16	*Anopheles annulipes*		1					**1**
Barren Box	21-Nov-16	*Culex annulirostris*			1				**1**
Hanwood	21-Nov-16	*Culex annulirostris*		3					**3**
Barren Box	21-Nov-16	*		1					**1**
Lake Wyangan	14-Nov-16	*Aedes sagax*	1						**1**
Lake Wyangan	14-Nov-16	*	1						**1**
Lake Wyangan	01-Nov-16	*Aedes theobaldi*		1					**1**
Lake Wyangan	01-Nov-16	*Anopheles annulipes*		1					**1**
		Total	**4**	**19**	**39**	**1**	**1**	**2**	**66**

*Detection via Honey-Baited Cards, the mosquito species cannot be determined. BFV = Barmah Forest virus, RRV = Ross River virus, SINV = Sindbis virus, KOKV = Kokobera virus, KUNV = Kunjin virus, TRUV = Trubanman virus.

Bourke: mosquito collections were 'low' for the entire season. There were no sentinel chickens operated this season.

Deniliquin: no mosquito collections were undertaken this season. There were no

seroconversions to MVEV or KUNV in the sentinel chickens.

Forbes: the first collection of the season from mid-October produced a 'high' yield, with *Culex australicus* being the most common species trapped. For the next few weeks until mid-December, total collections were 'very high' (and dominated by *Culex annulirostris*) and then 'high' until late December. For all collections from January 2017, mosquito numbers were 'low'. There were 13 arboviral isolates and these are listed in Table 10 above. There were no seroconversions to MVEV or KUNV in the sentinel chickens.

Griffith: a new trapping site began this year in Griffith; Lake Wyangan, which is only a few kilometres to the north west of the city and proved to be highly productive for mosquitoes. Trapping began in mid-October and over 7,000 mosquitoes were yielded from the first week, with the majority being *Culex australicus*. Typical catches at this time of the year would be less than 100 mosquitoes. Total collections rose to over 10,000 mosquitoes per week during late October and early November; in fact close to 30,000 mosquitoes were trapped during these two weeks! Collections of over 10,000 mosquito in one week occurred again in mid and late January. Total mosquito collections were 'very high' for almost every week of the season, even up to late March. There 66 arboviral isolates this season and these are detailed in Table 11 above. 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 mid-October, with and 'very high' totals yielded. Collections remained this level up until mid-November and were mostly 'high' for the remainder of the season. One very large collection of almost 6,000 mosquitoes were produced in mid-December. There were 15 arboviral isolates and these are detailed in Table 12 below. There were no seroconversions to MVEV or KUNV in the sentinel chickens.

Table 12. Arboviral isolations from Leeton, 2016-2017.

Site	Date Trapped	Mosquito Species	Virus		
			RRV	SINV	Total
Farm 347	17-Jan-17	*Culex annulirostris*		2	**2**
Farm 347	17-Jan-17	*		1	**1**
Almond Rd	09-Jan-17	*Culex annulirostris*	1		**1**
Almond Rd	09-Jan-17	*	1		**1**
Farm 347	13-Dec-16	*Culex annulirostris*	2	2	**4**
Farm 347	07-Dec-16	*	1		**1**
Farm 347	07-Dec-16	*Culex annulirostris*		1	**1**
Farm 347	29-Nov-16	*Culex annulirostris*	1		**1**
Farm 347	16-Nov-16	*Culex annulirostris*	1		**1**
Farm 347	16-Nov-16	*Anopheles annulipes*	1		**1**
Farm 347	16-Nov-16	*	1		**1**
		Total	**9**	**6**	**15**

*Detection via Honey-Baited Cards, the mosquito species cannot be determined. RRV = Ross River virus, SINV = Sindbis virus.

Macquarie Marshes: no mosquito collection were made this season. There were six

seroconversions KUNV in the sentinel chickens, with one positive from the bleed taken on 15/Jan/2017 and five positives from the bleed taken on 26/Jan/2017.

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

Mathoura: 'high' mosquito numbers were yielded from the first two collections of the season, in mid-October and early November. Thereafter mosquito numbers were 'low'. There were three arboviral isolates and these are detailed in Table 13 below. There were no seroconversions to MVEV or KUNV in the sentinel chickens, with only three bleeds for the season.

Table 13. Arboviral isolations from Mathoura, 2016-2017.

Site	Date Trapped	Mosquito Species	Virus	
			RRV	Total
Moama	06-Dec-16	*	1	**1**
Moama	08-Nov-16	*	1	**1**
Moama	08-Nov-16	*Aedes sagax*	1	**1**
		Total	**3**	**3**

*Detection via Honey-Baited Cards, the mosquito species cannot be determined. RRV = Ross River virus.

Menindee: only six mosquito collections were undertaken this season and numbers were mainly 'low'. There were no seroconversions to MVEV or KUNV in the sentinel chickens.

Wagga Wagga: as per the other inland sites, trapping began in mid-October and 'high' mosquito numbers were yielded. Collections remained 'high' until late November and thereafter declined to be mainly 'low' for the remainder of the season. There were no arboviral isolates this season. Sentinel chickens did not operate at Wagga Wagga.

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

Coastal Locations

Ballina: trapping continued at the two sites of North Creek Road and Pacific Pines. 'Very high' totals were yielded in the first week during early December and remained 'high' or greater throughout the season. Collections peaked in mid-April when over 6,000 mosquitoes were trapped over a two week period. During these weeks the main mosquito species captured was *Aedes multiplex*. No arboviral isolates were detected.

Coffs Harbour: trapping was undertaken at Caba Close and Mardells Road. The former site had some 'high' numbers, which dominated by *Culex quinquefasciatus*. IN contrast, Mardells Road had consistently 'low' yields. No arboviral isolates were detected.

Gosford: collections were 'high' for most of the season and peaked during early ot mid-February, with numbers of over 600 mosquito per trap. During these weeks, *Aedes vigilax* predominated. No arboviral isolates were detected.

Lake Macquarie: collections were undertaken from three sites: Belmont Lagoon, Teralba and Dora Creek. Mosquito numbers were 'medium' to 'high' for much of the season, with collections peaking in mid-March with a total of over 1,000 mosquitoes trapped. Freshwater species dominated in these collections. No arboviral isolates were detected.

Port Macquarie: Trapping was undertaken at three sites; North Haven, Partridge Creek, and Stevens Street. Mosquito numbers were mostly 'low' until late February. Collections peaked during March when *Aedes vigilax* dominated the catches. There was one arboviral detection via the FTA cards; SINV from Partridge Creek from the mosquitoes trapped on 10/Apr/2017.

Tweed Heads: trapping was undertaken at three sites; Koala Beach, Beltana Drive and Piggabeen Road. Mosquito numbers remained mostly 'low' until late December. Collections peaked during the last week of March, with almost 1,500 mosquitoes captured, dominated by a mix of *Aedes vigilax* and *Culex sitiens*. 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. No arboviral isolates were detected.

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. 'Low' numbers were yielded early in the season, however a 'high' collection was made in late December and remained 'high' until late January. A series of 'medium' catches were made until numbers rose again in early March and 'high' numbers were trapped for the remainder of the season until mid-April. No arboviral isolates were detected.

Georges River: trapping was again undertaken at the four sites of Alfords Point, Lugarno, Illawong, and Picnic Point. Mosquito numbers were consistently 'high' or greater with a series of 'very high' collections at varying times through the season. Typically, *Aedes vigilax* dominated in these large collections. There were 33 arboviral detections and these are listed in Table 14 below.

Hawkesbury: trapping was undertaken at four main sites, including at Wheeney Creek, Yarramundi, McGraths Hill and Ebenezer. As per normal, Wheeney Creek yielded the greatest collections with a series of 'high' mosquito yields through early to mid-April, with collections dominated by *Aedes* Marks No. 51 (a relative of *Aedes procax*). Some 'high' catches were made at the other sites, but numbers were mainly 'low' for most weeks. No arboviral isolates were detected.

Table 14. Arboviral isolations from Georges River, 2016-2017.

Site	Date Trapped	Mosquito Species	Virus			
			RRV	EHV	STRV	Tot
Illawong	20-Apr-17	*Aedes notoscriptus*		1		**1**
Illawong	20-Apr-17	*Aedes procax*		1		**1**
Alfords Point	26-Mar-17	*	1			**1**
Alfords Point	26-Mar-17	*Aedes vigilax*	1			**1**
Alfords Point	26-Mar-17	*Coquillettidia linealis*	3			**3**
Picnic Point	26-Mar-17	*Aedes vigilax*	1			**1**
Picnic Point	26-Mar-17	*Coquillettidia linealis*	1			**1**
Picnic Point	26-Mar-17	*		1		**1**
Picnic Point	19-Mar-17	*Aedes vigilax*	1			**1**
Picnic Point	19-Mar-17	*Aedes vigilax*			1	**1**
Picnic Point	19-Mar-17	*	1			**1**
Alfords Point	13-Mar-17	*Aedes vigilax*	2			**2**
Illawong	13-Mar-17	*Aedes vigilax*	3			**3**
Picnic Point	13-Mar-17	*Aedes vigilax*			4	**4**
Picnic Point	13-Mar-17	*Aedes vigilax*	2			**2**
Picnic Point	13-Mar-17	*	1			**1**
Illawong	07-Mar-17	*		1		**1**
Picnic Point	02-Mar-17	*Aedes vigilax*	2			**2**
Illawong	02-Mar-17	*Aedes vigilax*		1		**1**
Illawong	02-Mar-17	*	1			**1**
Alfords Point	29-Dec-16	*Aedes alboannulatus*	1			**1**
Alfords Point	29-Dec-16	*	1			**1**
Illawong	08-Dec-16	*	1			**1**
		TOTAL	**23**	**5**	**5**	**33**

*Detection via Honey-Baited Cards, the mosquito species cannot be determined. RRV = Ross River virus, SINV = Sindbis virus, EHV = Edge Hill virus, KOKV = Kokobera virus, STRV = Stratford virus.

Penrith: trapping was undertaken at the three sites of Emu Plains, Muru Mittigar, Glenmore Park and Werrington. Muru Mittigar produced the greatest mosquito catches with mostly 'high' numbers collected from mid-March to late April. The other sites tended to have 'low' numbers. 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. Most weeks produced 'high' mosquito numbers with the biggest collection occurring during the Christmas week with over 4,500 trapped. 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 both of RRV and BFV.
	**The Bushland Mosquito**, ***Aedes procax***. Common throughout coastal NSW. This species breeds in bushland freshwater ground. 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***. The most 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 the main 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 1000'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.

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. This is an emerging disease and is increasingly being recognised in NSW, with around 3-400 cases annually. However, serological over diagnosis of this condition through the non-specificity of the commercial kit has been a major issue. Despite being first isolated from an inland region, cases of BFV disease tend to occur mainly in coastal regions in NSW. The main vector in NSW is *Aedes vigilax* although other species are involved, notably *Aedes procax*. In 2010-2011 there was a small epidemic of BFV (but largest to date for the inland 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). 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 isolated 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 deaths resulting. *Culex annulirostris* appears to be the main vector.

Murray Valley Encephalitis (MVEV): 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 the recent season of 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.

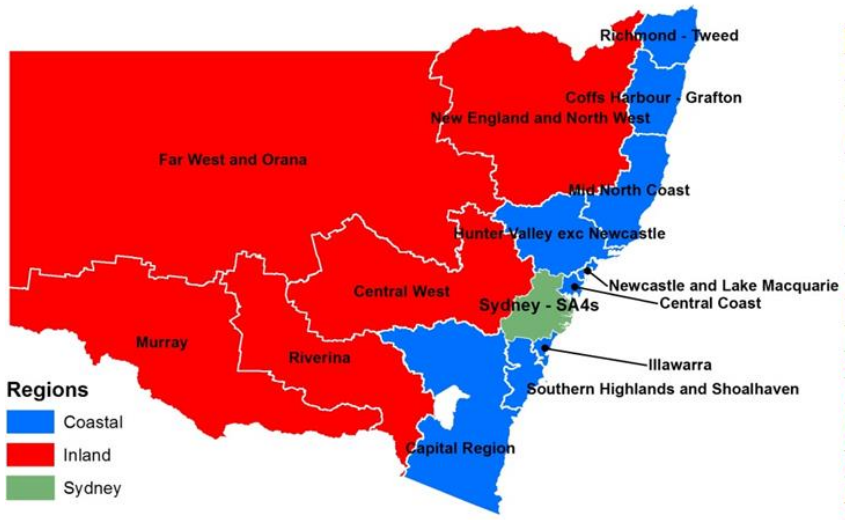
Stratford virus (STRV): there have been very few documented symptomatic patients, only three described to date and symptoms included fever, arthritis and lethargy. The virus has mostly been isolated from coastal NSW, particularly from the saltmarsh mosquito, *Aedes vigilax*, although recent isolates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