THE NEW SOUTH WALES ARBOVIRUS SURVEILLANCE & MOSQUITO MONITORING PROGRAM

2014-2015 Annual Report

Culex annulirostris

S. DOGGETT, J. HANIOTIS, J. CLANCY, C. WEBB & C. TOI

Mosquito & Arbovirus Surveillance Laboratory,
Medical Entomology Department, CIDMLS,
ICPMR, Pathology West,
Westmead Hospital, Westmead, NSW 2145.

L. HUESTON, H. LIM, & D.E. DWYER

Arbovirus Laboratory, Clinical Virology, CIDMLS,
ICPMR, Pathology West,
Westmead Hospital, Westmead, NSW 2145.

PathologyWest ICPMR Westmead

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EXECUTIVE OVERVIEW

- **For the 2014-2015 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 November and April.
- **The climatic conditions** leading up to the 2014-2015 season for the inland were of elevated temperatures coupled with below average rainfall for the last six months of 2014. Both Forbes and Nicholls hypotheses were not suggestive of a potential MVEV epidemic for the season. For the coast, conditions were mostly similar, however heavy rainfall occurred through December and January.
- **For the inland**, despite the dry weather, the high temperatures late in the year resulted in an early seasonal start, with over 71,000 mosquitoes trapped, more than double the previous season. There were 12 arboviral detections; 5BFV, 5RRV, and 1STRV. There were no seroconversions in the sentinel chickens.
- **Human notifications from the inland** of RRV and BFV totalled 271 (260RRV & 11BFV), which was close to the long term average of 305. There were no human cases of flavivirus infection reported.
- **As of October 2015**, neither the Forbes nor the Nicholls hypotheses are suggestive of possible MVEV activity for the season of 2014-2015. The current El Nińo event is expected to persist to early 2016 and should result in reduced rain.
- **For the coast**, the significantly warmer conditions over the last half of 2014 coupled with spring tides and early summer rains lead to several sites experiencing an early rise in vector numbers. As a result, mosquito numbers were well up this season, particularly of freshwater breeding species such as *Culex annulirostris*. There were 41 isolates, including 6BFV, 29RRV, 4EHV, and 2STRV.
- **Coastal disease notifications saw a record RRV epidemic**; the season experienced the highest number of cases since RRV statistics began to be recorded in 1985. There were a total of 1,392 cases, including 1,225 RRV and 167 BFV, and this was double the average of 711. The statistical local area that produced the highest case load was Tweed, with 212 notifications (204RRV & 8BFV), followed by Port Macquarie (131 notifications: 114RRV & 17BFV), Bryon (112: 98RRV & 14BFV), Kempsey (86: 75RRV and 11BFV), Lismore (73: 62RRV and 11BFV), and Lake Macquarie (64: 63RRV and 1BFV).
- **Sydney**, also experienced an early rise in mosquito numbers which resulted in collections totalling around twice that of the previous season. There were 20 arboviral isolates, including 2BFV, 15RRV and 3EHV, with most (13) being from Georges River. Human notifications were more than double the average of 77, with a total of 196 reports including 186RRV and 10BFV.
- **New methodologies** to increase the sensitivity of the surveillance system continue to be successfully trialled and the honey-baited cards system was routinely incorporated into the program as of the 2014-2015 season.
- **The NSW Arbovirus Surveillance Web Site** http://medent.usyd.edu.au/arbovirus/ continued to expand and now has over 345MB, and has 2,510+ pages.
- **"*A Guide to Mosquitoes of Australia*"** is a new textbook on mosquitoes due for release in early 2016 produced by staff of Pathology West.

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NSW ARBOVIRUS SURVEILLANCE AND MOSQUITO MONITORING PROGRAM 2014-2015

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 2014-2015 season follow.

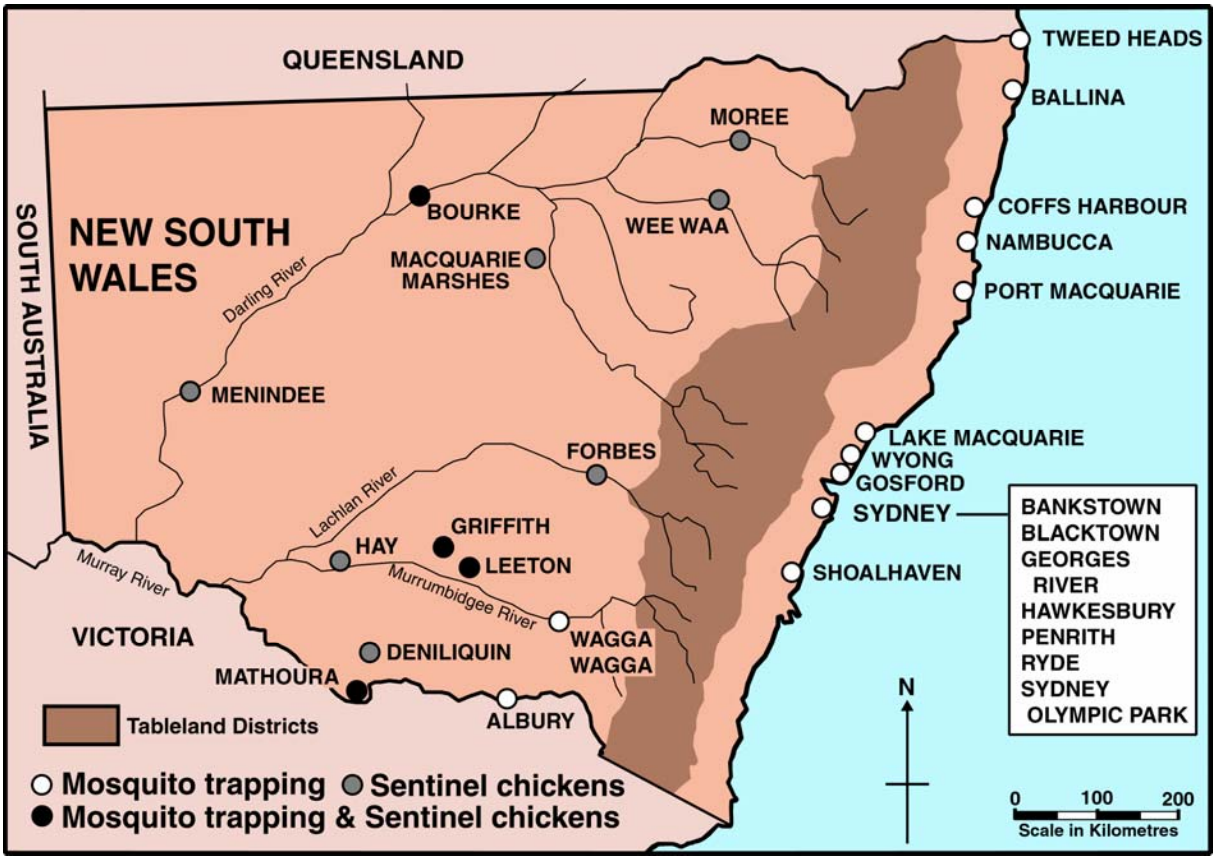


Fig 1. Mosquito trapping locations and Sentinel Chicken sites, 2014-2015.

MONITORING LOCATIONS

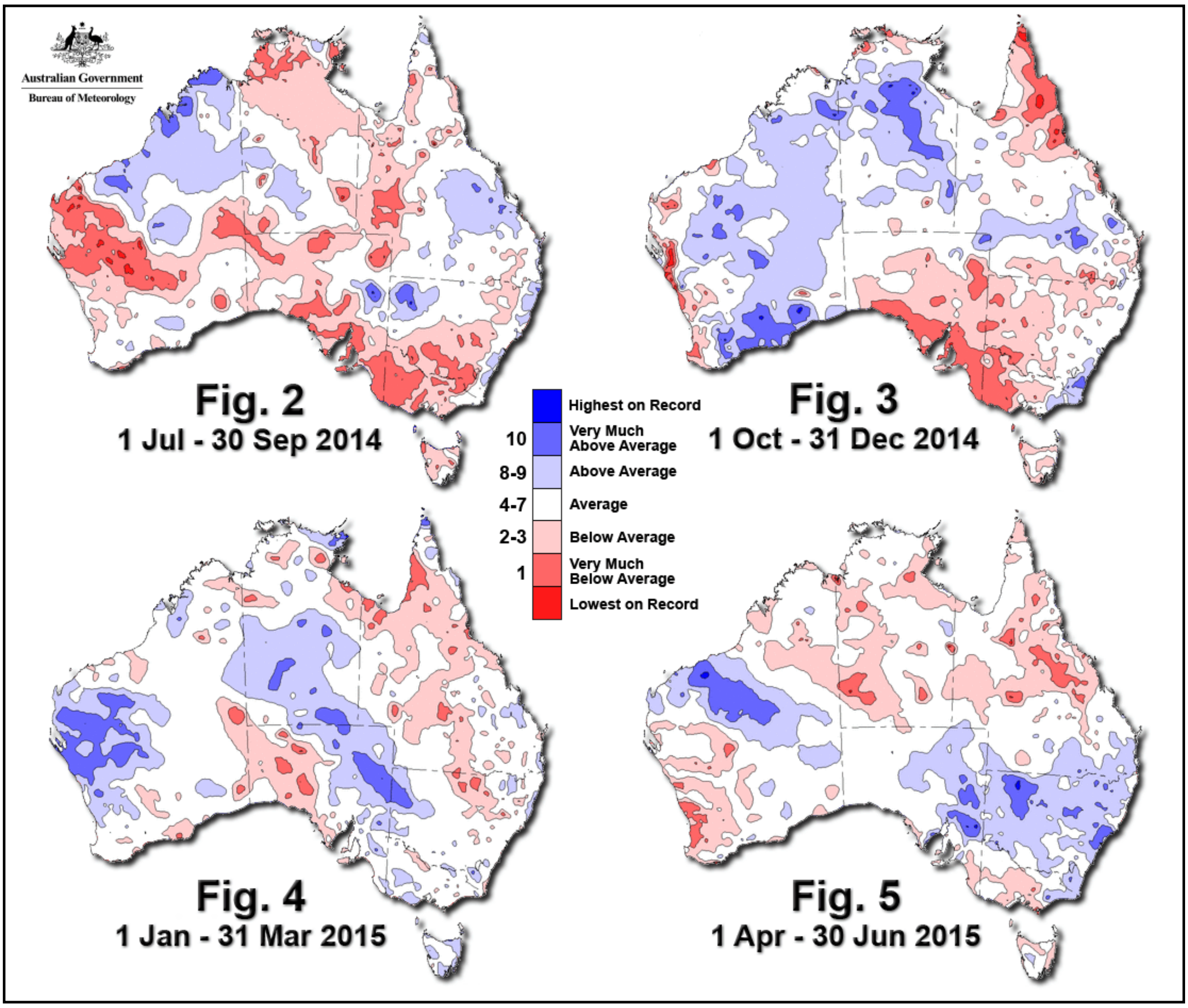
http://medent.usyd.edu.au/arbovirus/location/locations.htm

For 2014-2015, mosquito-trapping sites were operated at 6 inland, 7 coastal and 7 Sydney locations. Chicken sentinel flocks were located at 11 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 2014, Oct-Dec 2014, Jan-Mar 2015 & Apr-Jun 2015. The stronger the red, the drier the conditions. Conversely, the stronger the blue, the wetter the conditions. *Modifed from the Australian Bureau of Meterology, 2015*.

During the first quarter of 2014 (i.e. January to March), above average rainfall fell across the inland, however the coastal strip was mostly dry, with below average rainfall especially along the north coast. For the second quarter of 2014 (April to June), the south west of the state had above normal rainfall, while the north east had below average rainfall. The inland during the third quarter of 2014 (July to September) experienced very low precipitation patterns especially in the Murray/Murrumbidgee basins, although much of the coast had above average rainfall (Figure 2). The dry inland conditions persisted into the last quarter of 2014 (October to December) and the entire western region had very low rainfall amounts, while the coast experienced normal rainfall patterns (Figure 3). The first quarter of 2015 (January to March) had mostly average rainfall for much of the state (Figure 4), although was above average in January. The entire state had above average rainfall through the second quarter of 2015 (April to June, Figure 5). Currently a strong El Niño episode is persisting, which will lead to reduced rainfall over eastern Australia. It is expected that the El Niño will maintain into early 2016.

Temperatures for the last half of 2014 were above average by 2-3 degrees, with temperatures well above (4-6^{o}C) average during October and November. December was slightly above average (1-2^{o}C). January had slightly cooler than normal conditions with the above average rainfall, and weather patterns during the remainder of the mosquito season tended to be hotter than 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 2013) or the first quarter of the current year (January-March 2014) <u>and</u> the last quarter of the current year (October-December 2014), 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 for all of the catchment basins for the last quarter of 2013, was above decile 7 in only one catchment basin in the first quarter of 2014, and above decile 7 in only one of the catchment basins for the last quarter of 2014, thus the Forbes hypothesis was not fulfilled for 2014-2015 (Table 1). Additionally, decile 7 or above rainfall did not occur across all the catchment basins during the first quarter of 2015, therefore according to Forbes', there should be a lower risk of an MVEV epidemic for the upcoming 2015-2016 season.

Table 1. Rainfall indices for the main catchment basins of eastern Australia as per Forbes hypothesis, relevant to the 2013-2014 and 2014-2015 seasons.

Catchment Basin	Oct-Dec 2013	Jan-Mar 2014	Oct-Dec 2014	Jan-Mar 2015
Darling River	0.41	0.75	0.80	0.65
Lachlan/Murrumbidgee/ Murray Rivers	0.49	1.26	0.97	1.05
Northern Rivers	0.83	0.91	0.94	0.67
North Lake Eyre system	0.52	0.83	1.07	0.67

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 2014, the SO values were respectively: 1009.90mm, 1013.63mm and 1011.53mm (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 have resulted from over-wintering

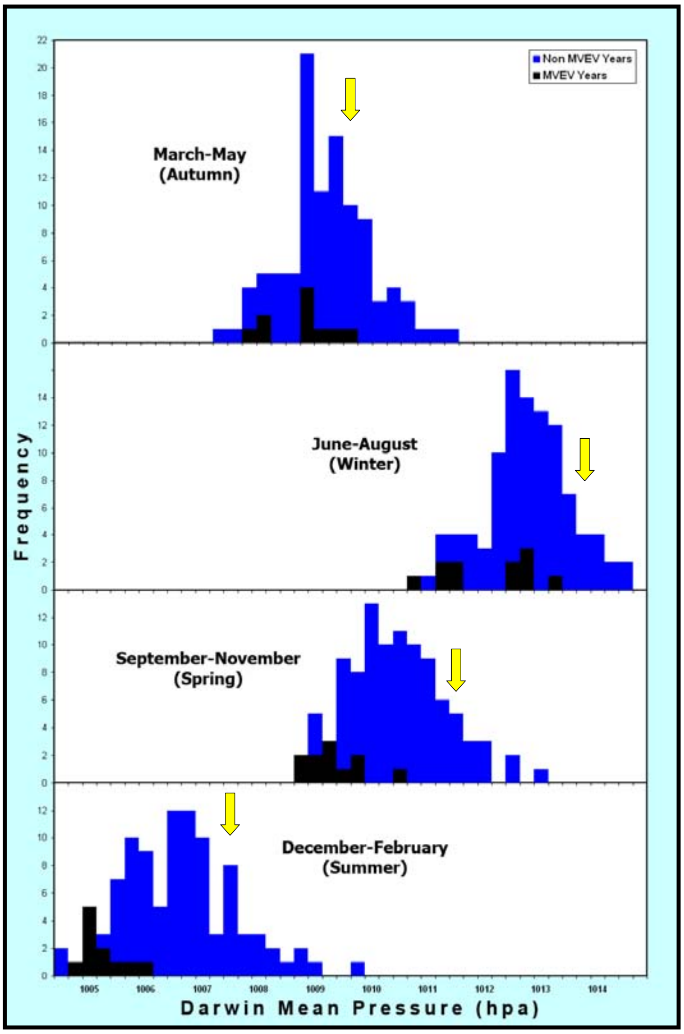


Figure 6. The SO by seasons prior to MVEV active years, according to Nicholls (1986), updated up to Spring 2014. The black bars represent the pre-MVEV active seasons. The yellow arrows indicate the respective SO values relevant to the 2014-2015 season.

mosquitoes.

As of October 2015, the autumn Nicholls' value is 1010.83mm, the winter value 1014.37 and incomplete spring value is 1014.57 (Table 2). All of these are outside the range of values during past MVEV outbreak years, suggesting a reduced risk for 2015-2016.

Table 2. The seasonal atmospheric pressures (in mm) according to Nicholls' hypothesis, relevant to the 2015-2016 season.

	Autumn 2015	Winter 2015	Spring 2015*
2014 Values	1010.83	1014.37	1014.57
Pre past MVEV seasons	<1009.74	<1012.99	<1009.99

*Data for Sep/15 only

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 Vector 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), Pathology West, 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 monitoring results (with comments on the collections) were placed on the NSW Arbovirus Surveillance Web site, and generally were available within 1-2 days of sample receipt into the laboratory. Access to each location's result is from: **http://medent.usyd.edu.au/arbovirus/results/results.htm**.

Results

Overall, 213,401 mosquitoes representing 57 species were collected in NSW during 2014-2015, which was more than double upon the previous season. *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 and the complete mosquito monitoring results are available on the NSW Arbovirus Surveillance web site. A brief description of the most important vectors is provided in Appendix 2.

Inland

The total of 88,111 mosquitoes comprising 16 species was over double the previous season total of 43,252 trapped in 2013-2014. *Culex annulirostris* was the dominant species yielded at most sites and comprised 76.8% of the total inland collections. *Anopheles annulipes* (21.0%) was the next most common species.

Coastal

In total, 71,780 mosquitoes comprising 49 species were collected from coastal NSW and this was almost three times the previous season's collection. The most common species collected were *Culex annulirostris* (23.8%), *Culex sitiens* (15.5%), *Aedes vigilax* (12.9%), *Aedes multiplex* (11.8%), *Aedes notoscriptus* (8.7%), and *Verrallina funerea* (6.6%). For most years, *Aedes vigilax* is usually by far the most predominant species and generally comprises 50-60% of the coastal collections.

Metropolitan Sydney

A total of 51,473 mosquitoes, comprising 37 species, was collected from metropolitan Sydney and this was around double the previous season's total collection. *Aedes vigilax* (51.7% of the total Sydney mosquitoes trapped) was the most common species, followed by *Culex annulirostris* (17.1%), *Aedes notoscriptus* (8.2%), and *Culex sitiens* (5.7%).

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) by means of a suite of targeted multiplexed, real-time RT-PCR assays using a high saturating fluorescent dye. Viral RNA was extracted using the EZ1® Virus Mini Kit (Qiagen), reverse transcribed, and amplified on the Corbett™ Rotor-Gene 6000.

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). Captured mosquitoes were tested for arboviruses as above, while for the FTA cards, viral RNA was extracted from the FTA card eluates and tested by real-time RT-PCR using Pan-Flavivirus (Moureau G*, et al.* 2007, Hall-Mendelin *et al.* 2010) and Alphavirus primers. Amplified products were definitively identified by targeted multiplex RT-PCR.

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, to the relevant Public Health Unit, and posted on the NSW Arbovirus Surveillance Web Site (under 'Mosquito/Chicken Results', and under each location's surveillance results).

Results

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

From the mosquitoes processed, there were 53 arboviral detections; 12 from the inland (Table 3) and 41 from the coast (Table 4).

Table 3. Arbovirus isolates from inland NSW, 2014-2015.

LOCATION	Date Trapped	Mosquito Species	Virus			
			BFV	RRV	STRV	Total
GRIFFITH	11-Feb-15	*Culex annulirostris*			1	1
GRIFFITH	19-Jan-15	*	1			1
GRIFFITH	11-Feb-15	*	2			2
GRIFFITH	18-Feb-15	*Culex annulirostris*		1		1
GRIFFITH	10-Mar-15	*	1			1
LEETON	23-Jan-15	*		1		1
LEETON	11-Feb-15	*	1			1
LEETON	3-Mar-15	*Anopheles annulipes*		1		1
LEETON	10-Mar-15	*		2		2
MACQUARIE MARSHES	6-Mar-15	*		1		1
		TOTAL	**5**	**6**	**1**	**12**

Table 4. Arbovirus isolates from coastal NSW, 2014-2015.

LOCATION	Date Trapped	Mosquito Species	Virus				
			BFV	RRV	EHV	STRV	Total
BANKSTOWN	17-Dec-14	*		1			1
BANKSTOWN	27-Jan-15	*Aedes vigilax*	1				1
GEORGES RIVER	11-Feb-15	*		1			1
GEORGES RIVER	17-Feb-15	*Aedes vigilax*	1				1
GEORGES RIVER	17-Feb-15	*		1			1
GEORGES RIVER	24-Feb-15	*Aedes multiplex*			1		1
GEORGES RIVER	3-Mar-15	*Aedes procax*			1		1
GEORGES RIVER	9-Mar-15	*		3			3
GEORGES RIVER	18-Mar-15	*		4			4
GEORGES RIVER	31-Mar-15	*		1			1
GOSFORD	20-Jan-15	*	1				1
GOSFORD	4-Mar-15	*		1			1
HAWKESBURY	3-Mar-15	*Aedes procax*		2			2
HAWKESBURY	3-Mar-15	*Aedes* sp. Marks 51		1			1
HAWKESBURY	3-Mar-15	*		1			1
HOMEBUSH	9-Feb-15	*Aedes vigilax*			1		1
HOMEBUSH	24-Feb-15	*		1			1
HOMEBUSH	9-Mar-15	*		2			2
HOMEBUSH	18-Mar-15	*		1			1
LAKE MACQUARIE	20-Jan-15	*	1				1
LAKE MACQUARIE	10-Feb-15	*	1				1
LAKE MACQUARIE	17-Feb-15	*	1				1
LAKE MACQUARIE	10-Mar-15	*		1			1
PORT MACQUARIE	23-Apr-15	*			1		1
PORT MACQUARIE	5-May-15	*				1	1
TWEED HEADS	23-Jan-15	*		2			2
TWEED HEADS	9-Feb-15	*Aedes vigilax*				1	1
TWEED HEADS	24-Feb-15	*		1			1
TWEED HEADS	10-Mar-15	*		3			3
TWEED HEADS	5-May-15	*		1			1
TWEED HEADS	8-Apr-15	*		1			1
		TOTAL	6	29	4	2	41

BFV = Barmah Forest virus, RRV = Ross River virus, EHV = Edge Hill virus, STRV = Stratford, *detection via FTA card.

SENTINEL CHICKEN PROGRAM

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

Location of flocks

The 2014-2015 season began on 5th November 2014 with the first bleed and ended on 14th May 2015 with the last. A total of eleven flocks each containing up to 15 Isa Brown pullets was deployed, with one flock each at Bourke, Deniliquin, Forbes, Griffith, Hay, Leeton, Macquarie Marshes, Menindee, Moama (near Mathoura), 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 150 pullets and no deaths were recorded. A total of 3,030 samples was received from the ten flocks in NSW over the six-month period in 2014-2015. This represented 6,060 ELISA tests (excluding controls and quality assurance samples), with each specimen being tested for MVEV and KUNV antibodies. There were no seroconversions to MVEV or KUNV in the sentinel chickens.

HUMAN NOTIFICATIONS

The notification of human arboviral infections is based on laboratory notifications, which define cases as being '*confirmed*', '*presumptive*', '*inconclusive*' or '*negative*' (Mackenzie *et al*. 1993). A '*confirmed*' infection is where there is at least a fourfold rise or fall in antibodies between paired sera, with the first blood sample begin taken early in the disease phase (the 'acute' sample) and the second sample taken during convalescence of the illness (the 'convalescent' sample). The detection of the virus by isolation or through molecular techniques also constitutes a '*confirmed*' infection. A '*presumptive*' infection is where there is IgM antibody in the acute sera, or moderate or high antibody (such as IgG) with IgM antibodies. An '*inconclusive*' infection has little to no IgM antibody in the acute sample or stable antibody levels in two convalescent samples without IgM antibodies. A '*negative*' infection has no specific arbovirus antibody.

The notifications are assessed against the respective surveillance case definitions for each infection as described in the NSW Health Public Health Unit Disease Control

Guidelines (NSW Ministry of Health) for each condition, available at: http://www.health.nsw.gov.au/Infectious/controlguideline/Pages/default.aspx. Only cases classified as 'confirmed' are reported.

The notification of human infections with these arboviruses is required under the NSW Public Health Act 2010 and is managed by Health Protection NSW and the NSW Public Health Units, which are part of the NSW Ministry of Health.

Table 6. Arbovirus notifications according to former Area Health Service, July 2014 - June 2015.

Month	CS	NS	WS	WE	SW	CC	HU	IL	SE	NR	MN	NE	MA	MW	FW	GM	SA	Total
RRV	14	52	33	50	10	88	250	33	27	483	345	55	45	55	29	76	26	**1671**
BFV	1	0	1	5	2	5	8	8	1	61	67	2	2	1	3	3	18	**188**
Total	**15**	**52**	**34**	**55**	**12**	**93**	**258**	**41**	**28**	**544**	**412**	**57**	**47**	**56**	**32**	**79**	**44**	**1859**

CS = Central Sydney, NS = Northern Sydney, WS = Western Sydney, WE = Wentworth, SW = South Western Sydney, CC = Central Coast, HU = Hunter, IL = Illawarra, SE = South Eastern Sydney, NR = Northern Rivers, MN = Mid North Coast, NE = New England, MA = Macquarie, MW = Mid Western, FW = Far Western, GM = Greater Murray, SA = Southern Area.

Table 6 contains the number of laboratory notifications of human RRV and BFV infection by former Area Health Service (AHS) for NSW. The former AHSs data were used, rather than the current, to allow for a comparison of notification trends over time. The majority of notifications are *'presumptive'* infections. As a result there are likely to be significant errors in the data given the high false positive rate of commercial kits (20% false positives, L. Hueston, *pers. comm*.), the degree of cross-reactivity of closely related arboviruses, the persistence of IgM for long periods (18 to 48 months) in genuine infections, and the fact that antibody is produced regardless of clinical disease (L. Hueston, *pers. comm*.). In an investigation of serologically diagnosed BFV cases from the mid-north coast of NSW, it was found that there was a significant amount of over-diagnosis (Cashman *et al*. 2008), which continued some time after this publication (Doggett 2014). Some laboratories have reported a 95% false positive rate with the commercial kit and this has been now removed from the marketplace. Thus any epidemiological interpretation of the BFV notifications over recent years must be viewed with a high degree of uncertainty.

The total number of RRV and BFV notifications for the period July 2014 to June 2015 was 1,859 and included 188BFV and 1,671RRV. This season was well above the long term average of 1,090 and in fact, the RRV notifications represent the largest recorded outbreak to date since notifications began to be reported in 1985.

The coastal region accounted for 1,392 (74.9% of the state total) of the BFV and RRV notifications, which was around double the seasonal average of 711. For RRV, alone, the coast experienced four times the average number of cases. The 271 notifications (14.6% of the state total) from the inland were slightly below the average of 305. Within the Sydney region, there were 196 cases reported, over double the seasonal average of 77 notifications. The overall reduced number of BFV notifications for the state (188 down from the average of 348) was probably due to the withdrawal from the market of the commercial serological assay.

From the coast, the Northern Rivers and Mid-North Coast Area Health Services received the highest notifications (544 and 412 respectively), with the Hunter having 258. Combined, these three areas accounted for 65.3% of all the arbovirus notifications for the state. From the inland, the Greater Murray AHS had the highest number of notifications (76), with the vast majority being RRV (73 notifications).

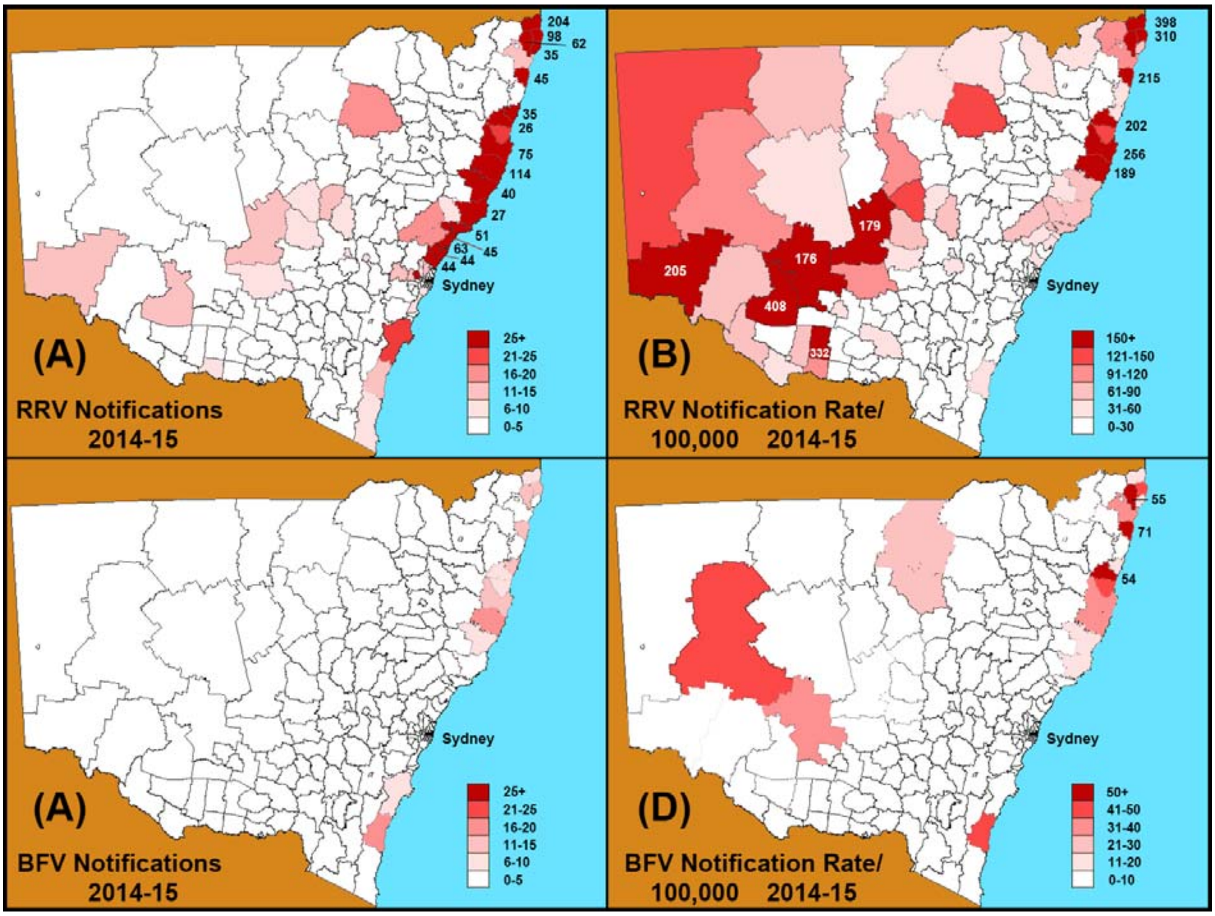


Figure 7. Notifications and notification rates of RRV and BFV by Statistical Local Areas for NSW for Jul 2014 to Jun 2015. (A) RRV notifications. (B) RRV notification rate/100,000 population. (C) BFV notifications. (D) BFV notification rate/100,000 population. Note that different scales are used on the notifications and rates graphs.

Figure 7 depicts the notifications and notification rates of RRV and BFV by Statistical Local Area (SLA) for NSW during the 2014-2015 mosquito season.

There were no human locally acquired flavivirus seroconversions reported.

DISCUSSION

The Inland. Early at the start of this decade there was been extensive arbovirus activity following increased rainfall patterns. During the season of 2010-2011, there were large vector numbers, a major outbreak of KUNV in horses, concomitant MVEV activity, elevated RRV notifications, and an outbreak of BFV. The following season (2011-2012) saw elevated mosquito numbers again, with further MVEV/KUNV activity.

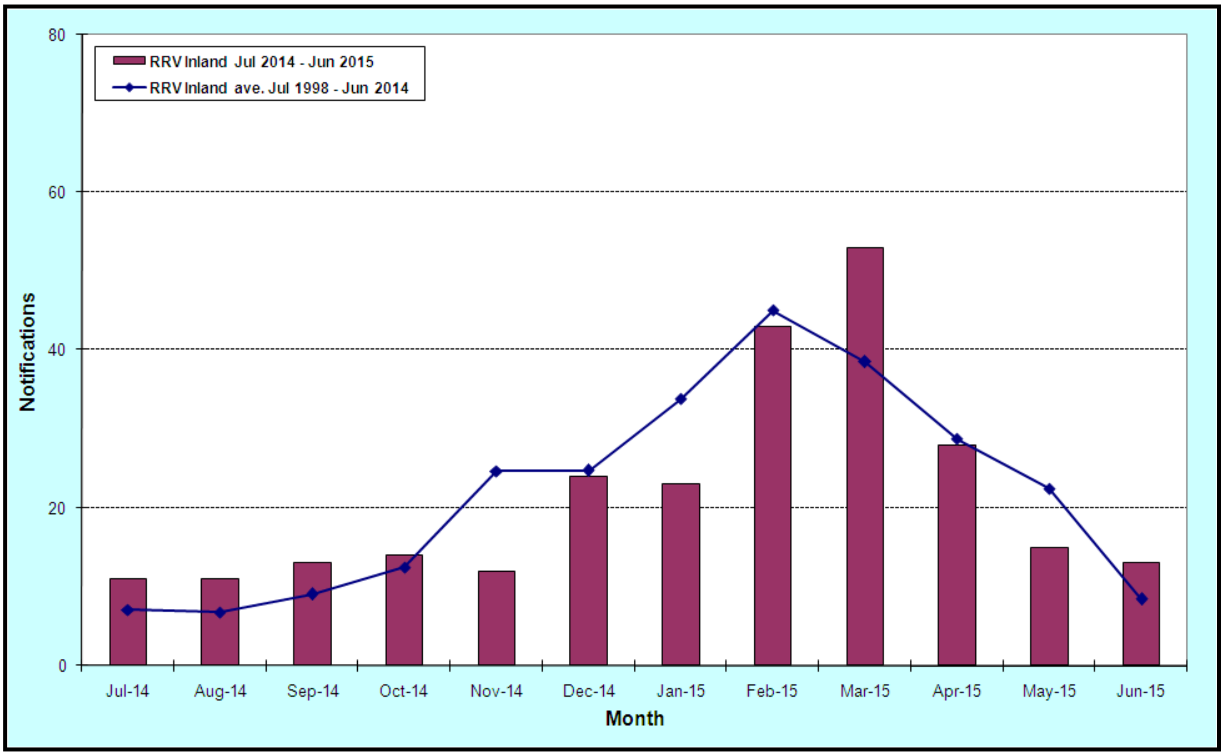


Figure 8. Notifications of RRV per month from inland NSW. The bars are for 2014-2015 season and the line represents the long term average.

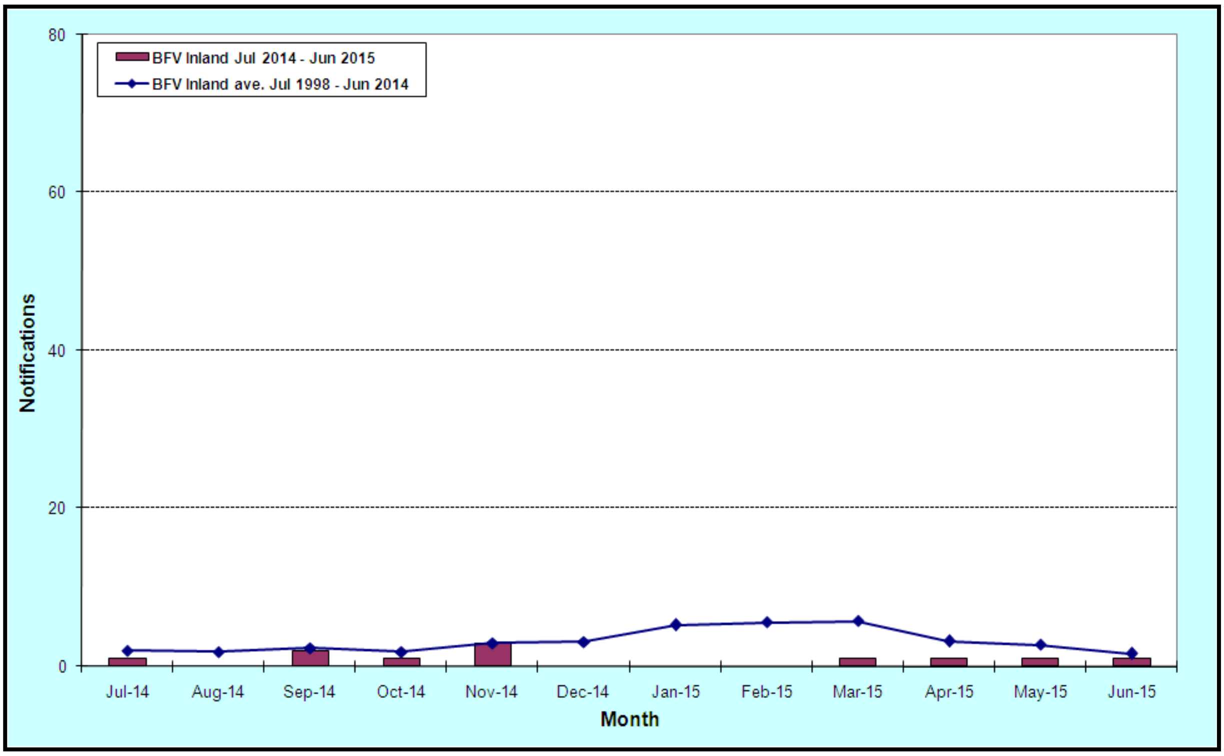


Figure 9. Notifications of BFV per month from inland NSW. The bars are for 2014-2015 season and the line represents the long term average.

The following two seasons (2012-2013 and 2013-2014) were much drier with rainfall levels below average. As a consequence, there was relatively minimal arboviral activity during these seasons. For 2014-2015, the last three months of the year were exceptionally warm, with maximum temperatures 3-4^{o}C above average and minimum temperatures 1-2^{o}C above normal. As a result, mosquito numbers were well up early in the season and considerably greater than usual, with the total number of mosquitoes trapped being double that of the previous season. This was despite the fact that rainfall patterns were below average for most of the second half of 2014. January did experience above average rainfall, however no widespread increase in mosquito numbers were observed as a consequence. Perhaps the conditions experienced over last season, may be an indicator to the future as a consequence of global warming with climate change.

Relatively few arboviral detections were made this season; some 5BFV, 6RRV and 1 STRV, with no seroconversions in the sentinel chickens. Despite this, notifications were only slightly below normal (Figures 8 & 9). The SLAs that produced most inland RRV cases (Figure 7a) included Narrabri (19 notifications), Dubbo (14), Wentworth (14), Hay (12), Lachlan (12), Tamworth (11), Wagga Wagga (11), Orange (10), and Parkes (10). The diversity of regions across the state with notifications demonstrates that the RRV was much more widespread this season with activity less concentrated to certain SLAs, as seen in previous years. The few arboviral detections in the mosquito populations reflect that the surveillance sites were located in areas of lower activity for this season. The highest notification rates (Figure 7b) were from the SLAs of Hay (408 cases/100,000 population), Jerilderie (332), Wentworth (205), Lachlan (179), Carrathool (176), Narrabri (139), Unincorporated (137), Narromine (131), Bland (117), and Warren (103). The inland is a region of low endemicity for BFV and very few cases were reported (11).

Currently the two main climatic models for MVEV are not suggestive of an MVEV epidemic for 2015-2016. However, as there has been considerable MVEV activity over recent years, there is the ongoing potential risk of vertical transmission of the virus through *Aedes* mosquitoes. Over the two seasons of 2011-2013, there were high collections of floodwater *Aedes* collected, notably *Aedes theobaldi* and *Aedes vittiger*. Viruses such as MVEV are known to survive in the eggs of floodwater *Aedes* species, which is a mechanism by which the virus can be maintained from one mosquito season to the next (Broom *et al*. 1995). This may have happened in late 2003 when there was one seroconversion in the sentinel chicken flock to MVEV at Menindee despite two concurrent seasons of dry weather (Doggett *et al*. 2004), and again in the recent season with the one MVEV seroconversion at Deniliquin. It is probable that some of the eggs of these floodwater species can remain viable for years to even decades. This means that as the MVEV activity was in our recent history, the risk of MVEV recurring over the next few years must be considered as a possibility.

Currently a strong El Niño episode is occurring and the Bureau of Meteorology is predicting this to persist until early 2016. Typically, El Niño’s are associated with drier conditions for eastern Australia and this should result in reduced vector production across inland regions and lessen the risk for possible MVEV activity. However, for many inland sites, the amount of water available for irrigation practices is usually more deterministic for overall vector abundance, especially in the Riverina.

The Coast. Like the inland, the coastal region was significantly warmer than usual over the last half of 2014, with several sites experiencing an earlier rise in vector abundance, particularly with *Aedes vigilax* following the spring high tides. The heavy coastal rains through December and January resulted in large mosquito collections, almost three times that of the previous season. With this heavy rain, the predominate species was *Culex annulirostris*, while *Aedes vigilax* populations were well below average, comprising only around 13% of the total collections, where usually this figure is around 50-60%.

Table 7. Notifications of BFV & RRV infection per virogeographic regions of NSW, per season from 1995-1996 to 2014-2015 (*after Doggett 2004, Doggett & Russell 2005*).

Season	BFV				RRV			
	Coastal Cases[1]	Inland Cases[2]	Sydney[3]	Total	Coastal Cases[1]	Inland Cases[2]	Sydney[3]	Total
94/95	233	8	7	**248**	163	45	14	**222**
95/96	141	9	3	**153**	399	511	32	**942**
96/97	155	19	16	**190**	731	566	250	**1,547**
97/98	103	14	2	**119**	162	129	41	**332**
98/99	208	26	8	**242**	575	522	117	**1,214**
99/00	158	22	6	**186**	359	341	43	**743**
00/01	367	18	3	**388**	432	218	115	**765**
01/02	371	14	11	**396**	135	73	6	**214**
02/03	407	21	6	**434**	395	57	10	**462**
03/04	303	26	6	**335**	417	176	41	**634**
04/05	394	33	9	**436**	327	87	23	**437**
05/06	536	58	20	**614**	730	419	119	**1,268**
06/07	504	47	38	**589**	428	196	52	**676**
07/08	471	49	17	**537**	638	453	105	**1,196**
08/09	355	38	10	**403**	614	275	63	**952**
09/10	246	41	6	**293**	511	493	119	**1,123**
10/11	299	112	38	**424**	264	349	25	**638**
11/12	256	38	7	**301**	237	250	32	**519**
12/13	364	36	23	**423**	297	130	43	**470**
13/14	224	23	9	**256**	304	144	47	**495**
14/15	167	11	10	**188**	1225	260	186	**1671**
Total	**6262**	**663**	**255**	**7155**	**9343**	**5694**	**1483**	**16520**
Ave[4]	**305**	**33**	**12**	**348**	**406**	**272**	**65**	**742**

[1]Represents the former Area Health Services of CC, HUN, ILL, MNC, NR and SA. [2]Represents the former Area Health Services of FW, GM, MAC, MW and NE. [3]Represents the former Area Health Services of CS, NS, SES, SWS, WEN and WS. [4]This is the nineteen season average of 1994/95 to 2013/14.

This season saw the largest RRV outbreak since notifications began being actively documented in 1985. The 1,392 human cases of infection reported from the coastal region was almost double the average of 711, and was dominated by RRV cases (1,225RRV versus 167BFV, Table 7, Figures 10 & 11). In terms of notifications for SLAs along the coast, Tweed, with 212 had the highest number of reports (204RRV & 8BFV), followed by Port Macquarie (131 notifications: 114RRV & 17BFV), Bryon

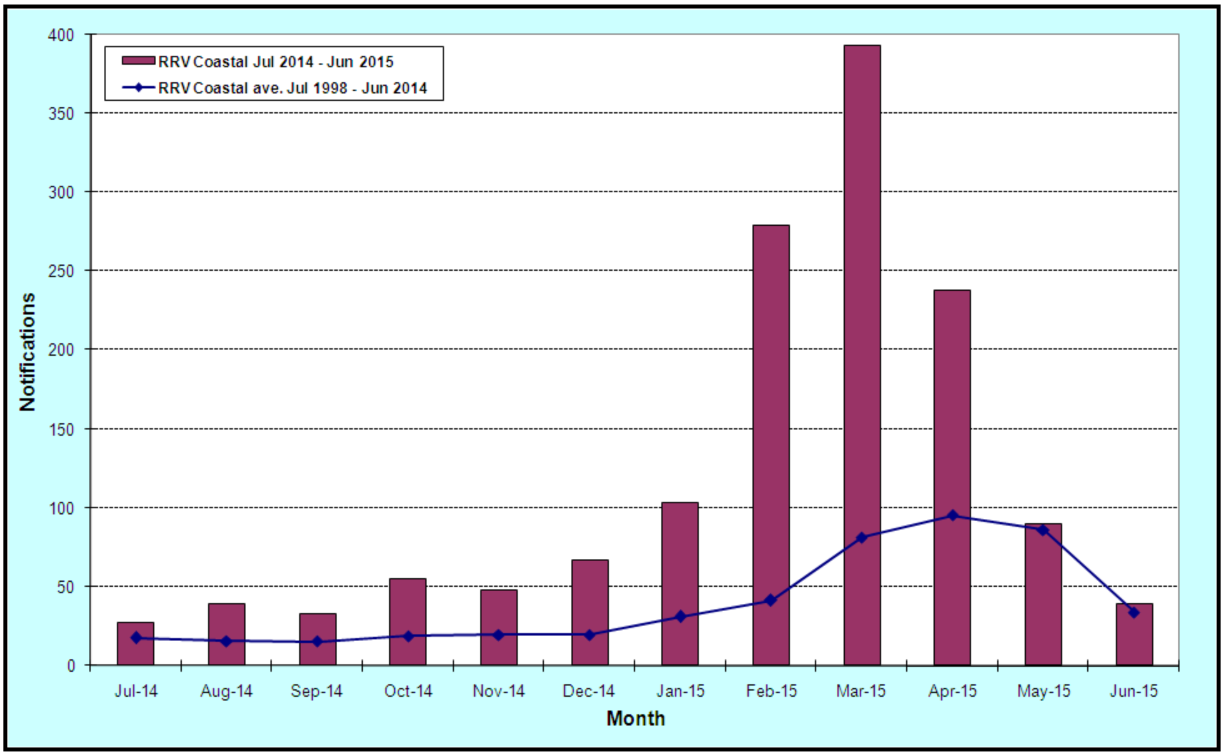


Figure 10. Notifications of RRV per month from coastal NSW. The bars are for 2014-2015 season and the line represents the long term average.

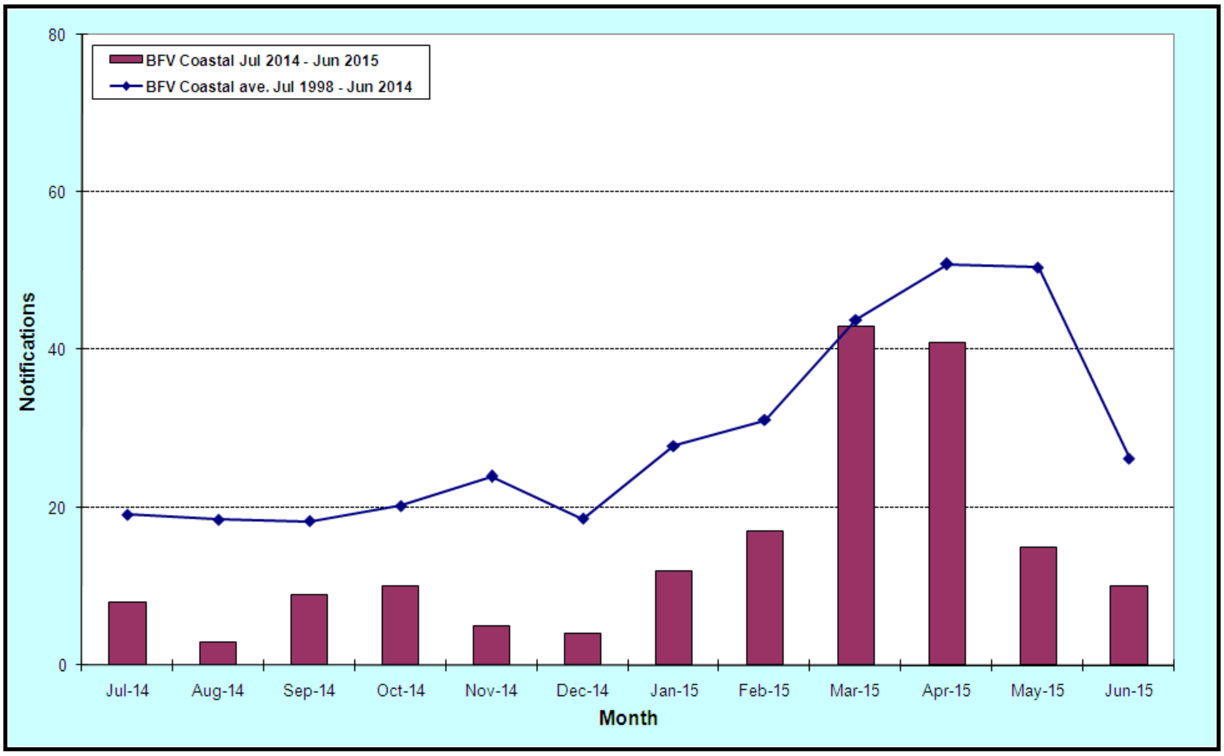


Figure 11. Notifications of BFV per month from coastal NSW. The bars are for 2014-2015 season and the line represents the long term average.

(112: 98RRV & 14BFV), Kempsey (86: 75RRV and 11BFV), Lismore (73: 62RRV and 11BFV), and Lake Macquarie (64: 63RRV and 1BFV, Figures 7a&c). In relation to notification rates (Figures 7b&d), Tweed was the highest for the coast with 398/100,000, followed by Byron (310), Lismore (292), Kempsey (256), Clarence

Valley (215), Bellingen (202), Port Macquarie (189), Nambucca (123), and Kyogle (116). There were no human flaviviruses notified from the region. It would appear that the season that was kicked off early with the elevated temperatures accompanied by the high spring tides and this allowed for a build up of arbovirus cycles. These factors may be responsible for the magnitude of the RRV outbreak. Again, this season may be a pointer to the future when impacts of climate change become more apparent.

There were a total of 41 isolates (6BFV, 29RRV, 4EHV and 2STRV), which is around normal, and this may be considered fewer than expected in light of the major RRV outbreak. The surveillance sites however, have been historically positioned for the monitoring of saltmarsh mosquitoes (especially *Aedes vigilax*), rather then freshwater species. As *Aedes vigilax* collections were so low, it is not surprising that the number of isolates were not greater. A review of the surveillance locations based around the locations of human infections is recommended.

In contrast to the inland, an El Nińo event can be favourable for *Aedes vigilax* production. This species requires the saltmarsh habitat to dry out for egg maturation and continual rainfall can actually lead to reduced larval numbers. A drier season may result in increased vector production, which would normally lead to a possible rise in arboviral notifications. However as last season had such intense RRV activity, herd immunity effects means that another outbreak would seem unlikely.

Sydney. Like elsewhere in the state, the above average temperatures heralded an early start to the mosquito season at the Sydney sites. For example, over 1,400 mosquitoes were trapped at the Haslams Creek of Homebush Bay in mid-November and 1,300 mosquitoes trapped in the first week of December at Alfords Point (Georges River). Both collections were dominated by *Aedes vigilax*. The early season rise resulted in overall mosquito numbers that was well up, in fact around double that of the previous season.

There were 20 arboviral isolates, including 2BFV, 15RRV and 3EHV, with most (13) being from Georges River. Of the mosquito species, three of the isolates were from *Aedes procax* (2RRV & 1EHV), two from *Aedes vigilax* (1BFV & 1 EHV), with one each from *Aedes multiplex* (EHV) and *Aedes* Marks no. 51 (RRV).

Georges River is the one Sydney site that continually produces large numbers of isolates from mosquitoes. Unlike the more urbanised trapping locations, the trapping sites at Georges River are surrounded by national parks, which contain numerous potential vertebrate hosts for the viruses. With the addition of major vectors in large numbers, notably *Aedes vigilax*, there is a real risk of arboviral infection to the local residents.

Unsurprisingly with all the RRV activity along the coast, notifications from Sydney were more than double the norm. The 196 human notifications included 186RRV & 10BFV (Table 6). How many of the Sydney reported human cases were locally acquired is unknown and it is likely that many of the patients became infected elsewhere in the state in the more hyperendemic regions. The SLAs with the highest number of notifications included Penrith (29 notifications: 27RRV & 2BFV), Blue Mountains (17: 14RRV & 3BFV), Kuringai (14RRV), Hills Shire (14RRV), Warringah (14RRV), and Hornsby (10RRV).

PASSIVE MOSQUITO TRAP & HONEY-BAITED FTA CARDS FOR ARBOVIRUS SURVEILLANCE IN NSW: FURTHER INVESTIGATIONS

Introduction. The use of sentinel animals for arbovirus surveillance posses a number of challenges; the placement of animals in optimal locations is often not possible, there are ethical implications in using animals, while van den Hurk & colleagues (2012) point out that some testing laboratories have issues with cross reactions in the serological tests. Thus alternative technologies not employing animals would appear to have several advantages.

A method that has recently been under investigation is the use of passive mosquito traps (PT, these do not have a motor) that have strips of specialised paper (FTA® cards) placed on the inside that are coated with honey. Mosquitoes enter the trap, feed on the honey and in the process expectorate (spit) out viruses. The viruses are then trapped on the paper which has been manufactured to capture viral nucleic acid. The paper is subsequently tested via a range of molecular based assays to determine which viruses are present. The PTs have already been demonstrated as being more sensitive than sentinel animals for the detection of flaviviruses in the field (Hall-Mendelin *et al*. 2010, van den Hurk *et al*. 2012), have the benefit of being a rapid technique for arbovirus identification, with isolates usually being identified within two days of samples coming into the laboratory, plus the technique has labour saving potentials as not every mosquito has to be identified prior to testing. The main disadvantages with this systems is that it is impossible to determine which mosquitoes are transmitting the viruses, and as a virus is not isolated, it is not available for further studies such as vector competence investigations.

Before implementing the PTs on a routine basis as part of the NSW Arbovirus Surveillance Program, it was necessary to undertake a comparison with current technologies for evaluation purposes. An initial limited comparison of the mosquito trapping capability of the PTs vs Encephalitis Vector Surveillance trap (EVS; these are the traps in current use) was undertaken during the mosquito season of 2012-2013, and further investigations were undertaken in the previous season of 2013-2014. The results of these were presented in the respective annual reports (Doggett *et al*. 2013, 2014).

From our initial results, the indications are that the honey-baited card system is considerably more sensitive at detecting alphaviruses then traditional cell culture and hence cards where placed in all traps for the 2014-2015 season. However, due to limited flavivirus activity in recent years, the relative sensitivity of the two assay systems at detecting this group of arboviruses has yet to be fully elucidated. Thus for the season of 2014-2015, continuing evaluation of the sensitivity of honey-baited FTA cards versus cell culture was again undertaken, mostly at inland locations where the threat of serious flavivirus activity is greater.

FTA cards versus cell culture. Honey-baited FTA cards were placed into the EVS traps and operated overnight as per normal. The trapped mosquitoes were identified and processed for arboviruses via cell culture, while the FTA cards were processed via PCR, with both procedures as described in the methods. A total of 681 traps were used in this evaluation and the results are presented in Table 8 below. Overall, the FTA cards were more than twice as sensitive at detecting arboviruses compared with cell culture. However in this comparison, cell culture was more sensitive at detecting the flaviviruses. Thus virus isolation via cell culture of the trapped mosquitoes will still continue at inland sites in conjunction with the use of FTA cards. For coastal sites, only FTA cards will be employed for arbovirus surveillance in the future.

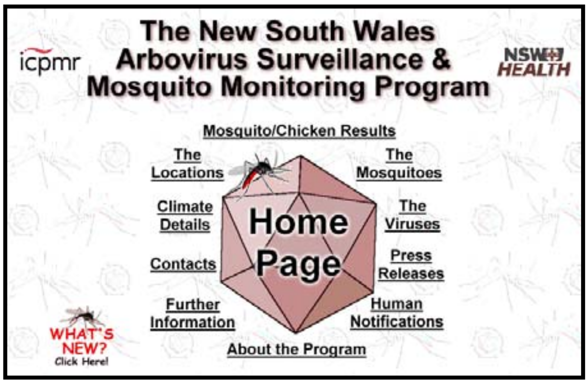
Table 8. Comparison of arboviral detection between the FTA cards and Cell Culture (CC).

	FTA			CC					
No. Traps	**RRV**	**BFV**	**Total**	**RRV**	**BFV**	**EHV**	**KOKV**	**STRV**	**Total**
681	23	4	**27**	5	2	3	1	1	**12**

THE NEW SOUTH WALES ARBOVIRUS SURVEILLANCE WEB SITE

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

The NSW Arbovirus Surveillance web site was established in early 1999 to facilitate the rapid dissemination of surveillance results (Doggett *et al.*, 1999b). An additional important function is to provide information on mosquitoes and the arboviruses they transmit. Over the last year, the site has continued to grow to the current size of 345MB, and has 2,510+ pages of information.

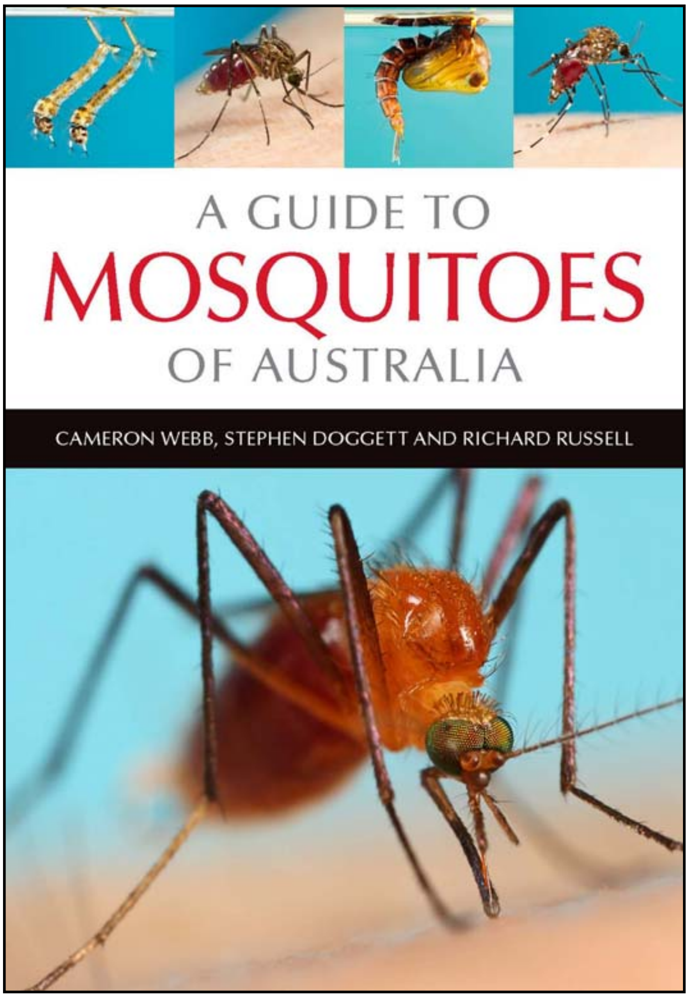


Added to the site since the last annual report includes:

- Archived data for the 2014-2015 season,
- Monthly rainfall summaries, with long-term averages,
- Monthly rainfall and temperatures maps,
- Daily high tides,
- Monthly SOI updates.

NEW PUBLICATION & COLLABORATIVE RESEARCH

The NSW Arbovirus Surveillance Program principal aim is to act as an early warning system for arbovirus activity. However, the Program is involved in collaborative projects with a number of researchers across the country, and internationally, acts as a reference laboratory for referral of mosquito advice.



A recent key project has been the development of a new mosquito text, "***A Guide to Mosquitoes of Australia***", which is being published by CSIRO Publications. The book is aimed at the secondary/tertiary level and highlights the biodiversity of mosquitoes, in particular their habitats and ecology. Around 100 mosquito species are described in detail, which includes all the major vector and pest species in Australia. All up, over 10,000 mosquito photographs were taken for the ***Guide***, with specimens derived from the NSW Arbovirus Surveillance Program. Release date is expected in February 2016, with an estimated price of around $40.00.

Research collaboration over the recent season included:

- Dr Chris Hardy, Biodiversity, Ecosystem Knowledge and Services, CSIRO; mosquito samples for DNA barcoding and identification.
- Ms Jana Batovska, Biosciences Research, Vic Agriculture; mosquito samples for DNA barcoding.
- Prof. Roy Hall, University of Queensland; insect specific flavivirus detection and viral genotyping.
- Assoc. Craig Williams, University of South Australia; detection of arboviruses via FTA cards.

Additionally, the Department of Medical Entomology provided consultation services for the confirmation of exotic mosquito specimens for New Zealand Biosecurity, and the Greater Los Angeles Mosquito District (the latter following the detection of *Aedes notoscriptus* in California). Staff from Entomology provided several key presentations at the Western Australia Department of Health biennial mosquito course.

Appendix 1. LOCATION-BY-LOCATION SUMMARY

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

Inland Locations

Albury: mosquito numbers were mostly 'low' throughout the season with only the occasional 'medium' collection, and the one 'high' catch (from mid-December). There were no arboviral isolates 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 from the trapped mosquitoes nor any seroconversions to MVEV or KUNV in the sentinel chickens.

Deniliquin: no mosquito collections were undertaken this season. 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: Collections at both sites were consistently 'very high' from mid-December until late February. Numbers peaked during early February with collections of over 6,000 during consecutive weeks at Barren Box. There were six arboviral isolates; this includes four from Barren Box, with one BFV (via FTA cards) and one KOKV (ex *Culex annulirostris*) from 11/Feb/15, one RRV from 18/Feb/15 (ex *Culex annulirostris*), and one RRV from 10/Mar/15. There were two BFV from Hanwood, one each from 18/Jan/15 and 11/Feb/15. There were no seroconversions to MVEV or KUNV in the sentinel chickens.

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

Leeton: mosquito numbers from both sites tended to be above average for most of the season. Collections peaked in the first week of January with one trap at Farm 347 yielding over 14,000 mosquitoes. There were five arboviral isolates. This includes four from Almond Road; one RRV from 23/Jan/15, one BFV from 11/Feb/15, one RRV from 3/Mar/15, and one RRV from 10/Mar/15. The one isolate from Farm 347 was trapped on 10/Mar/15. The isolate from the 3/Mar/15 came from *Anopheles annulipes*, whereas the rest were via the FTA cards. There were no seroconversions to MVEV or KUNV in the sentinel chickens.

Macquarie Marshes: six mosquito collections were made throughout the season and numbers were always 'low'. Despite this, there was one detection of RRV via the FTA cards from 6/Mar/15. 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.

Mathoura: trapping was again undertaken at Picnic Point and at Moama from where the sentinel chicken flock was located. The former site yielded the greater mosquito numbers, with several ‘high’ collections between January and mid-March, whereas mosquito collections at Moama were mostly ‘low’. There were no arboviral isolates nor any seroconversions to MVEV or KUNV in the sentinel chickens.

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

Wagga Wagga: trapping was undertaken at two sites and mosquito collections were ‘low’ for most of the season, with only the occasional ‘medium’ numbers during mid to late summer. 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. Both sites produced mosquito numbers that were ‘high’ or greater, with North Creek Road having ‘very high’ collections through March to early April. No arboviral isolates were detected.

Coffs Harbour: trapping was undertaken at Marcia Street and Christmas Bells Road. Collections at Marcia Street were variable throughout the season, with several ‘high’ traps during late February and March. Christmas Bells Road produced the greater mosquito numbers, with a series of ‘very high’ yields in March, with species dominated by *Culex annulirostris*. No arboviral isolates were detected.

Gosford: two sites at Gosford were again monitored this year: Empire Bay and Killcare Heights. For Empire Bay, collections were consistently ‘high’ throughout the season. Killcare tended to produce fewer mosquito numbers, although there were large collections of *Aedes vigilax* early in the season. There were two arboviral detections from the FTA cards at Killcare including one BFV from 20/Jan/15 and one RRV from 4/Mar/15.

Lake Macquarie: collections were undertaken from three sites: Belmont Lagoon, Teralba and Dora Creek. Mosquito numbers were consistently ‘low’ from all three sites until mid-February, where there were some ‘high’ collections in March. There were four arboviral detections from the FTA cards. This included one BFV from Terabla (20/Jan/15), one BFV from Belmont (/10Feb/15), one BFV from Dora Creek (17/Feb/15), and one RRV from Dora Creek (10/Mar/15).

Port Macquarie: Trapping was undertaken at three sites; Yarranabee Road, Partridge Creek, and Stevens Street. The latter site produced the greatest numbers, with a series of ‘high’ collections from mid-January to late March, being dominated by *Culex annulirostris*. Partridge Creek also tended to have ‘high’ numbers during this period, but collections were smaller. Yarranabee Road produced the smallest collections. There were two arboviral detections via the FTA cards. This included one EHV from Yarranadee Road (23/Apr/15) and one STRV from Partridge Creek

(5/May/15).

Port Stephens: no surveillance was undertaken this season.

Tweed Heads: trapping was undertaken at three sites; Koala Beach, Beltana Drive and Piggabeen Road. All sites tended to catch ‘high’ mosquito numbers for most of the season, although Beltana Road yielded the greatest collections. Numbers at this site even reached ‘very high’ during late February with *Culex sitiens* being the predominate mosquito species collected. This species also dominated at Piggabeen Road and the most common mosquito at Koala Beach was *Culex annulirostris*. There were nine arboviral detections and these are listed in Table 9 below.

Table 9. Arboviral Isolates from Tweed Heads, 2014-2015.

LOCATION - Site	Date Trapped	Mosquito Species	Virus		
			RRV	STRV	Total
TWEED HEADS - Beltana	23-Jan-15	*	1		**1**
TWEED HEADS - Piggabeen	23-Jan-15	*	1		**1**
TWEED HEADS - Beltana	9-Feb-15	*Aedes vigilax*		1	**1**
TWEED HEADS - Koala Beach	24-Feb-15	*	1		**1**
TWEED HEADS - Beltana	10-Mar-15	*	1		**1**
TWEED HEADS - Koala Beach	10-Mar-15	*	1		**1**
TWEED HEADS - Piggabeen	10-Mar-15	*	1		**1**
TWEED HEADS – Beltana	8-Apr-15	*	1		**1**
TWEED HEADS - Piggabeen	5-May-15	*	1		**1**
		TOTAL	**8**	**1**	**9**

*Positive via FTA cards

Wyong: trapping was undertaken at three sites: Ourimbah, Halekalani and Charmhaven, with the latter two sites tending to produce ‘low’ mosquito numbers. Ourimbah yielded on average, ‘medium’ collections, dominated by *Aedes notoscriptus*. 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. Mosquito numbers were consistently ‘high’ from the start of trapping in early December to early February. There were two arboviral detections at Deepwater including one RRV detected via FTA cards from the collection made on 17/Dec/14 and one BFV from *Aedes vigilax* trapped on 27/Jan/15.

Blacktown: Collections were made at two sites; Nurrangingy Reserve and Ropes Crossing. The latter site mainly produced ‘low’ mosquito numbers, while Nurrangingy Reserved yielded consistently ‘high’ collections through February and March, with *Culex annulirostris* dominating. No arboviral isolates were detected.

Georges River: trapping was undertaken at the four sites of Alfords Point, Lugarno, Illawong, and Picnic Point. Most of the collections were ‘high’ in number at Alfords Point and Picnic Point, which dominated by *Aedes vigilax*. The other two sites tended to produce ‘medium’ collections with *Aedes notoscriptus* being more commonly trapped due to the urban location of these sites. There 13 arboviral detections and there are listed in Table 10 below.

Table 10. Arboviral Isolates from Georges River, 2014-2015.

LOCATION - Site	Date Trapped	Mosquito Species	Virus			
			BFV	RRV	EHV	Total
GEORGES RIVER - Alfords Point	11-Feb-15	*		1		**1**
GEORGES RIVER - Alfords Point	17-Feb-15	*Aedes vigilax*	1			**1**
GEORGES RIVER - Picnic Point	17-Feb-15	*		1		**1**
GEORGES RIVER - Picnic Point	24-Feb-15	*Aedes multiplex*			1	**1**
GEORGES RIVER - Lugarno	3-Mar-15	*Aedes procax*			1	**1**
GEORGES RIVER - Picnic Point	9-Mar-15	*		1		**1**
GEORGES RIVER - Deepwater	9-Mar-15	*		1		**1**
GEORGES RIVER - Lugarno	9-Mar-15	*		1		**1**
GEORGES RIVER - Alfords Point	18-Mar-15	*		1		**1**
GEORGES RIVER - Deepwater	18-Mar-15	*		1		**1**
GEORGES RIVER - Illawong	18-Mar-15	*		1		**1**
GEORGES RIVER - Lugarno	18-Mar-15	*		1		**1**
GEORGES RIVER - Picnic Point	31-Mar-15	*		1		**1**
		TOTAL	**1**	**10**	**2**	**13**

*Positive via FTA cards

Hawkesbury: trapping was undertaken four sites on various weeks, including at Wheeney Creek, Yarramundi, Sackville and McGraths Hill. Most sites tended to produce ‘low’ mosquito numbers although Wheeney Creek yielded ‘high’ catches through mid-February to early March. There were four RRV detections from the Wheeney Creek collection made on 3/Mar/15, including two from *Aedes procax*, one from *Aedes* Marks no. 51, and one from the FTA cards.

Penrith: trapping was undertaken at the three sites of Emu Plains, Muru Mittigar and Glenmore Park. For most of the season, mosquito numbers tended to be ‘low’ to ‘medium’, although Muru Mittigar yielded ‘high’ numbers in January and April. *Aedes notoscriptus* was the main species trapped during these times. No arboviral isolates were detected.

Ryde: trapping was undertaken by the Medical Entomology staff from the two sites of Wharf Road and Lambert Park. The former site produced the greater mosquito collections with mainly ‘high’ numbers dominated by *Culex sitiens*. Lambert Park tended to have ‘medium’ yields with some ‘high’ collections in late March dominated by *Culex annulirostris*. No arboviral isolates were detected.

Sydney Olympic Park: mosquito monitoring at this location included the long-term locations of Narawang and Haslams Creek, as well as the new site of Newington. Collections were consistently ‘high’ throughout most of the season, with Aedes vigilax dominating the larger collections. There were five arboviral detections. This included one EHV from *Aedes vigilax* trapped at Haslams Creek on 9/Feb/15, and four RRV detected via FTA cards. The latter included detections at Newington Nature Reserve (one each 24/Feb/15 & 9/Mar/15), one at Haslams Creek (9/Mar/15), and one at Nurrawang Wetlands (18/Mar/15).

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 (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 on 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
GODSEND	Graphical Online Data Surveillance and Evaluation for Notifiable Diseases
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
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)

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