THE NEW SOUTH WALES ARBOVIRUS SURVEILLANCE & MOSQUITO MONITORING PROGRAM

2013-2014 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, L. McINTYRE, H. LIM, & D.E. DWYER

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

PathologyWest ICPMR Westmead

NSW Government Health Pathology

ISBN 1-74080-154-7

© 2014. Department of Medical Entomology,
Institute of Clinical Pathology & Medical Research,
Pathology West, Westmead Hospital,
Westmead, NSW Australia 2145.

EXECUTIVE OVERVIEW

- **For the 2013-2014 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 2013-2014 season for the inland were of well below average rainfall for the last six months of 2013. In contrast, rainfall was above average for most of the inland during the first six months of 2014. The Forbes hypothesis was not suggestive of a potential MVEV epidemic for the 2013-2014 season, however the Nichols' theory was not exclusive of possible activity. For the coast, conditions were mostly similar, however the dry conditions continued for the north coast into the first three months of 2014.
- **For the inland**, the dry conditions produced fewer mosquito numbers with a total trapped of around 100,000, being about 30,000 down upon the previous season. There were eight arboviral detections; 3RRV and 1STRV from Griffith, and 3RRV and 1BFV from Leeton. There were four seroconversions in the sentinel chickens including 1MVEV from Deniliquin (from the bleed taken on 31/Mar/14) and 1KUNV each from Forbes (11/Feb/14), Griffith (12/Feb/14) and Leeton (30/Mar/14).
- **Human notifications from the inland** of RRV and BFV totalled 167 (144RRV & 23BFV), which was close to half the long term average of 311. There were no human cases of flavivirus infection reported.
- **As of September 2014**, the Forbes hypothesis is not suggestive of possible MVEV activity for the season of 2014-2015, however the Nicholls hypothesis does not exclude the risk.
- **For the coast**, with the continual dry conditions mosquito numbers were below average. There was overall 58 isolates, including 12BFV, 33RRV, 2EHV, and 11STRV.
- **Coastal disease notifications** of RRV and BFV totalled 528 cases, including 304 RRV and 224 BFV, and this was below the average of 720. The statistical local area that produced the highest case load was Tweed, with 71 notifications (28RRV & 43BFV), followed by Hastings (45 notifications: 28RRV & 17BFV), Bryon (36: 14RRV & 22BFV), and Port Stephens (35: 22RRV and 13BFV).
- **For Sydney**, mosquito collections were fewer this season, and as a consequence, overall mosquito numbers were down. There was a record level of arboviral activity detected with 34 isolates, including 8BFV, 15RRV and 11STRV, with most (29) being from Georges River. Despite the high number of arboviral detections, human notifications were below the average of 78, with a total of 56 reports including 47RRV and 9BFV.
- **New methodologies** to increase the sensitivity of the surveillance system continue to be successfully trialled. The rapid molecular based system of virus identification via honey-baited cards will be 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 321MB, and has 2,440+ pages.

TABLE OF CONTENTS

NSW ARBOVIRUS SURVEILLANCE AND MOSQUITO MONITORING PROGRAM 2013-2014

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 2013-2014 season follow.

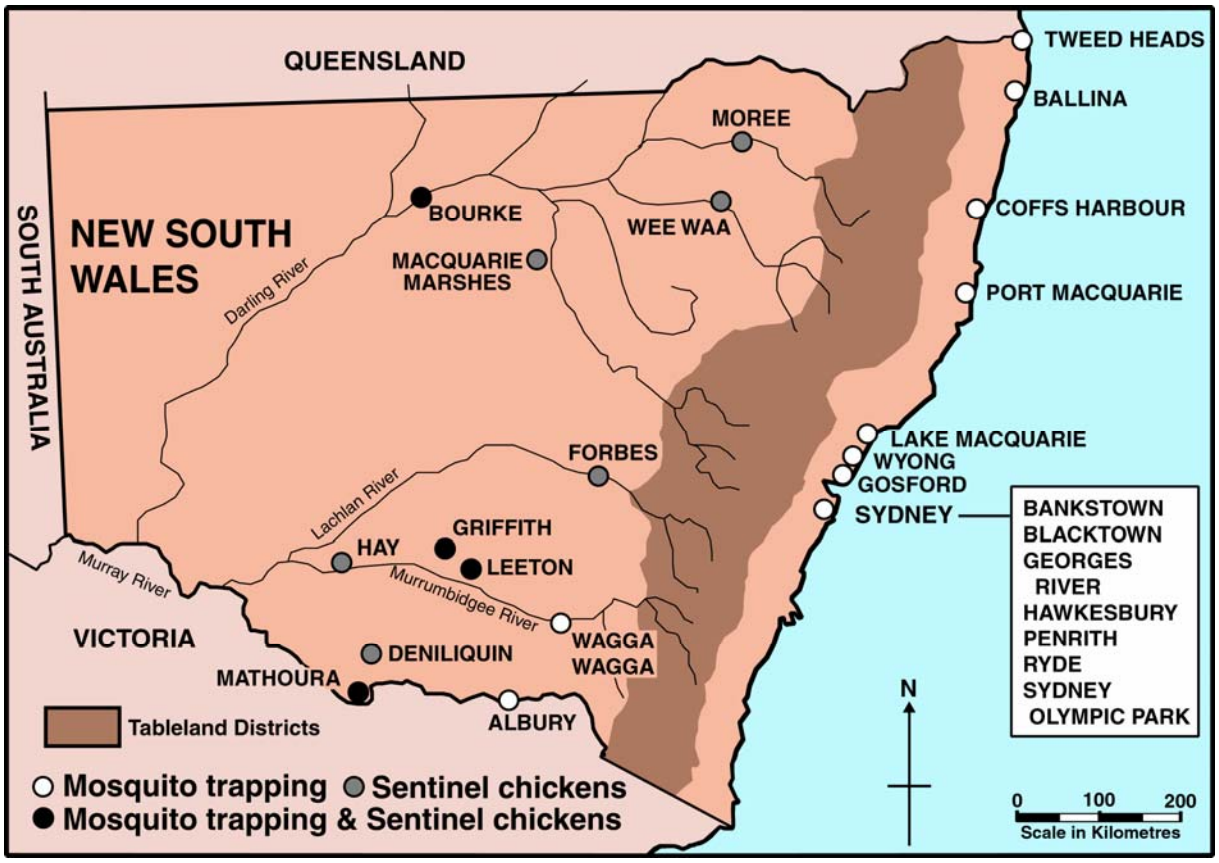


Fig 1. Mosquito trapping locations and Sentinel Chicken sites, 2013-2014.

MONITORING LOCATIONS

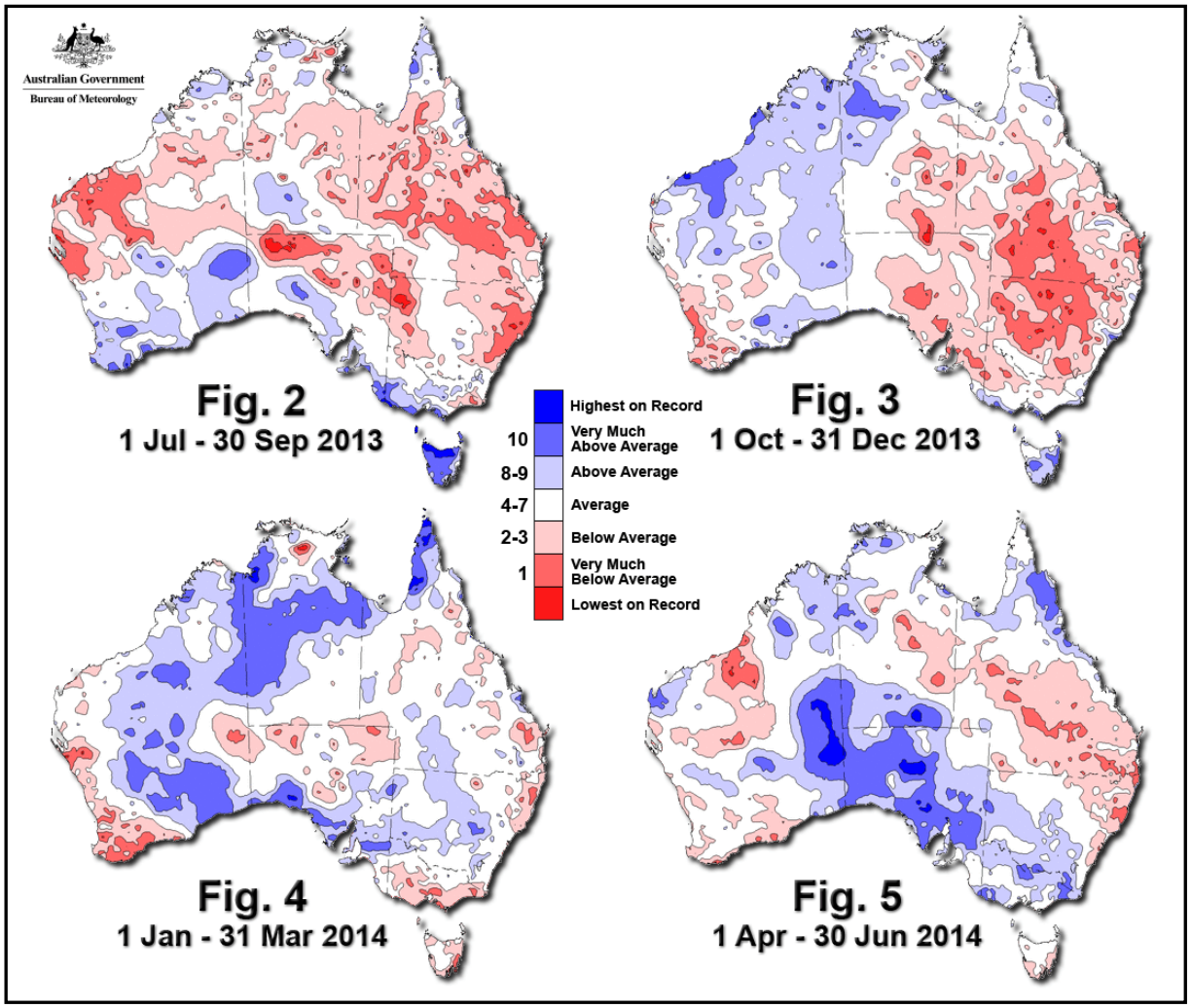
http://medent.usyd.edu.au/arbovirus/location/locations.htm

For 2013-2014, mosquito-trapping sites were operated at 6 inland, 7 coastal and 7 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 2013, Oct-Dec 2013, Jan-Mar 2014 & Apr-Jun 2014. The stronger the red, the drier the conditions. Conversely, the stronger the blue, the wetter the conditions. *Modifed from the Australian Bureau of Meterology, 2014*.

During the first quarter of 2013 (i.e. Jan – Mar), average rainfall was experienced across the inland, however the coastal strip was very wet, with very much above average rainfall. Most parts of the state had normal rainfall during the second quarter of 2013. The third quarter of 2013, was extremely dry across the state, with many areas experiencing very much below average precipitation (Figure 2). This was followed by an even drier quarter where some regions of NSW even experienced record low rainfall (Figure 3). In the first three months of 2014, above average rainfall fell across the inland, however the coastal strip was still mostly dry, with below average rainfall especially along the north coast (Figure 4). For the second quarter of 2014, the south west of the state had above normal rainfall, while the north east had below average rainfall (Figure 5).

Temperatures for the last half of 2013 were above average by 2-3 degrees, which continued into January and February. March temperatures were below average for central regions of the state, while for April southern areas were cooler than normal, and northern regions experienced above average temperatures.

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 2012) or the first quarter of the current year (January-March 2013) and the last quarter of the current year (October-December 2013), 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 2012, was above decile 7 in only one catchment basin in the first quarter of 2013, and not above decile 7 in any of catchment basins for the last quarters of 2013, thus the Forbes hypothesis was not fulfilled for 2013-2014 (Table 1). Additionally, decile 7 or above rainfall did not occur across all the catchment basins during the first quarter of 2014, therefore according to Forbes', there should be a lower risk of an MVEV epidemic for the upcoming 2014-2015 season.

Table 1. Rainfall indices for the main catchment basins of eastern Australia as per Forbes hypothesis, relevant to the 2012-2013 and 2013-2014 seasons.

Catchment Basin	Oct-Dec 2012	Jan-Mar 2013	Oct-Dec 2013	Jan-Mar 2014
Darling River	0.48	0.91	0.41	0.75
Lachlan/Murrumbidgee/ Murray Rivers	0.50	1.02	0.49	1.26
Northern Rivers	0.64	0.63	0.83	0.91
North Lake Eyre system	0.73	0.41	0.52	0.83

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 2013, the SO values were respectively: 1009.23mm, 1012.27mm and 1010.00mm (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 mosquitoes.

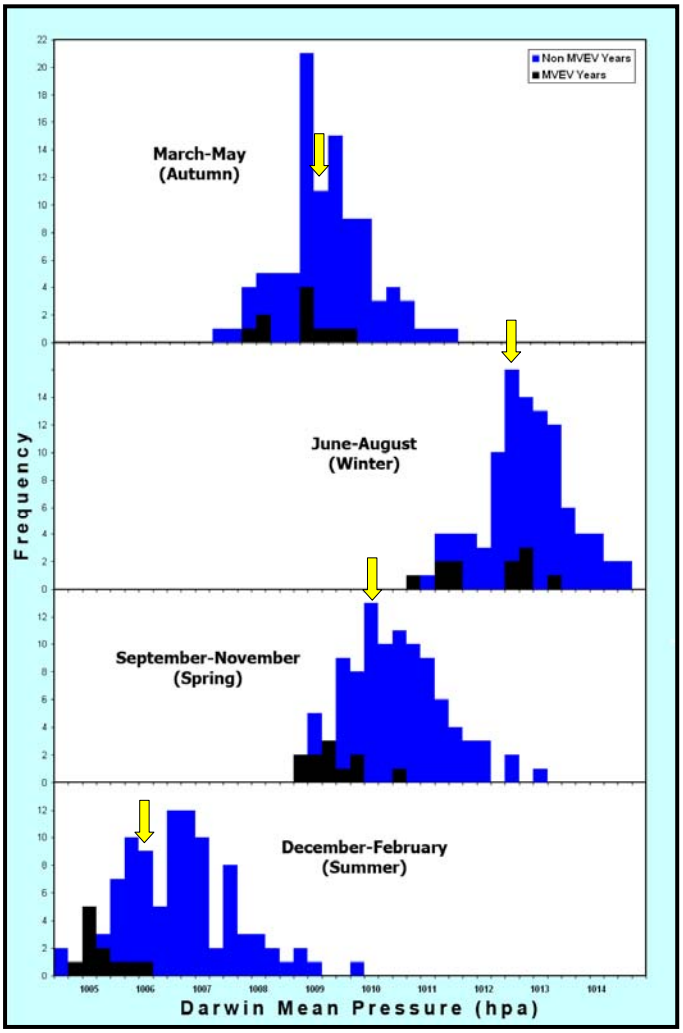


Figure 6. The SO by seasons prior to MVEV active years, according to Nicholls (1986), updated up to Spring 2013. The black bars represent the pre-MVEV active seasons. The yellow arrows indicate the respective SO values relevant to the 2012-2013 season.

As of August 2014, the autumn Nicholls' value is 1009.90mm and the incomplete winter value is 1012.50. Both of these are within the range of values during past MVEV outbreak years, suggesting a potential risk for 2013-2014.

Table 2. The seasonal atmospheric pressures (in mm) according to Nicholls' hypothesis, relevant to the 2013-2014 season.

	Autumn 2013	Winter 2013	Spring 2013
2013 Values	1009.23	1012.27	1010.00
Pre past MVEV seasons	<1009.74	<1012.99	<1009.99

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

MOSQUITO MONITORING

Methods

Mosquitoes were collected overnight in dry-ice baited Encephalitis 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, 98,893 mosquitoes representing 47 species were collected in NSW during 2013-2014, which was around 20% less 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 43,252 mosquitoes comprising 17 species was well below the previous season total of 128,309 trapped in 2012-2013. *Culex annulirostris* was the dominant species yielded at most sites and comprised 62.5% of the total inland collections. *Anopheles annulipes* (27.5%) was the next most common species.

Coastal

In total, 26,330 mosquitoes comprising 30 species were collected from coastal NSW and this was below the previous season's total. The most common species collected were *Culex sitiens* (27.2%), *Aedes vigilax* (23.1%), *Aedes notoscriptus* (17.9%), and *Verrallina funerea* (8.3%). 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 26,330 mosquitoes, comprising 30 species, was collected from metropolitan Sydney and this was around a third less of the previous season's total collection. The decrease was largely due to the reduced trapping at Bankstown and Ryde. *Aedes vigilax* (61.7% of the total Sydney mosquitoes trapped) was the most common species, followed by *Culex annulirostris* (11.2%), *Aedes notoscriptus* (8.3%), and *Anopheles annulipes* (4.7%).

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 64 arboviral detections; eight from the inland (Table 3) and 58 from the coast (Table 4).

Table 3. Arbovirus isolates from inland NSW, 2013-2014.

LOCATION	Date Trapped	Mosquito Species	Virus			
			BFV	RRV	STRV	Total
LEETON	2-Apr-14	*Aedes theobaldi*		1		**1**
LEETON	12-Mar-14	*		2		**2**
GRIFFITH	11-Mar-14	*	1			**1**
GRIFFITH	11-Mar-14	*		1		**1**
GRIFFITH	14-Jan-14	*Culex annulirostris*			1	**1**
GRIFFITH	26-Mar-14	*		1		**1**
GRIFFITH	17-Mar-14	*		1		**1**
		TOTAL	**1**	**6**	**1**	**8**

SENTINEL CHICKEN PROGRAM

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

Location of flocks

The 2013-2014 season began on 3rd November 2013 with the first bleed and ended on 14th April 2014 with the last. A total of ten flocks each containing up to 15 Isa Brown pullets was deployed, with one flock each at Bourke, Deniliquin, Forbes, Griffith, Hay, Leeton, Macquarie Marshes, Moama (near Mathoura), Moree and Wee Waa (Figure 1).

Table 4. Arbovirus isolates from coastal NSW, 2013-2014.

LOCATION	Date Trapped	Mosquito Species	Virus				
			BFV	RRV	EHV	STRV	Total
GEORGES RIVER	14-Jan-14	*Aedes notoscriptus*				3	**3**
GEORGES RIVER	21-Jan-14	*Aedes notoscriptus*				1	**1**
GEORGES RIVER	21-Jan-14	*Aedes vigilax*				2	**2**
GEORGES RIVER	29-Jan-14	*Aedes notoscriptus*				3	**3**
GEORGES RIVER	29-Jan-14	*Aedes vigilax*	1			1	**2**
GEORGES RIVER	4-Feb-14	*Aedes notoscriptus*				1	**1**
BANKSTOWN	18-Feb-14	*	1				**1**
GEORGES RIVER	18-Feb-14	*Aedes vigilax*		3			**3**
GEORGES RIVER	18-Feb-14	*	1	1			**2**
GEORGES RIVER	25-Feb-14	*Aedes vigilax*	1				**1**
BANKSTOWN	4-Mar-14	*		1			**1**
GEORGES RIVER	4-Mar-14	*Aedes vigilax*		3			**3**
GEORGES RIVER	4-Mar-14	*	2	1			**3**
TWEED	5-Mar-14	*		1			**1**
GEORGES RIVER	11-Mar-14	*	1	1			**2**
TWEED	11-Mar-14	*		2			**2**
GEORGES RIVER	17-Mar-14	*		1			**1**
PORT MACQUARIE	17-Mar-14	*	1	2			**3**
TWEED	17-Mar-14	*		1			**1**
BALLINA	18-Mar-14	*		1			**1**
COFFS HARBOUR	18-Mar-14	*		1			**1**
GEORGES RIVER	18-Mar-14	*Aedes vigilax*	1				**1**
PORT STEPHENS	18-Mar-14	*Coquillettidia linealis*		1			**1**
PORT STEPHENS	25-Mar-14	*		2			**2**
PORT STEPHENS	25-Mar-14	*Aedes vigilax*			1		**1**
PORT MACQUARIE	26-Mar-14	*		3			**3**
GOSFORD	1-Apr-14	*Aedes procax*	1				**1**
PORT STEPHENS	1-Apr-14	*Aedes procax*			1		**1**
PORT STEPHENS	8-Apr-14	*Aedes vigilax*	1				**1**
PORT STEPHENS	15-Apr-14	*Coquillettidia linealis*		1			**1**
PORT STEPHENS	15-Apr-14	*Coquillettidia xanthogaster*		2			**2**
BLACKTOWN	17-Apr-14	*Aedes procax*		1			**1**
BLACKTOWN	17-Apr-14	*Aedes vigilax*		2			**2**
PORT STEPHENS	23-Apr-14	*Culex annulirostris*	1				**1**
GEORGES RIVER	29-Apr-14	*		1			**1**
PORT STEPHENS	29-Apr-14	*Aedes vigilax*		1			**1**
		TOTAL	**12**	**33**	**2**	**11**	**58**

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

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 2 deaths were recorded. A total of 2,871 samples was received from the ten flocks in NSW over the six-month period in 2013-2014. This represented 5,742 ELISA tests (excluding controls and quality assurance samples), with each specimen being tested for MVEV and KUNV antibodies. There were four seroconversions and these are listed in Table 5 below.

Table 5. Seroconversions in the sentinel chicken flocks, 2013-2014.

LOCATION	Date Bled	MVEV	KUNV	Total
Forbes	11/Feb/14		1	1
Griffith	12/Feb/14		1	1
Leeton	30/Mar/14		1	1
Deniliquin	31/Mar/14	1		1
	TOTAL	**1**	**3**	**4**

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.

Table 6. Arbovirus notifications according to former Area Health Service, July 2013 - June 2014*.

Month	CS	NS	WS	WE	SW	CC	HU	IL	SE	NR	MN	NE	MA	MW	FW	GM	SA	Total
RRV	2	12	4	11	7	17	124	9	11	88	49	46	18	22	18	40	17	495
BFV	2	2	0	3	1	17	48	5	1	105	46	8	1	6	3	5	3	256
Total	**4**	**14**	**4**	**14**	**8**	**34**	**172**	**14**	**12**	**193**	**95**	**54**	**19**	**28**	**21**	**45**	**20**	**751**

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. *Data from 'GODSEND'.

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 appears to be continuing (Doggett 2014). Some laboratories are now reporting a 95% false positive rate with the commercial kit. Thus any epidemiological interpretation of the BFV notifications must be viewed with a high degree of uncertainty.

The total number of RRV and BFV notifications for the period July 2013 to June 2014 was 751 and included 256BFV and 495RRV. This season was well below the long term average of 1,078. The coastal region accounted for 528 (70% of the state total) of the BFV and RRV notifications, which was below the seasonal average of 720. The 167 notifications (22.2% of the state total) from the inland were around half the average of 311. Within the Sydney region there were 56 cases reported, well below the seasonal average (78 notifications). The reduced number of BFV notifications for the state (256 down from the average of 394) was probably due to corrections with the commercial serological assay.

From the coast, the Northern Rivers and Hunter Health Services received the highest notifications (193 and 172 respectively), with the Mid-North Coast having 95. Combined, these three areas accounted for 61.3% of all the arbovirus notifications for the state. From the inland, the New England AHS had the highest number of notifications (54), although it is likely that many of these patients acquired their infections from the coastal region.

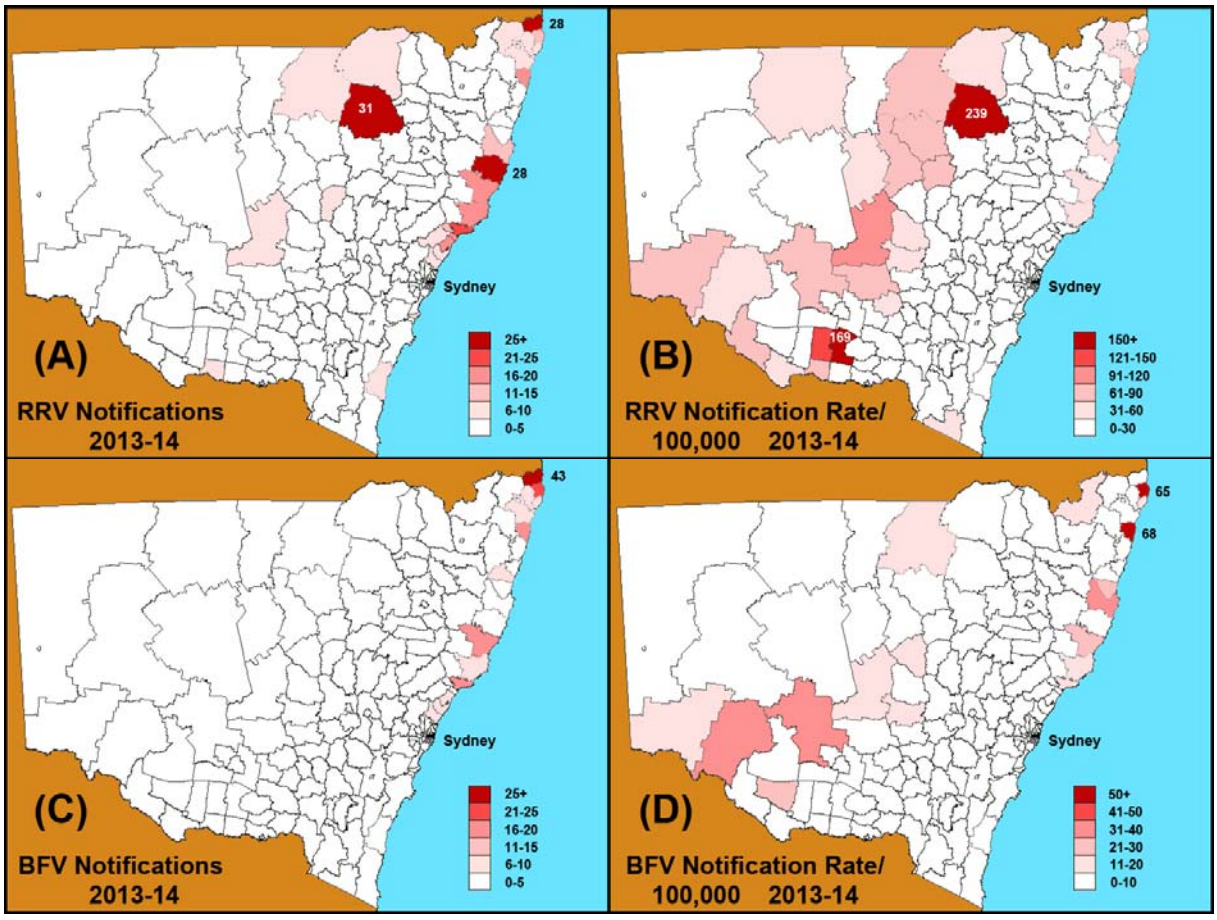


Figure 7. Notifications and notification rates of RRV and BFV by Statistical Local Areas for NSW for Jul 2013 to Jun 2014. (A) RRV notifications. (B) RRV notification rate/100,000 population. (C) BFV notifications. (D) BFV notification rate/100,000 population. Note that different scales are used on the notifications and rates graphs. Data from 'GODSEND'.

Figure 7 depicts the notifications and notification rates of RRV and BFV by Statistical Local Area (SLA) for NSW during the 2013-2014 mosquito season.

There were no human locally acquired flavivirus seroconversions reported.

DISCUSSION

The Inland. Over recent seasons across the inland there has been extensive arbovirus activity, which was associated with extreme rainfall patterns and increased vector production. In 2010-2011, over 200,000 mosquitoes were trapped with some 105 arboviral isolates, 21 sentinel chickens seroconversions, a major outbreak of KUNV in horses, increased RRV notifications, and an outbreak of disease from BFV. For 2011-2012, mosquito collections totalled over 170,000, there were some 67 arboviruses isolated, 15 seroconversions in the sentinel chickens, however human notifications were around average.

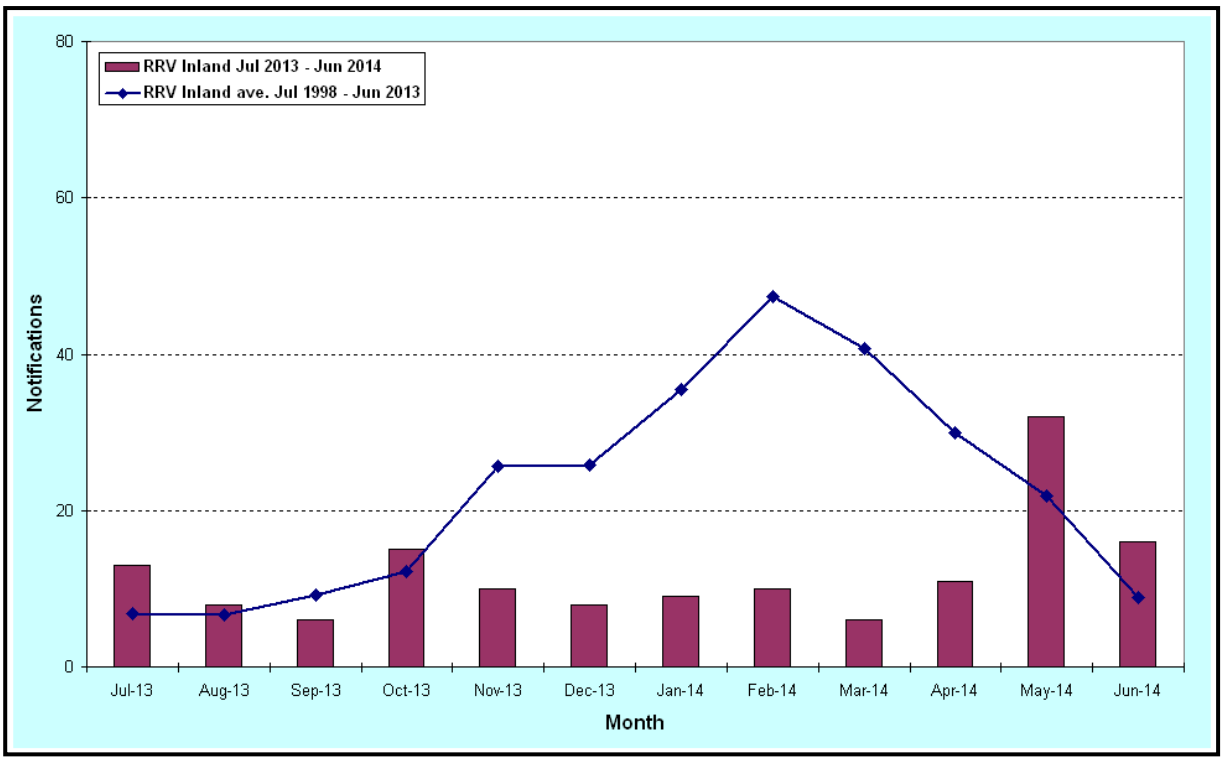


Figure 8. Notifications of RRV per month from inland NSW. The bars are for 2013-2014 season and the line represents the long term average. Data from 'GODSEND'.

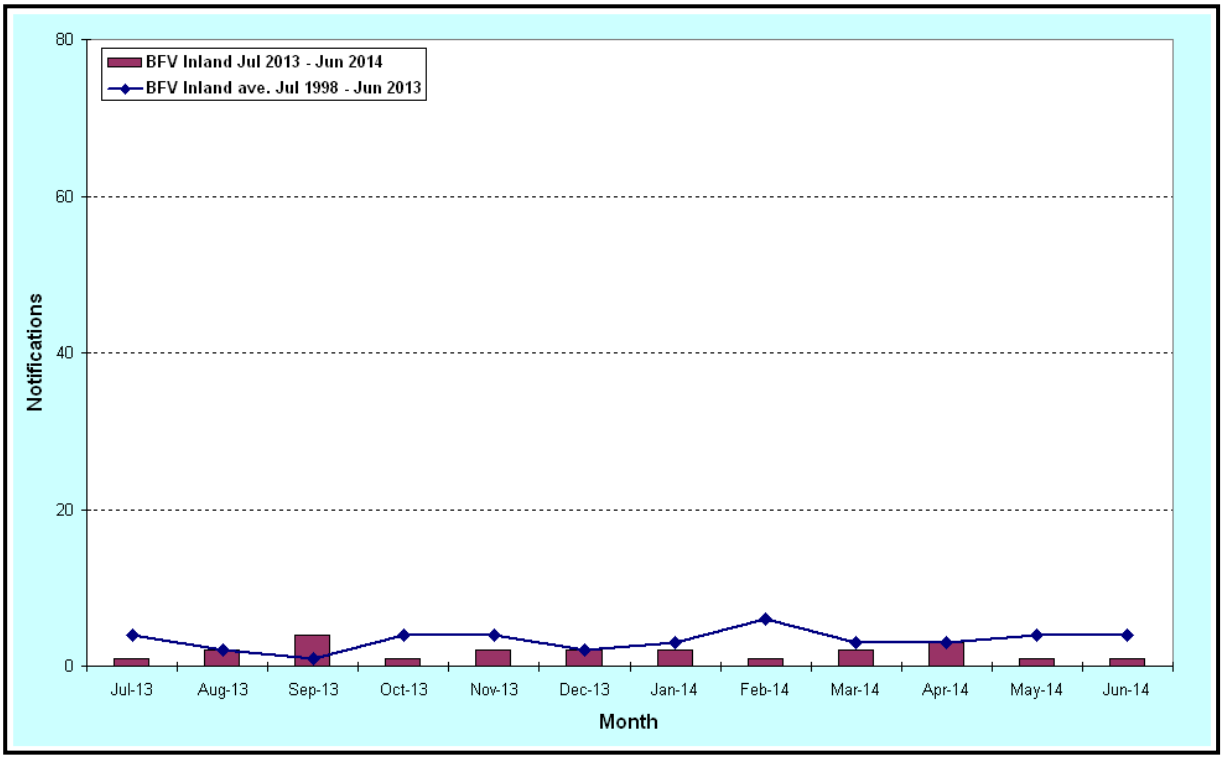


Figure 9. Notifications of BFV per month from inland NSW. The bars are for 2013-2014 season and the line represents the long term average. Data from 'GODSEND'.

In contrast, the succeeding two seasons were much drier. Both in 2012-2013 and again in the recent season of 2013-2014, the second half of the proceeding year (i.e.

2013 for 2013-2014), precipitation was below to well below average. As a consequence, there was relatively minimal arboviral activity in both seasons. For 2013-2014, mosquito numbers considerably down upon recent years (in fact less than half the number where trapped compared with 2010-2011) and human notifications were around half the long term average. Additionally, there were few arboviral isolations and while there were some late season flavivirus seroconversions in the sentinel chickens, these largely occurred when vector numbers had declined and probably represented little risk to the community.

As mentioned above, human notifications were around half the long term average (Figures 8 & 9, Table 7). The SLAs that produced most inland RRV cases (Figure 7a) included Narrabri (32 notifications, which was also the highest over the previous three seasons), Lachlan (8), Moree (7) and Walgett (7). The highest rates (Figure 7b) were from the SLAs of Narrabri (247 cases/100,000 population), Jerilderie (130), Lachlan (121), Wakool (111), and Walgett (102). Eight of the top ten highest SLA notification rates for RRV in the state this season were from the inland, indicating the greater risk of arboviral disease for communities from inland regions of NSW.

The inland is a region of low endemicity for BFV and few cases were reported (23). Despite this figure being close to average, there is considerable misdiagnosis of BFV and there are probably very few true local cases.

Currently the two main climatic models for MVEV epidemics are contrary. Thus while the Forbes hypothesis is not suggestive of an MVEV epidemic for 2014-2015 the Nichols is indicating the opposite. Also, as there has been considerable MVEV activity over recent years, there is the 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.

Presently the Bureau of Meteorology is predicting a possible El Nińo event for 2014, which is typically associated with drier conditions for eastern Australia. This should result in reduced vector production across inland regions and lessen the risk for possible MVEV activity.

The Coast. With the period of low precipitation during the preceding winter and spring, and the summer months of 2013-2014 also being relatively dry, it was no surprise that mosquito numbers were down upon recent years. The 58 isolates, while being above average for the coast, were boosted in number by the new more sensitive arboviral detection technology, along with the intense trapping program at Port Stephens. The latter involved a comparison of the FTA cards with arboviral

isolation via traditional cell culture. Taking these into account, the number of isolations would have been much lower than normal and the reduced isolations were reflected in the below average number of human notifications.

Table 7. Notifications of BFV & RRV infection* per virogeographic regions of NSW, per season from 1995-1996 to 2013-2014 (*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**
Total	**6,095**	**652**	**245**	**6,967**	**8,118**	**5,434**	**1,297**	**14,849**
Ave[4]	309	33	12	353	411	278	66	755

[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 2012/13. *Data from 'GODSEND'.

Despite the late seasonal rainfall in March which resulted in increased vector numbers, this came too late to influence arbovirus activity.

Overall, there were 528 human cases of arboviral infection reported from the coastal region being down upon the average of 720 (Table 7), and included 304RRV and 224BFV. There were no human flaviviruses notified from the region. In terms of notifications for SLAs along the coast, Tweed, with 71 had the highest number of reports (28RRV & 43BFV), followed by Hastings (45 notifications: 28RRV & 17BFV), Bryon (36: 14RRV & 22BFV), and Port Stephens (35: 22RRV and 13BFV, Figures 7a&c). In relation to notification rates (Figures 7b&d), Urana was the highest for the coast with 169/100,000, followed by Maclean (139), and Byron (106).

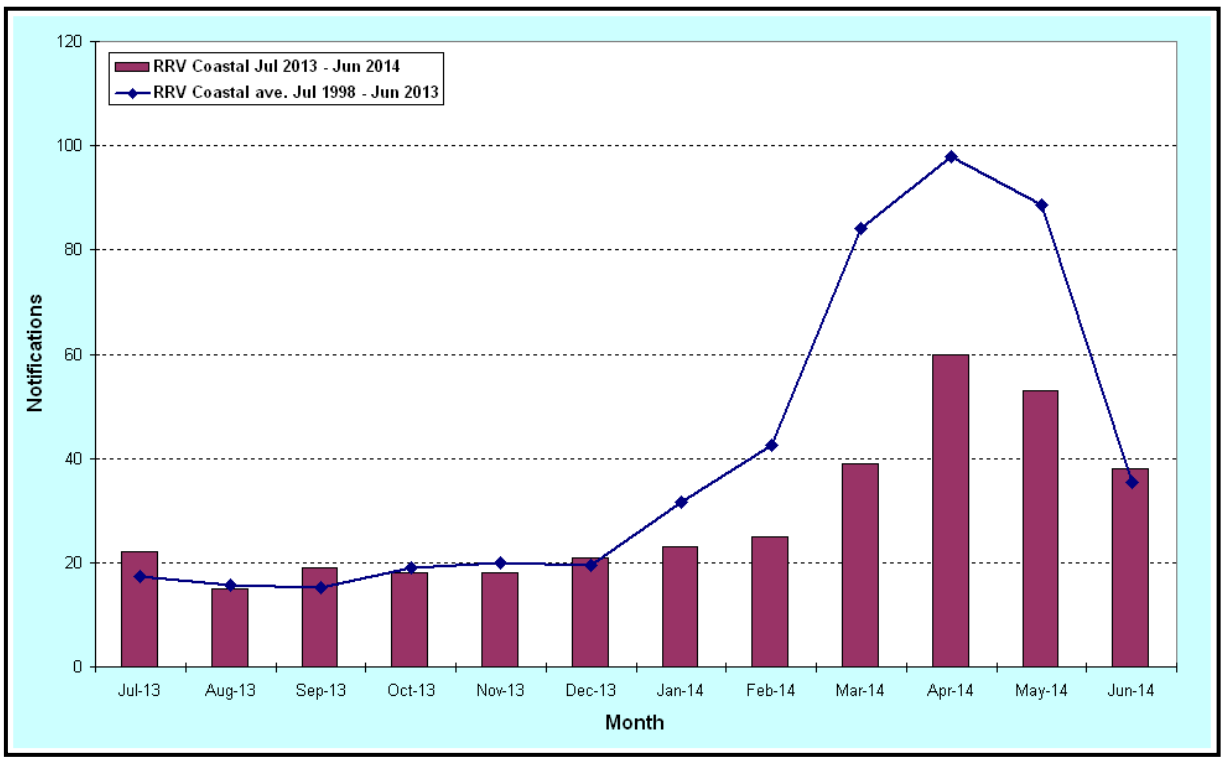


Figure 10. Notifications of RRV per month from coastal NSW. The bars are for 2013-2014 season and the line represents the long term average. Data from 'GODSEND'.

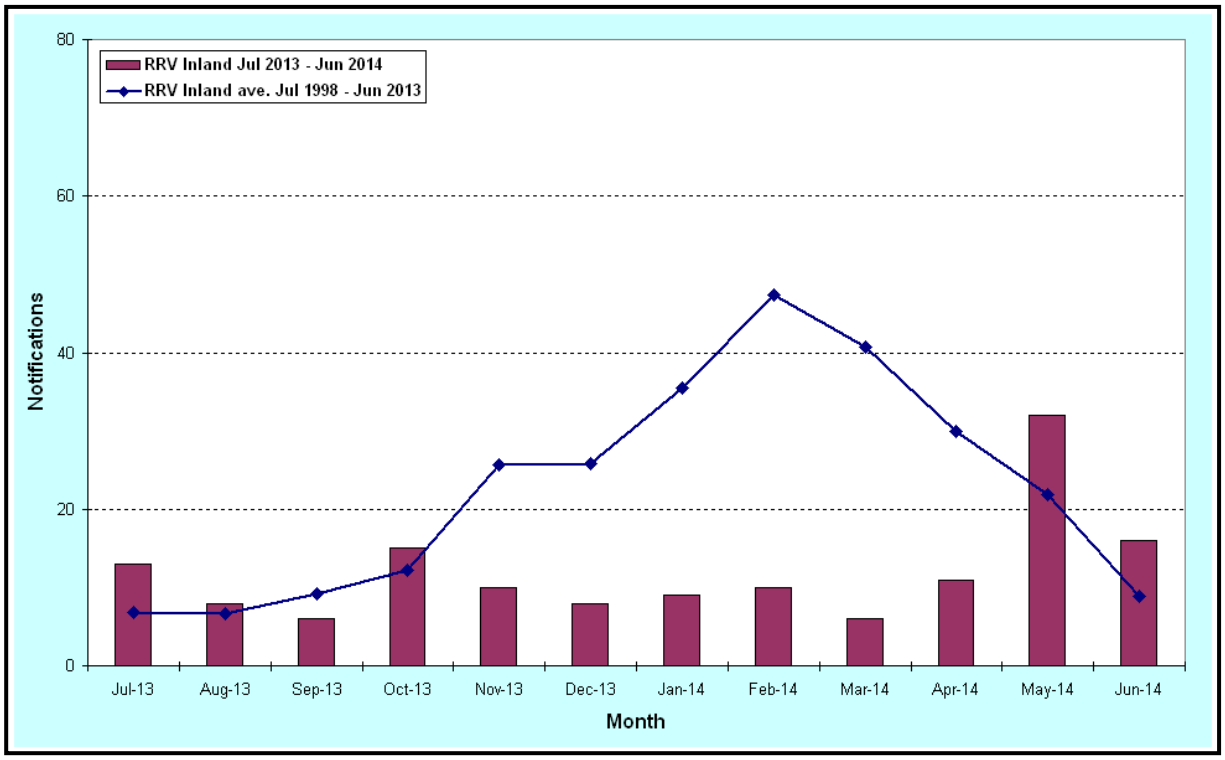


Figure 11. Notifications of BFV per month from coastal NSW. The bars are for 2013-2014 season and the line represents the long term average. Data from 'GODSEND'.

It was noted in previous annual reports that there were many notifications of BFV during the cooler months of the year, which made no epidemiological sense as

mosquito activity is minimal during the winter months (Doggett *et al*. 2014). It is now clear that many of these cases do not represent recent infections and the over diagnoses related to issues in specificity of the commercial BFV serological kit. The kit was withdrawn from the marketplace for a period in late 2013 and corrected, and these factors would partially account for the reduced BFV notifications over the recent season. Unfortunately, high numbers of RRV winter notifications are now occurring as evident in Figure 10. In a recent publication from Western Australia (Selvey *et al*., 2014) it was found that winter notifications probably do not represent infections from the cooler months and may be due to delays in testing, or possible false positives due to issues with the commercial kit (as had occurred with the BFV kit). It is probable that the NSW cases fall into a similar category and the winter reported cases are not occurring at that time of the year.

In contrast to the inland, the possible 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 and a possible rise in arboviral notifications.

Sydney. For the Sydney region, overall mosquito numbers were down largely due to Ryde and Bankstown undertaking reduced trapping. Despite this, there were numerous arboviral isolates from the Sydney region; in fact this was the most active season to date with some 34 isolates (Table 4). Of these, 29 were from Georges River (Table 10), with 7BFV, 11RRV and 11STRV. Of the mosquito species, 11 of the isolates were from *Aedes vigilax* and 8 from *Aedes notoscriptus*. The high number of arboviral detections prompted the local public health unit to release mosquito warnings and human notifications were no greater than normal.

Typically Sydney has been a region of historically low arboviral activity as the presence of native vertebrate reservoirs are required for perpetuation of natural arboviral cycles. In the case of the Georges River, the region is surrounded by national parks and the combination of reservoirs and vectors, has seen this area as a hot-spot of arboviral activity in recent years, with numerous isolates, yet surprisingly relatively few human cases. The elucidation of the factors behind the low local notifications may provide insights into how the arboviral burden could be minimised in the wider community.

There were 56 human notifications (47RRV & 9BFV) with cases being well below the normal of 78 (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.

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 2013 and presented in the previous annual report (Doggett *et al*. 2013).

EVS vs PTs. Opportunistic testing was undertaken at Homebush to compare the sensitivity of EVS traps vs the PTs at trapping mosquitoes over four consecutive nights using four of each trap type per night. The results are presented in Figure 12 below whereby the PTs barely captured 8% of the EVS traps. In light of the consistency between this and last year's results, the PTs will no longer be investigated for use in the NSW Arbovirus Surveillance Program.

FTA cards versus cell culture. Honey-baited FTA cards were placed into the EVS traps at a limited number of sites as part of a pilot study, and the traps were 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 222 traps were used in this evaluation and the results are presented in Table 8 below. Overall, the FTA cards were around three times more sensitive at detecting arboviruses compared with cell culture. Interestingly, despite both methodologies testing the same batch of mosquitoes, there was little concordance between the two technologies and on only three occasions did both techniques detect the same virus within the batch of mosquitoes.

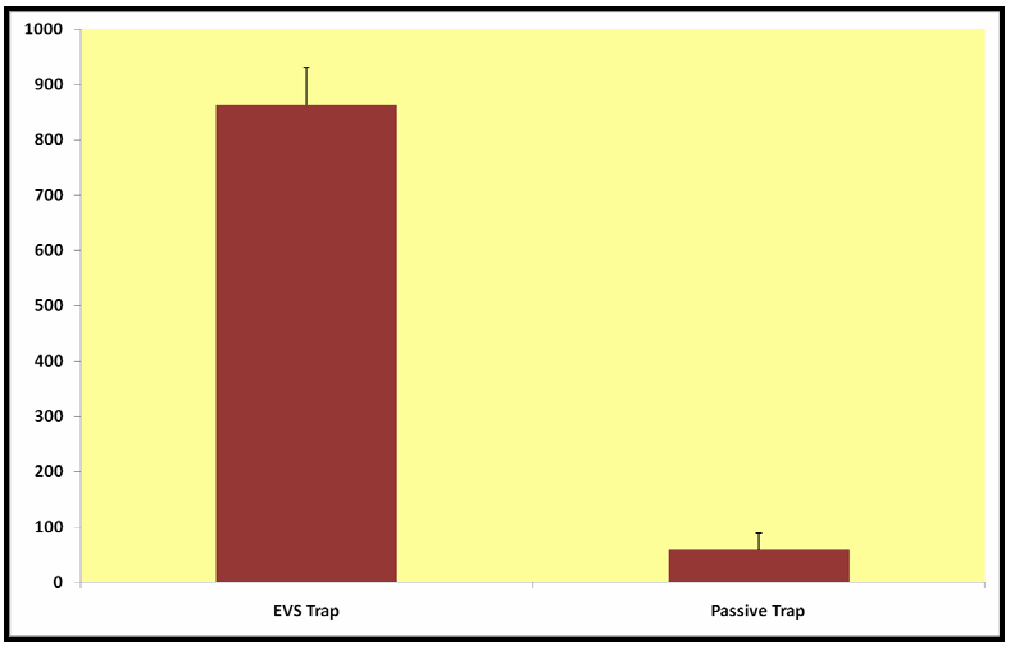


Figure 12. Comparison of the EVS vs PTs at trapping mosquitoes at Homebush Bay, (4 traps each over 4 nights).

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

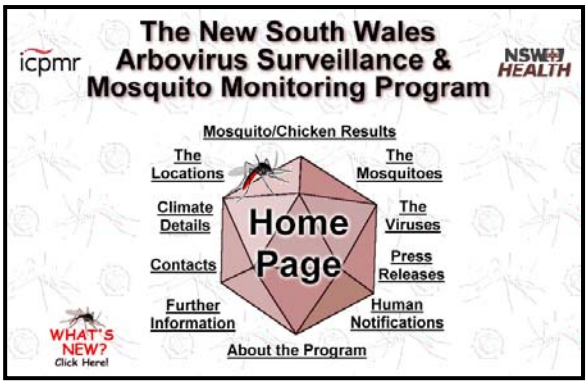
	FTA			CC			
No. Traps	**RRV**	**BFV**	**Total**	**RRV**	**BFV**	**EHV**	**Total**
222	45	8	**53**	12	4	2	**18**

In light of the benefits in speed of service delivery (i.e. time to virus identification) and sensitivity, the intention is to place FTA cards in all EVS traps next season. As the cards are still a relatively new technology and that the FTA cards did not detect any flaviviruses, virus isolation of the trapped mosquitoes will still continue at inland sites. For coastal sites, only FTA cards will be employed for arbovirus surveillance in the future.

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 321MB, and has 2,440+ pages of information.



Added to the site since the last annual report includes:

- Archived data for the 2013-2014 season,
- Monthly rainfall summaries, with long-term averages,
- Monthly rainfall and temperatures maps,
- Daily high tides,
- Monthly SOI updates.

Appendix 1. LOCATION-BY-LOCATION SUMMARY

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

Inland Locations

Albury: mosquito numbers were 'low' throughout the season with only the one 'medium' collection, and no 'high' catches. 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 was one MVEV seroconversion in the sentinel chickens from the bleed taken on 31/Mar/14.

Forbes: no mosquito collections were undertaken this season. There was one KUNV seroconversion in the sentinel chickens from the bleed taken on 11/Feb/14.

Griffith: Collections at both sites were consistently 'very high' from mid January until late February, with greater mosquito numbers being trapped at Barren Box. There were five arboviral isolates; this includes three from Hanwood, with one each of STRV (14/Jan/14), BFV (11/Mar/14), and RRV (11/Mar/14), and two RRV from Barren Box (one each 17/Mar/14 and 26/Mar/14). There was one KUNV seroconversion in the sentinel chickens from the bleed taken on 12/Feb/14.

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 Farm 347 were above average for the entire season and mostly 'very high' from mid-January to early March. Almond Rd typically had lower catches yielding 'high' collections for most of the season. There were three arboviral isolates; one RRV from *Aedes theobaldi* trapped on 2/Apr/14 at Farm 347, and two RRV detected via FTA cards on the 12/Mar/14 with one each from Farm 347 and Almond Road. There was one KUNV seroconversion in the sentinel chickens from the bleed taken on 30/Mar/14.

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

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

Mathoura: trapping was again undertaken at Picnic Point and at Moama from where the sentinel chicken flock was located. Mosquito collections at both sties were mainly 'low' until late February where there was subsequently a series of 'high' catches until mosquito numbers declined in mid-April. There were no arboviral isolates nor any seroconversions to MVEV or KUNV in the sentinel chickens.

Menindee: no surveillance was undertaken at Menindee this season.

Wagga Wagga: trapping was undertaken at two sites and mosquito collections were 'low' for the entire 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. North Creek Road generally produced greater mosquito numbers and was 'high' for most of the season with numbers peaking in early April. Similarly Pacific Pines also mainly trapped 'high' mosquito collections, albeit in small numbers, with one 'very high' catch in late April. There was one detection of RRV via the FTA cards at Lennox Heads from 18/Mar/14.

Byron Bay: no mosquito trapping was undertaken this season.

Coffs Harbour: trapping was undertaken at Moonee Beach, and initially at Park Roa, with this trap later being moved to Boambee Road. Mosquito numbers were mostly 'low' to 'medium', with the occasional 'high' trap being dominated by either *Aedes notoscriptus* or *Culex quinquefasciatus*. No arboviral isolates were yielded.

Gosford: two sites at Gosford were again monitored this year: Empire Bay and Killcare Heights. For Empire Bay, collections were mainly 'low' to 'medium' up until mid-March, which were then followed by a series of 'high' collections until late April. In contrast, except for one 'high' collection in mid-March, mosquito numbers at Killcare were mainly 'low'. There was one isolate of BFV from *Aedes procax* trapped at Empire Bay on 1/Apr/14.

Lake Macquarie: collections were undertaken from three sites: Belmont Lagoon, Teralba and Dora Creek. Mosquito numbers were consistently 'low' from all three sites until late March when there was a spike in the collections with 'high' numbers trapped at Belmont and Dora Creek until early April. No arboviral isolates were yielded.

Port Macquarie: Trapping was undertaken at three sites; Yarranabee Road, Partridge Creek, and Stevens Street. All three sites demonstrated a similar trend; mostly 'low' collections until late March, then a spike with 'high' numbers over two weeks and a subsequent decline in ensuing weeks. There were three detections of RRV via the FTA cards from 26/Mar/14, with one detection from all three sites.

Port Stephens: opportunistic testing was undertaken from March to May at the Heatherbrae Botanical Gardens. This was part of a comparison in the examining the sensitivity of arbovirus detection through isolation via traditional cell culture against arboviral nucleic detection via the use of honey-baited FTA cards. Mosquito numbers were consistently 'high' to 'very high' and 11 arboviral detections were made, which are listed in Table 9 below.

Tweed Heads: trapping was undertaken at three sites; Koala Beach, Beltana Drive

and Piggabeen Road. Koala Beach produced the lowest catch with consistently 'low' to 'medium' collections. Piggabeen Road yielded 'high' catches through late November and early December, then 'low' to 'medium' through to mid-April, which was then followed by series of 'high' catches for the end of April. Beltana Road yielded the highest catches for Tweed and where mostly 'high' throughout the season, with the higher collections being dominated by *Aedes vigilax*. There were three RRV detections via the FTA cards; two from mosquitoes trapped on 11/Mar/14 (one each from Beltana Drive and Koala Beach) and one from 17/Mar/14 (Piggabeen Road).

Table 9. Arboviral Isolates from Port Stephens, 2013-2014.

LOCATION	Date Trapped	Mosquito Species	Virus			
			BFV	RRV	EHV	Total
PORT STEPHENS	18-Mar-14	*Coquillettidia linealis*		1		**1**
PORT STEPHENS	25-Mar-14	*		2		**2**
PORT STEPHENS	25-Mar-14	*Aedes vigilax*			1	**1**
PORT STEPHENS	1-Apr-14	*Aedes procax*			1	**1**
PORT STEPHENS	8-Apr-14	*Aedes vigilax*	1			**1**
PORT STEPHENS	15-Apr-14	*Coquillettidia linealis*		1		**1**
PORT STEPHENS	15-Apr-14	*Coquillettidia xanthogaster*		2		**2**
PORT STEPHENS	23-Apr-14	*Culex annulirostris*	1			**1**
PORT STEPHENS	29-Apr-14	*Aedes vigilax*		1		**1**
		TOTAL	**2**	**7**	**2**	**11**

Wyong: trapping was undertaken at three sites: Ourimbah, Halekalani and Charmhaven, with the latter site only producing 'low' mosquito numbers. Ourimbah yielded on average, 'medium' collections, while Halekalani catches where mostly 'low'. No arboviral isolates were yielded.

Sydney Locations

Bankstown: Collections this season were almost exclusively undertaken at Deepwater, a site known for intense local *Aedes vigilax* production. Mosquito numbers were consistently 'high' throughout the season. There were two arboviral detections at Deepwater via the FTA cards including one RRV from mosquitoes trapped on 4/Mar/14 and one BFV from 18/Mar/14.

Blacktown: Collections were made at two sites; Nurrangingy Reserve and Ropes Crossing. The latter site mainly produced 'low' mosquito numers, while Nurrangingy Reserved yielded several 'high' collections, with *Culex annulirostris* dominating. There were three isolates made from mosquitoes trapped on 17/Apr/14 at Nurrangingy reserves, this includes two RRV from *Aedes vigilax* and one RRV from *Aedes procax*.

Georges River: trapping was again undertaken at the same three sites of Alfords Point, Lugarno and Illawong. Most of the collections were 'high' in number, with a series of 'very high' collections in mid-summer and these were always dominated by

Aedes vigilax. There 29 arboviral detections and there are listed in Table 10 below.

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 December. No arboviral isolates were yielded.

Table 10. Arboviral Isolates from Georges River, 2013-2014.

LOCATION - Site	Date Trapped	Mosquito Species	Virus			
			BFV	RRV	STRV	Total
GEORGES RIVER - Illawong	14-Jan-14	*Aedes notoscriptus*			3	**3**
GEORGES RIVER - Alfords Point	21-Jan-14	*Aedes notoscriptus*			1	**1**
GEORGES RIVER - Illawong	21-Jan-14	*Aedes vigilax*			2	**2**
GEORGES RIVER - Illawong	29-Jan-14	*Aedes notoscriptus*			3	**3**
GEORGES RIVER - Illawong	29-Jan-14	*Aedes vigilax*	1		1	**2**
GEORGES RIVER - Lugarno	4-Feb-14	*Aedes notoscriptus*			1	**1**
GEORGES RIVER - Alfords Point	18-Feb-14	*Aedes vigilax*		3		**3**
GEORGES RIVER - Illawong	18-Feb-14	*	1	1		**2**
GEORGES RIVER - Illawong	25-Feb-14	*Aedes vigilax*	1			**1**
GEORGES RIVER - Alfords Point	4-Mar-14	*Aedes vigilax*		3		**3**
GEORGES RIVER - Lugarno	4-Mar-14	*	2	1		**3**
GEORGES RIVER - Illawong	11-Mar-14	*	1	1		**2**
GEORGES RIVER - Alfords Point	17-Mar-14	*		1		**1**
GEORGES RIVER - Alfords Point	18-Mar-14	*Aedes vigilax*	1			**1**
GEORGES RIVER - Lugarno	29-Apr-14	*		1		**1**
		TOTAL	**7**	**11**	**11**	**29**

Penrith: trapping was undertaken at the three sites of Glenmore Park, Muru Mittigar and Emu Heights. For most of the season, mosquito numbers were 'low', with the one 'high' collection from Muru Mittigar in mid-March. No arboviral isolates were yielded.

Ryde: routine trapping has now ceased. A one off collection was made in mid-January with 'high' numbers being yielded from Wharf Road. No arboviral isolates were yielded.

Sydney Olympic Park: mosquito monitoring at this location has been occurring for a number of years and two sites (Narawang and Haslams Creek) were regularly included in the processing for arbovirus surveillance. Collections were consistently 'high' throughout the season from both sites, although collections tended to be greater at Haslams Creek. No arboviral isolates were yielded.

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 misdiagnosis of this condition appears to be common 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)

ACKNOWLEDGMENTS

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

Kishen Lachireddy & Prof. Wayne Smith (Environmental Health Branch, NSW Health); Tracey Oakman, James Allwood, Tony Burns & Warren Matthews (Murrumbidgee & Southern LHDs); Dr Thérèse Jones, David Ferrall, Ingo Steppat, Gerard van Yzendoorn & Jason Harwood (Far West & Western LHDs); Dr David Durrheim, Philippe Porigneaux, Glenn Pearce (Hunter New England LHD); Paul Corben, Kerryn Lawrence, David Basso, Greg McAvoy, Greg Bell & Geoff Sullivan (Mid North Coast & Northern NSW LHDs), Dr Peter Lewis, John James, Sam Curtis, Adam McEwen (Northern Sydney & Central Coast LHDs); Prof. Mark Ferson, Santo Cannata (South Eastern Sydney LHD); Dr Vicky Sheppard, Helen Ptolemy (Western Sydney & Nepean Blue Mountains LHD); Dr Stephen Conaty, Graham Burgess (South Western Sydney & Sydney LHDs); Lindsay Mack & Lauriston Muirhead (Albury City Council, Albury); Graham Plumb, Jenny Ryan, Rachael Jenner & Kristy Bell (Ballina Shire Council, Ballina); Robyn Young & Steve Stirton (Bankstown City Council, Bankstown); Adam Small & Andrew Said (Blacktown City Council, Blacktown); Jackie Davis (Birrang Enterprise Development Co., Bourke); Jon Rushforth, Michael Bingham & Nina Bertram (Byron Bay Shire Council, Mullumbimby), Rosemary Roche (Coffs Harbour City Council), Cassie Vitucci & Renae Foggiato (Griffith Shire Council, Griffith); Laura Craddock, Dianne Tierney, Edward White, Andrea Horan & Christine Mitchell (Hawkesbury Council); Derek Poulton & Keith Lainson (Lake Macquarie City Council, Speers Point); Dionisio Pantano (Leeton Shire Council, Leeton); Collin Doyle (Menindee); David Dundee (Murray Shire Council, Mathoura); Belinda Comer, Claire Rozema & David Durie (Penrith City Council, Penrith); Gith Striid (Ryde City Council, Top Ryde); Karen Willems (on behalf of the Sydney Olympic Park Authority); Clive Easton & Brian Falkner (Tweed Shire Council, Murwillumbah); Jason May (Wyong Shire Council, Wyong).

The chicken handlers included: Phillip Gaffney (Bourke), Adam Garlandm (Deniliquin), Mathew Teale (Forbes), Renae Foggiato & Cassie Vitucci (Griffith), Ken Rosser (Hay), Kendal Clydsdale (Leeton), Linda McLellan (Macquarie Marshes), David Dundee (Moama), Lester Rodgers (Moree), and Rohan Jayamaha (Wee Waa). The laboratory staff within CIDMLS are acknowledged, particularly Laurence McIntyre, Jennifer Goder & Heang Lim.

Human case numbers and epidemiological information were obtained through 'GODSEND', the Graphical Online Data Surveillance and Evaluation for Notifiable Diseases, which is part of NSW Ministry of Health's Notifiable Diseases Database. The input of Dr Ross Matthews, Director of Animal Care, Westmead Hospital in the continuation of the chicken surveillance program is greatly appreciated. We are grateful to the Arbovirus Laboratory, Department of Microbiology, University of Western Australia, particularly Dr Cheryl Johansen for the supply of monoclonal antibodies for antigen detection. Prof. Scott Ritchie & Mick Townsend provided training in the use of the passive mosquito traps as well as trapping equipment. The Sydney Olympic Park Authority funds the Department undertake mosquito surveillance in the Homebush area. Our apologies to anyone inadvertently omitted.

REFERENCES

Broom A.K., Lindsay M.D.A., Johansen C.A., Wright A.E. and MacKenzie J.S. (1995). **Two possible mechanisms for survival and initiation of Murray Valley encephalitis virus activity in the Kimberley region of Western Australia**. *American Journal of Tropical Medicine & Hygiene*, 53: 95-99.

Bureau of Meteorology, Australia. (2014). **Rainfall Maps**. http://www.bom.gov.au/cgi-bin/climate/rainmaps.cgi, *accessed 11/Jul/2014.*

Cashman P. Hueston L., Durrheim D., Massey P. Doggett S. and Russell R. (2008). **Barmah Forest virus serology; implications for diagnosis and public health action**. *Communicable Diseases Intelligence*, 32(2): 263-266.

Coffey L.L., Page B., Greninger A.L., Herring B., Russell R.C., Doggett S., Haniotis J., Wang C., Deng X., and Delwart E.L. (2013). **Enhanced arbovirus surveillance with deep sequencing: identification of novel rhabdoviruses and bunyaviruses in Australian mosquitoes**. in prep.

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

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

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

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

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

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

Doggett S., Clancy J., Haniotis J., Webb C., Russell R.C., Hueston L., McIntyre L., Lim H. and Dwyer D.E. (2013). **The NSW Arbovirus Surveillance and Mosquito Monitoring Program, 2012-2013**. *ICPMR, Westmead.* 32pp. ISBN 1-74080-148-2.

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

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

Doggett S., Russell R. and Dwyer D. (1999). **NSW Arbovirus Surveillance Web Site**. *NSW Public Health Bulletin,* 10: 7.

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

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

Hall-Mendelin S., Ritchie S.A., Johansen C.A., Zborowski P., Cortis G., Dandridge S., Hall R.A. and Van den Hurk A.F. (2010). **Exploiting mosquito sugar feeding to detect mosquito-borne pathogens**. *PNAS*, 107(25): 11255-11259.

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

Mackenzie J.S., Broom A.K., Calisher C.H. *et al.* (1993). **Diagnosis and reporting of arbovirus infections in Australia**. *Communicable Diseases Intelligence*, 17(10): 202-206.

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

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

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

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

Selvey L.A., *et al*. (2014). **Ross River virus infection in the Greater Perth metropolitan area – has there been an increase in cases in the winter months?** *Communicable Diseases Intelligence*, 38(2): E115-E122.

Van den Hurk A.F., Hall-Mendelin S., Johansen C.A., Warrilow D. and Ritchie S.A. (2012). **Evolution of mosquito-based arbovirus surveillance systems in Australia**. *Journal of Biomedicine and Biotechnology*, 2012:1-8. doi:10.1155/2012/325659.

Wagga Wagga: trapping was undertaken at two sites and mosquito collections were 'low' for the entire 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. North Creek Road generally produced greater mosquito numbers and was 'high' for most of the season with numbers peaking in early April. Similarly Pacific Pines also mainly trapped 'high' mosquito collections, albeit in small numbers, with one 'very high' catch in late April. There was one detection of RRV via the FTA cards at Lennox Heads from 18/Mar/14.

Byron Bay: no mosquito trapping was undertaken this season.

Coffs Harbour: trapping was undertaken at Moonee Beach, and initially at Coffs Harbour Resource Recovery Park with this trap later being moved to Boambee Road. Mosquito numbers were mostly 'low' to 'medium', with the occasional 'high' trap being dominated by either *Aedes notoscriptus* or *Culex quinquefasciatus*. No arboviral isolates were yielded. There was one RRV detected via the FTA cards from the mosquitoes trapped at Boambee Road on 18/Mar/14.

Gosford: two sites at Gosford were again monitored this year: Empire Bay and Killcare Heights. For Empire Bay, collections were mainly 'low' to 'medium' up until mid-March, which were then followed by a series of 'high' collections until late April. In contrast, except for one 'high' collection in mid-March, mosquito numbers at Killcare were mainly 'low'. There was one isolate of BFV from *Aedes procax* trapped at Empire Bay on 1/Apr/14.

Lake Macquarie: collections were undertaken from three sites: Belmont Lagoon, Teralba and Dora Creek. Mosquito numbers were consistently 'low' from all three sites until late March when there was a spike in the collections with 'high' numbers trapped at Belmont and Dora Creek until early April. No arboviral isolates were yielded.

Port Macquarie: Trapping was undertaken at three sites; Yarranabee Road, Partridge Creek, and Stevens Street. All three sites demonstrated a similar trend; mostly 'low' collections until late March, then a spike with 'high' numbers over two weeks and a subsequent decline in ensuing weeks. There were six arboviral detections via the FTA cards. This included three from mosquitoes trapped on 17/Mar/14 and included 2RRV (one each from Stevens Road and Partridge Creek) and 1BFV from Yarranabee Road. There were a further three RRV from the mosquitoes trapped on 26/Mar/14, with one detection from each of the three sites.

Port Stephens: opportunistic testing was undertaken from March to May at the Heatherbrae Botanical Gardens. This was part of a comparison in the examining the sensitivity of arbovirus detection through isolation via traditional cell culture against

arboviral nucleic detection via the use of honey-baited FTA cards. Mosquito numbers were consistently 'high' to 'very high' and 11 arboviral detections were made, which are listed in Table 9 below.

Tweed Heads: trapping was undertaken at three sites; Koala Beach, Beltana Drive and Piggabeen Road. Koala Beach produced the lowest catch with consistently 'low' to 'medium' collections. Piggabeen Road yielded 'high' catches through late November and early December, then 'low' to 'medium' through to mid-April, which was then followed by series of 'high' catches for the end of April. Beltana Road yielded the highest catches for Tweed and where mostly 'high' throughout the season, with the higher collections being dominated by *Aedes vigilax*. There were three RRV detections via the FTA cards; two from mosquitoes trapped on 11/Mar/14 (one each from Beltana Drive and Koala Beach) and one from 17/Mar/14 (Piggabeen Road).

Table 9. Arboviral Isolates from Port Stephens, 2013-2014.

LOCATION	Date Trapped	Mosquito Species	Virus			
			BFV	RRV	EHV	Total
PORT STEPHENS	18-Mar-14	*Coquillettidia linealis*		1		**1**
PORT STEPHENS	25-Mar-14	*		2		**2**
PORT STEPHENS	25-Mar-14	*Aedes vigilax*			1	**1**
PORT STEPHENS	1-Apr-14	*Aedes procax*			1	**1**
PORT STEPHENS	8-Apr-14	*Aedes vigilax*	1			**1**
PORT STEPHENS	15-Apr-14	*Coquillettidia linealis*		1		**1**
PORT STEPHENS	15-Apr-14	*Coquillettidia xanthogaster*		2		**2**
PORT STEPHENS	23-Apr-14	*Culex annulirostris*	1			**1**
PORT STEPHENS	29-Apr-14	*Aedes vigilax*		1		**1**
		TOTAL	**2**	**7**	**2**	**11**

Wyong: trapping was undertaken at three sites: Ourimbah, Halekalani and Charmhaven, with the latter site only producing 'low' mosquito numbers. Ourimbah yielded on average, 'medium' collections, while Halekalani catches where mostly 'low'. No arboviral isolates were yielded.

Sydney Locations

Bankstown: Collections this season were almost exclusively undertaken at Deepwater, a site known for intense local *Aedes vigilax* production. Mosquito numbers were consistently 'high' throughout the season. There were two arboviral detections at Deepwater via the FTA cards including one RRV from mosquitoes trapped on 4/Mar/14 and one BFV from 18/Mar/14.

Blacktown: Collections were made at two sites; Nurrangingy Reserve and Ropes Crossing. The latter site mainly produced 'low' mosquito numers, while Nurrangingy Reserved yielded several 'high' collections, with *Culex annulirostris* dominating. There were three isolates made from mosquitoes trapped on 17/Apr/14 at Nurrangingy reserves, this includes two RRV from *Aedes vigilax* and one RRV from

Aedes procax.

Georges River: trapping was again undertaken at the same three sites of Alfords Point, Lugarno and Illawong. Most of the collections were 'high' in number, with a series of 'very high' collections in mid-summer and these were always dominated by *Aedes vigilax*. There 29 arboviral detections and there are listed in Table 10 below.

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 December. No arboviral isolates were yielded.

Table 10. Arboviral Isolates from Georges River, 2013-2014.

	Date		Virus			
LOCATION - Site	Trapped	Mosquito Species	BFV	RRV	STRV	Total
GEORGES RIVER - Illawong	14-Jan-14	*Aedes notoscriptus*			3	**3**
GEORGES RIVER - Alfords Point	21-Jan-14	*Aedes notoscriptus*			1	**1**
GEORGES RIVER - Illawong	21-Jan-14	*Aedes vigilax*			2	**2**
GEORGES RIVER - Illawong	29-Jan-14	*Aedes notoscriptus*			3	**3**
GEORGES RIVER - Illawong	29-Jan-14	*Aedes vigilax*	1		1	**2**
GEORGES RIVER - Lugarno	4-Feb-14	*Aedes notoscriptus*			1	**1**
GEORGES RIVER - Alfords Point	18-Feb-14	*Aedes vigilax*		3		**3**
GEORGES RIVER - Illawong	18-Feb-14	*	1	1		**2**
GEORGES RIVER - Illawong	25-Feb-14	*Aedes vigilax*	1			**1**
GEORGES RIVER - Alfords Point	4-Mar-14	*Aedes vigilax*		3		**3**
GEORGES RIVER - Lugarno	4-Mar-14	*	2	1		**3**
GEORGES RIVER - Illawong	11-Mar-14	*	1	1		**2**
GEORGES RIVER - Alfords Point	17-Mar-14	*		1		**1**
GEORGES RIVER - Alfords Point	18-Mar-14	*Aedes vigilax*	1			**1**
GEORGES RIVER - Lugarno	29-Apr-14	*		1		**1**
		TOTAL	**7**	**11**	**11**	**29**

Penrith: trapping was undertaken at the three sites of Glenmore Park, Muru Mittigar and Emu Heights. For most of the season, mosquito numbers were 'low', with the one 'high' collection from Muru Mittigar in mid-March. No arboviral isolates were yielded.

Ryde: routine trapping has now ceased. A one off collection was made in mid-January with 'high' numbers being yielded from Wharf Road. No arboviral isolates were yielded.

Sydney Olympic Park: mosquito monitoring at this location has been occurring for a number of years and two sites (Narawang and Haslams Creek) were regularly included in the processing for arbovirus surveillance. Collections were consistently 'high' throughout the season from both sites, although collections tended to be greater at Haslams Creek. No arboviral isolates were yielded.