THE NEW SOUTH WALES ARBOVIRUS SURVEILLANCE & MOSQUITO MONITORING PROGRAM

2012-2013 Annual Report

Aedes rupestris

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EXECUTIVE OVERVIEW

- **For the 2012-2013 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 2012-2013 season for the inland were of well below average rainfall for the last quarter of 2012. Rainfall was average for the first quarter of 2013. Neither the Forbes nor the Nicholls hypotheses were suggestive of possible MVEV activity for the 2012-2013 season. For the coast, conditions were mostly similar, however for the north coast heavy precipitation fell during the first three months of 2013.
- **For the inland**, despite the very dry spring months, moderate mosquito numbers were collected with close to 130,000 being trapped. There were two arboviral detection; an EHV from Leeton and an 'unknown' from Griffith. No seroconversions to MVEV or KUNV were recorded in the sentinel chickens.
- **Human notifications from the inland** of RRV and BFV totalled 166 (130 RRV & 36 BFV), which was close to half the long term average of 320. There were no notified human cases of flavivirus infection.
- **As of July 2013**, the Forbes hypothesis is not suggestive of possible MVEV activity for the season of 2012-2013, however the Nicholls hypothesis does not exclude the possibility.
- **For the coast**, with the ongoing wet conditions during the summer months, numbers of *Aedes vigilax* were again quite low, only comprising around 10% of the overall mosquito collections. With reduced abundance of the major coastal vector, alphavirus activity was relatively minimal. There was a total of 17 isolates, including 1 RRV, 7 EHV, 7 STRV, and 2 unknowns. Most of the isolates were from freshwater breeding mosquitoes, notably *Aedes procax*.
- **Coastal disease notifications** of RRV and BFV totalled 562 cases, including 297 RRV and 364 BFV, and this was below the average of 724. The statistical local area that produced the highest case load was Maclean, with 67 notifications (32 RRV & 31 BFV).
- **For Sydney**, seven locations operated and mosquito numbers were much higher than previous years due to the inclusion of highly productive traps situated at Bankstown. There was a record level of arboviral activity detected with 20 isolates, including 7 EHV, 12 STRV and one unknown, but no human flavivirus case recognised. Human notifications were below the average of 79, with a total of 66 cases including 43 RRV and 23 BFV.
- **New methodologies** to increase the sensitivity of the surveillance system were trialled. This included a rapid molecular based system of virus identification and a passive mosquito trap system, which incorporates a novel method of viral detection.
- **Several new viruses** were identified by US colleagues, including Liao Ning (previously only known from China), Beaumont, North Creek, Murrumbidgee and Salt Ash viruses (all new). It is unknown if these have human health implications.
- **The NSW Arbovirus Surveillance Web Site** http://medent.usyd.edu.au/arbovirus/ continued to expand and now has over 293MB of information with 2,265+ pages.

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NSW ARBOVIRUS SURVEILLANCE AND MOSQUITO MONITORING PROGRAM 2012-2013

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 2012-2013 season follow.

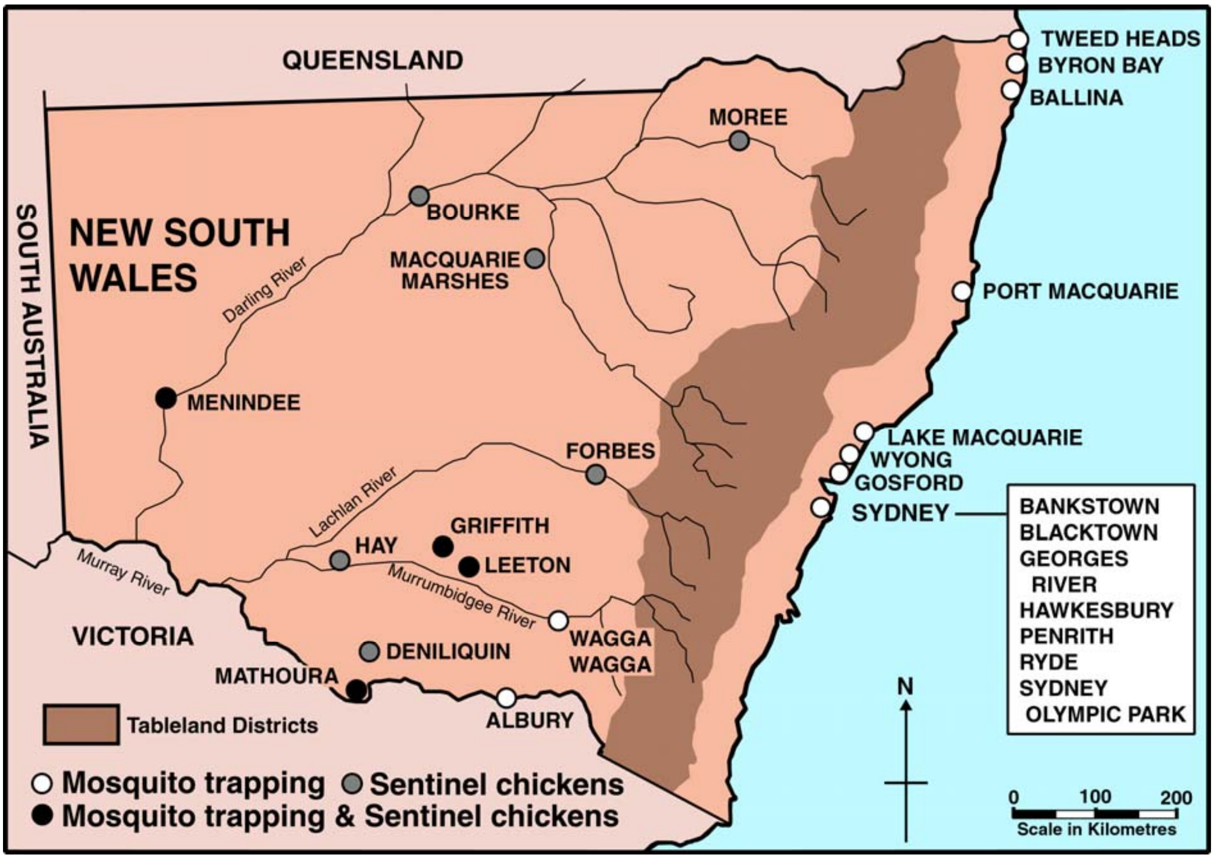


Fig 1. Mosquito trapping locations and Sentinel Chicken sites, 2012-2013.

MONITORING LOCATIONS

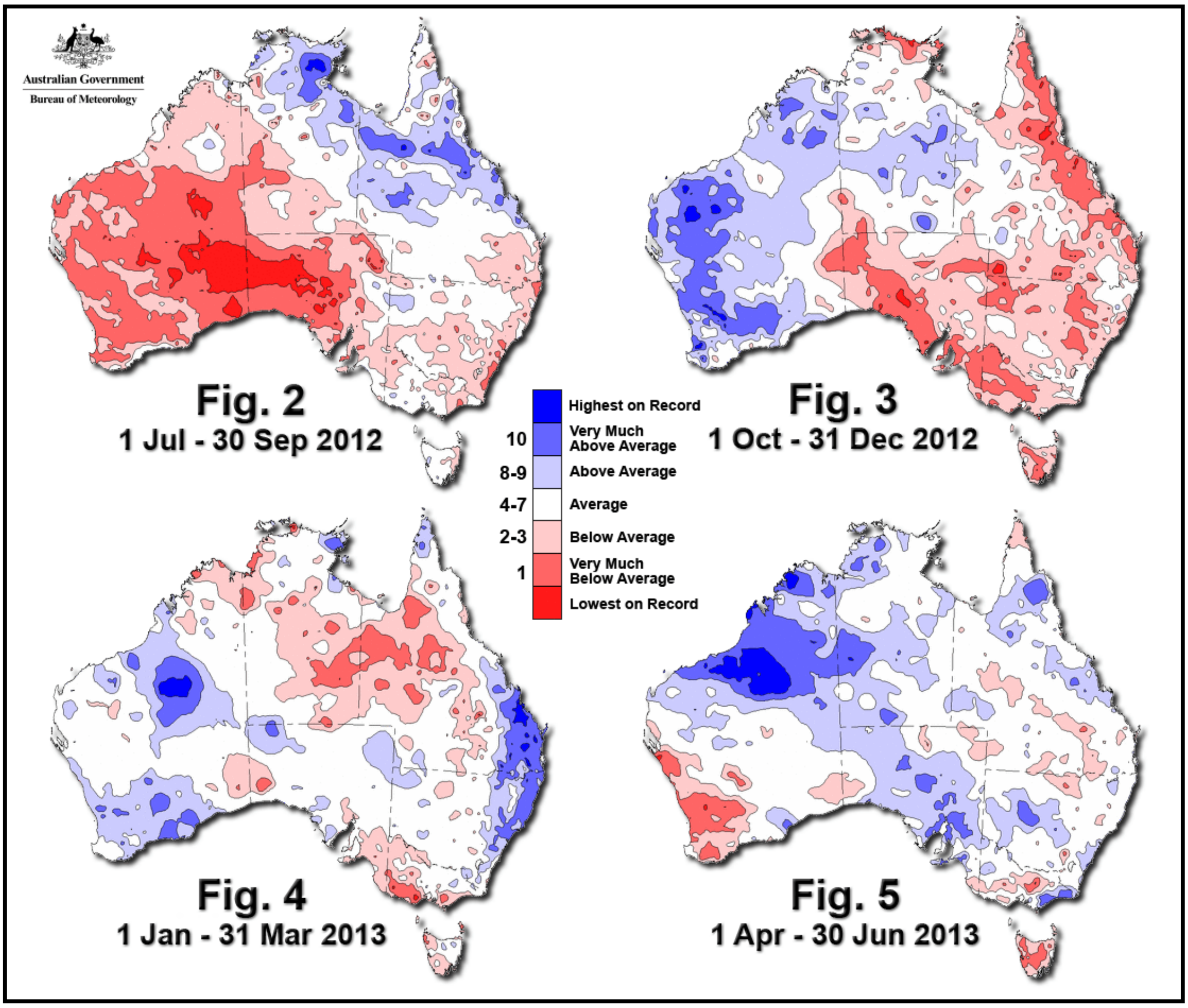
http://medent.usyd.edu.au/arbovirus/location/locations.htm

For 2012-2013, mosquito-trapping sites were operated at 7 inland, 7 coastal and 7 Sydney locations (Fig 1). Chicken sentinel flocks were located at 10 locations.

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 2012, Oct-Dec 2012, Jan-Mar 2013 & Apr-Jun 2013. The stronger the red, the drier the conditions. Conversely, the stronger the blue, the wetter the conditions. *Modifed from the Australian Bureau of Meterology, 2013*.

The first quarter of 2012 (i.e. Jan – Mar) was extremely wet for NSW, with most of the inland having very much above average precipitation and for some areas it was even the highest on record. Much of this rain fell during March and there was associated widespread flooding across the inland. The entire coastal strip also was very wet during these three months. The second quarter of 2012 experienced drier rainfall patterns and the inland had mostly below, to very much below, average precipitation. These dry conditions continued into the third (Figure 2) and fourth quarters (Figure 3) of 2012. In the first three months of 2013, average rainfall was experienced across the inland, however the coastal strip was very wet, experiencing very much above average rainfall (Figure 4). Most parts of the state had normal rainfall during the second quarter of 2013.

Temperatures for the last half of 2012 were above average, which continued into January. For February and March, temperatures along the north coast were well below average, while the inland had average conditions.

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 2011) or the first quarter of the current year (January-March 2012) <u>and</u> the last quarter of the current year (October-December 2012), 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 above decile 7 for all of the catchment basins for the last quarter of 2011, but not for the first and last quarters of 2012, thus the Forbes hypothesis was not fulfilled for 2012-2013 (Table 1). Additionally, decile 7 or above rainfall did not occur during the first quarter of 2013, therefore according to Forbes', there should be a lower risk of an MVEV epidemic for the upcoming 2013-2014 season.

Table 1. Rainfall indices for the main catchment basins of eastern Australia as per Forbes hypothesis, relevant to the 2011-2012 and 2012-2013 seasons.

Catchment Basin	Oct-Dec 2011	Jan-Mar 2012	Oct-Dec 2012	Jan-Mar 2013
Darling River	1.30	1.24	0.48	0.91
Lachlan/Murrumbidgee/ Murray Rivers	1.08	2.56	0.50	1.02
Northern Rivers	1.19	0.68	0.64	0.63
North Lake Eyre system	1.37	0.68	0.73	0.41

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 2012, the SO values were respectively: 1009.43mm, 1013.37mm and 1010.77mm (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.

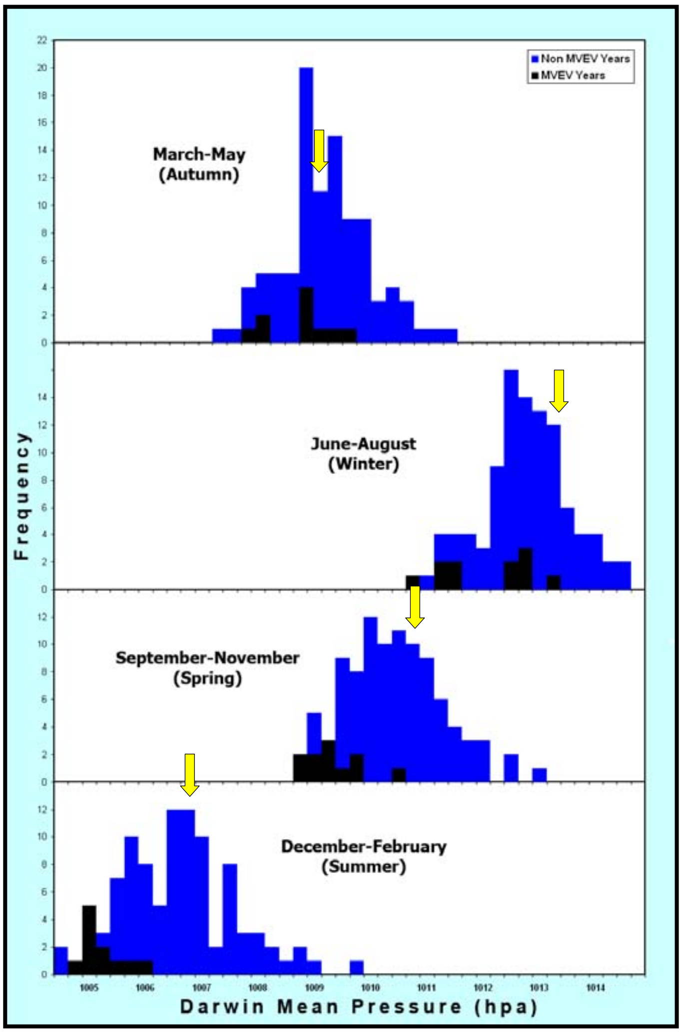


Figure 6. The SO by seasons prior to MVEV active years, according to Nicholls (1986), updated up to Autumn 2012. The black bars represent the pre-MVEV active seasons. The yellow arrows indicate the respective SO values relevant to the 2011-2012 season.

As of July 2013, the autumn Nicholls' value is 1009.23mm and the incomplete winter value is 1011.20. 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 2012-2013 season.

	Autumn 2012	Winter 2012	Spring 2012
2012 Value	1009.43	1013.37	1010.77
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 southeastern Australia.

MOSQUITO MONITORING

Methods

Mosquitoes were collected overnight in dry-ice baited Encephalitis Vector Surveillance 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, 206,218 mosquitoes representing 51 species were collected in NSW during

2012-2013, which was slightly down upon the previous season. *Culex annulirostris* was the most abundant and most important of the inland mosquito species during the summer months, whereas *Coquillettidia linealis*, *Aedes notoscriptus*, *Culex annulirostris, Aedes vigilax*, *Aedes procax*, *Culex sitiens,* 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.

Inland
The total of 128,309 was well below the previous season total of 170,005 trapped in 2011-2012. *Culex annulirostris* was the dominant species yielded at most sites and comprised 68.7% of the total inland collections. *Anopheles annulipes* (28.2%) was the next most common species.

Coastal
In total, 36,612 mosquitoes comprising 47 species were collected from coastal NSW and this was below the previous season's total collection mainly due to the cessation of trapping at Port Stephens. The most common species collected were *Aedes notoscriptus* (17.5%), *Verrallina funerea* (16.3%), *Aedes vigilax* (11.8%), *Culex sitiens* (10.2%), *Culex annulirostris* (9.3%), and *Aedes procax* (8.5%). 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 42,297 mosquitoes, comprising 32 species, was collected from metropolitan Sydney and this almost three times the previous season's total collection. The increase was largely due to the addition of very productive sites at Bankstown. *Aedes vigilax* (51.6% of the total Sydney mosquitoes trapped) was the most common species, followed by *Aedes notoscriptus* (11.3%), *Aedes procax* (8.3%), and *Culex annulirostris* (7.1%).

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 detecting 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, the mosquito grinds 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.

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 37 arboviral detections; 35 from the coast and two from the inland (Table 2).

Table 2. Arbovirus isolates from NSW, 2012-2013.

LOCATION	Date Trapped	Mosquito Species	Virus				Total
			RRV	EHV	STRV	Virus?	
BLACKTOWN	26-Feb-13	*Aedes procax*		1	1		**2**
WYONG	11-Mar-13	*Aedes vigilax*			1		**1**
BLACKTOWN	26-Feb-13	*Aedes procax*			1		**1**
BLACKTOWN	5-Mar-13	*Aedes procax*			1		**1**
HAWKESBURY	5-Mar-13	*Aedes* sp. Marks 51**				1	**1**
BLACKTOWN	12-Mar-13	*Aedes procax*			1		**1**
GRIFFITH	12-Mar-13	*Anopheles annulipes*				1	**1**
LEETON	19-Mar-13	Not known		1			**1**
BANKSTOWN	20-Mar-13	*Aedes procax*		1			**1**
HAWKESBURY	20-Mar-13	*Aedes procax*		1			**1**
HAWKESBURY	26-Mar-13	*Aedes* sp. Marks 51**			2		**2**
LAKE MACQUARIE	26-Mar-13	*Aedes procax*			1		**1**
WYONG	26-Mar-13	*Aedes procax*			1		**1**
BALLINA	25-Mar-13	*Aedes multiplex*		1	1		**2**
BYRON BAY	25-Mar-13	*Aedes procax*			2		**2**
PORT MACQUARIE	25-Mar-13	*Aedes procax*		1			**1**
PORT MACQUARIE	25-Mar-13	*Verrallina* sp. Marks 52*		1			**1**
TWEED	25-Mar-13	*Aedes vigilax*		1			**1**
GEORGES RIVER	2-Apr-13	*Aedes vigilax*			1		**1**
HAWKESBURY	2-Apr-13	*Aedes* sp. Marks 51**		1			**1**
LAKE MACQUARIE	2-Apr-13	*Aedes procax*			1		**1**
BYRON BAY	8-Apr-13	*Culex annulirostris*	1				**1**
HOMEBUSH	8-Apr-13	*Aedes procax*			1		**1**
PENRITH	8-Apr-13	*Aedes procax*			1		**1**
HAWKESBURY	10-Apr-13	*Aedes* sp. Marks 51**		1			**1**
WYONG	10-Apr-13	*Aedes vigilax*		1			**1**
GEORGES RIVER	14-Apr-13	*Aedes vigilax*		1			**1**
BYRON BAY	15-Apr-13	*Aedes procax*				1	**1**
GOSFORD	15-Apr-13	*Aedes procax*		1			**1**
BANKSTOWN	18-Apr-13	*Aedes procax*			1		**1**
BANKSTOWN	18-Apr-13	*Aedes vigilax*		1	2		**3**
		TOTAL	**1**	**14**	**19**	**3**	**37**

RRV = Ross River virus, EHV = Edge Hill virus, STRV = Stratford, Virus? = unknown (not BFV, RRV, SINV, EHV, KOKV, KUNV, MVEV, STRV). *closely related to *Verrallina funerea*. **closely related to *Aedes procax*.

SENTINEL CHICKEN PROGRAM

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

Location of flocks

The 2012-2013 season began on 29th October 2012 with the first bleed and ended on 30th April 2013 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, Menindee, Moama (near Mathoura) and Moree (Figure 1).

Methods

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

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

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

Results

The season began with 150 pullets and 13 deaths were recorded during the program, all at the one site due to fox attack. A total of 2,929 samples was received from the ten flocks in NSW over the six-month period in 2012-2013. This represented 5,858 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 recorded through the season.

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 5. Arbovirus notifications according to former Area Health Service, July 2012 - June 2013*.

Month	CS	NS	WS	WE	SW	CC	HU	IL	SE	NR	MN	NE	MA	MW	FW	GM	SA	Total
RRV	3	7	5	19	3	19	80	6	6	110	70	30	19	15	10	56	12	470
BFV	1	4	5	8	3	11	81	13	2	174	78	14	3	5	1	13	7	423
Total	**4**	**11**	**10**	**27**	**6**	**30**	**161**	**19**	**8**	**284**	**148**	**44**	**22**	**20**	**11**	**69**	**19**	**893**

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 5 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. Also note that 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. 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 2012 to June 2013 was 893 and included 423 BFV and 470 RRV. This season was well below the long term average of 1,122. The coastal region accounted for 661 (74% of the state total) of the BFV and RRV notifications, which was below the seasonal average of 724. The 166 notifications (18.6% of the state total) from the inland were around half the average of 320. Within the Sydney region there were 66 cases reported, well below the seasonal average (79 notifications).

From the coast, the Northern Rivers and Hunter Health Services received the highest notifications (284 and 161 respectively) with the Mid-North Coast having 148. Combined, these three areas accounted for 66.4% of all the arbovirus notifications for the state. From the inland, the Greater Murray AHS had the highest number of notifications (69).

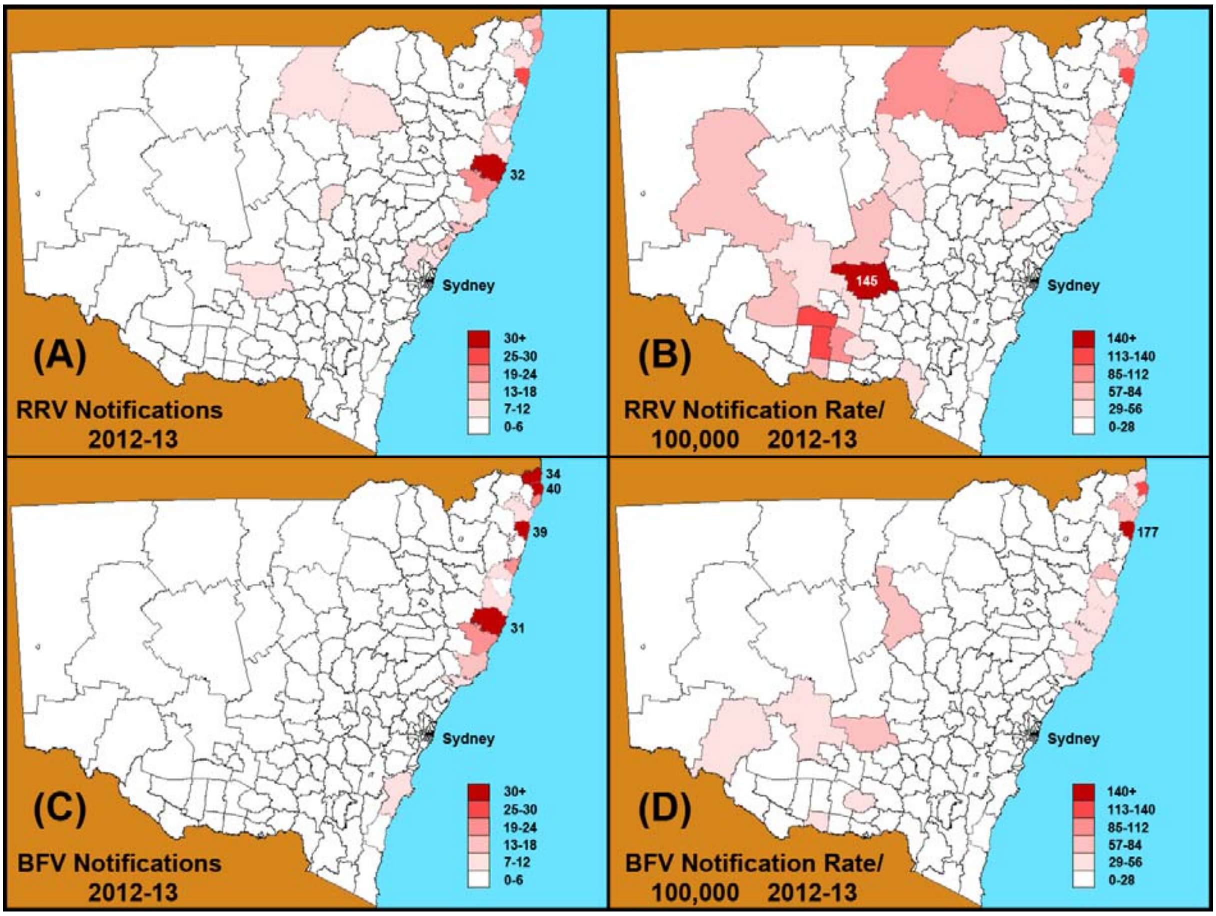


Figure 7. Notifications and notification rates of RRV and BFV by Statistical Local Areas for NSW for Jul 2012 to Jun 2013. (A) RRV notifications. (B) RRV notification rate/100,000 population. (C) BFV notifications. (D) BFV notification rate/100,000 population. Note that different scales are used on the 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 2012-2013 mosquito season.

There were no human locally acquired flavivirus seroconversions reported.

DISCUSSION

The Inland. Over the previous two seasons there was extensive arbovirus activity that 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.

For the recent season of 2012-2013, the climatic conditions were dominated by a very dry period from August through to early February. The late rainfall did prolong the mosquito season with large mosquito collections being trapped well into March,

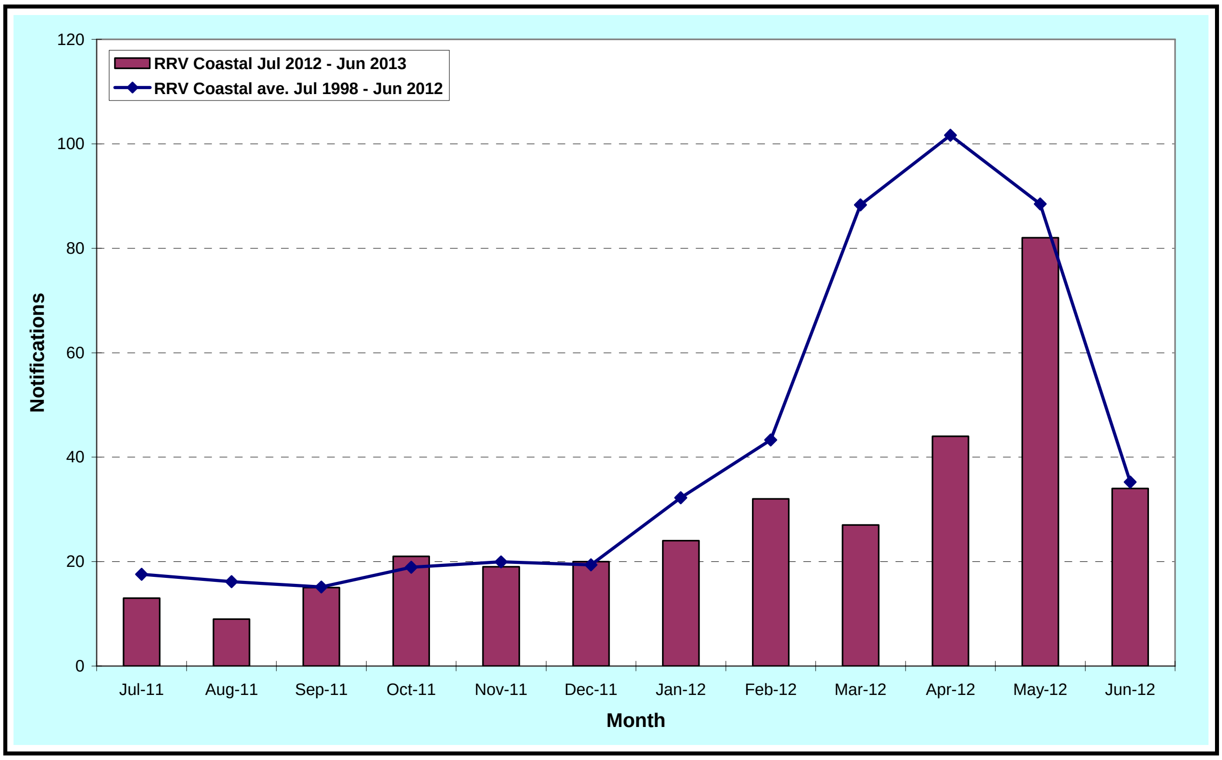


Figure 8. Notifications of RRV per month from inland NSW. The bars are for 2012-2013 season and the line represents the long term average. Data from 'GODSEND'.

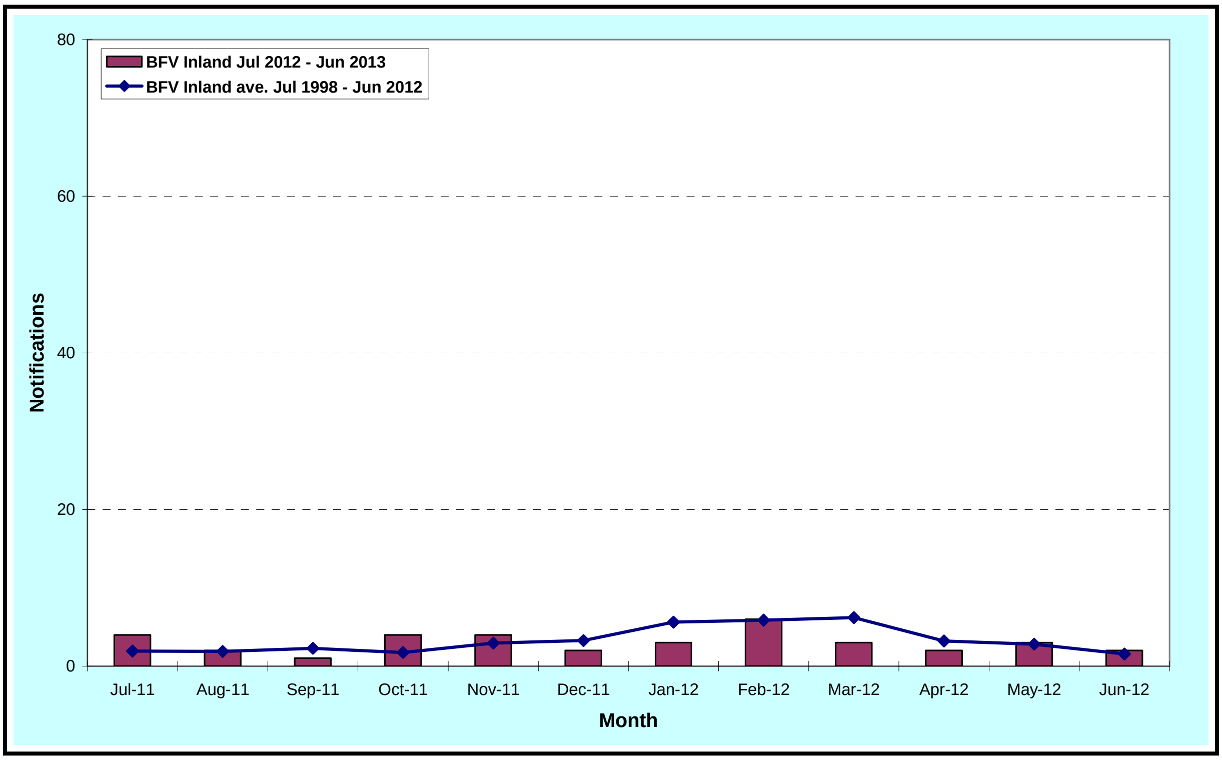


Figure 9. Notifications of BFV per month from inland NSW. The bars are for 2011-2012 season and the line represents the long term average. Data from 'GODSEND'.

when normally few are collected at this time of the year. Despite many high mosquito collections being made, arboviral isolations were very low, with the one EHV from Leeton in mid-March and an unknown from Griffith around the same time. Human notifications were also well down, totalling almost half the long term average (166 *cf*

310). 'Herd immunity' factors may help to explain the lower number of notifications for the recent season despite the quite high mosquito populations. Herd immunity is when there has been recent activity of an infectious agent within a community and as a result may individuals develop immunity. This can then reduce the potential and size of subsequent outbreaks.

Currently the two main climatic models for MVEV epidemics are contrary. Thus while the Forbes hypothesis is not suggestive of an MVEV epidemic for 2013-2014, 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). It is probably that some of the eggs of these floodwater species can remain viable for years. 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.

As mentioned above, human notifications were around half the long term average (Figures 8 & 9). The SLAs that produced most inland RRV cases (Figure 7a) included Narrabri (12 notifications, which was also the highest over the previous two seasons), Dubbo (11), Bland (9) and Walgatt (7). The highest rates (Figure 7b) were from the SLAs of Bland (145 cases/100,000 population), Jerilderie (140), Murrumbidgee (121), Walgatt (110) and Narrabri (100). Nine 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 (36). Despite this figure being close to average, there is considerable misdiagnosis of BFV and there are probably very few true local cases.

The Coast. Arbovirus activity along the coast continued to be again relatively low for the season of 2012-2013. Despite widespread activity from the flaviviruses EHV and STRV, the only isolate of RRV was from Byron. Human notifications were also well down; the 562 reported cases (364 BFV and 297 RRV) was around 23% lower that the long term average of 724 (Table 6). Notifications of RRV were down by around one quarter (297 RRV notifications this season compared with the average of 418, Figure 10), while BFV reports were slightly above average (364 versus the average of 306, Figure 11), with much fewer cases of both diseases reported during normal peak incidence. There were no human cases of EHV or STRV notified.

The graph of BFV notifications over this season (Figure 11) suggests that many newly acquired cases of human BFV occurred during the cooler months of the year. This data makes no epidemiological sense as during the cooler months of the year, mosquito vectors are less active and so the risk of viral transmission is minimal. As mentioned above, there appears to be very high false positive rates with the commercial BFV test. This was evident in one study that reviewed notified human cases of BFV from mid north coast NSW, where it was found that false positive rates were very high (19%), while only 4/37 (10.8%) of the patents had confirmed serology compatible with recent infection (Cashman *et al*. 2008). As mentioned earlier, with such a large error rate, epidemiological interpretation of the BFV notification data is virtually impossible.

Table 6. Notifications of BFV & RRV infection* per virogeographic regions of NSW, per season from 1995-1996 to 2012-2013 (*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**
Total	**5,871**	**629**	**236**	**6,711**	**7,814**	**5,290**	**1,250**	**14,354**
Ave[4]	306	33	12	349	418	287	67	771

[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 fourteen season average of 1994/95 to 2011/12. *Data from 'GODSEND'.

Like the previous season, despite the lower viral activity, rainfall on the coast was well above average during the early part of 2013. On going rainfall along the coast can result in reduced abundances of *Aedes vigilax*. Continual rainfall prevents the saltmarsh habitat from undergoing the usual tidal inundation and drying cycle which is so important for egg maturation in this mosquito species. As *Aedes vigilax* is normally the major coastal vector, the lower numbers are presumably one of the main reasons for the below average human notifications this season.

In terms of overall notifications for SLAs along the coast, Maclean had the greatest number with 67 (39 BFV & 28 RRV), followed by Hastings (31 BFV & 32 RRV) and

Byron (40 BFV & 19 RRV, Figures 7a&c). In relation to notification rates (Figures 7b&d), Maclean was the highest for the coast with 316/100,000, followed by Byron (178), and Bellingen (142).

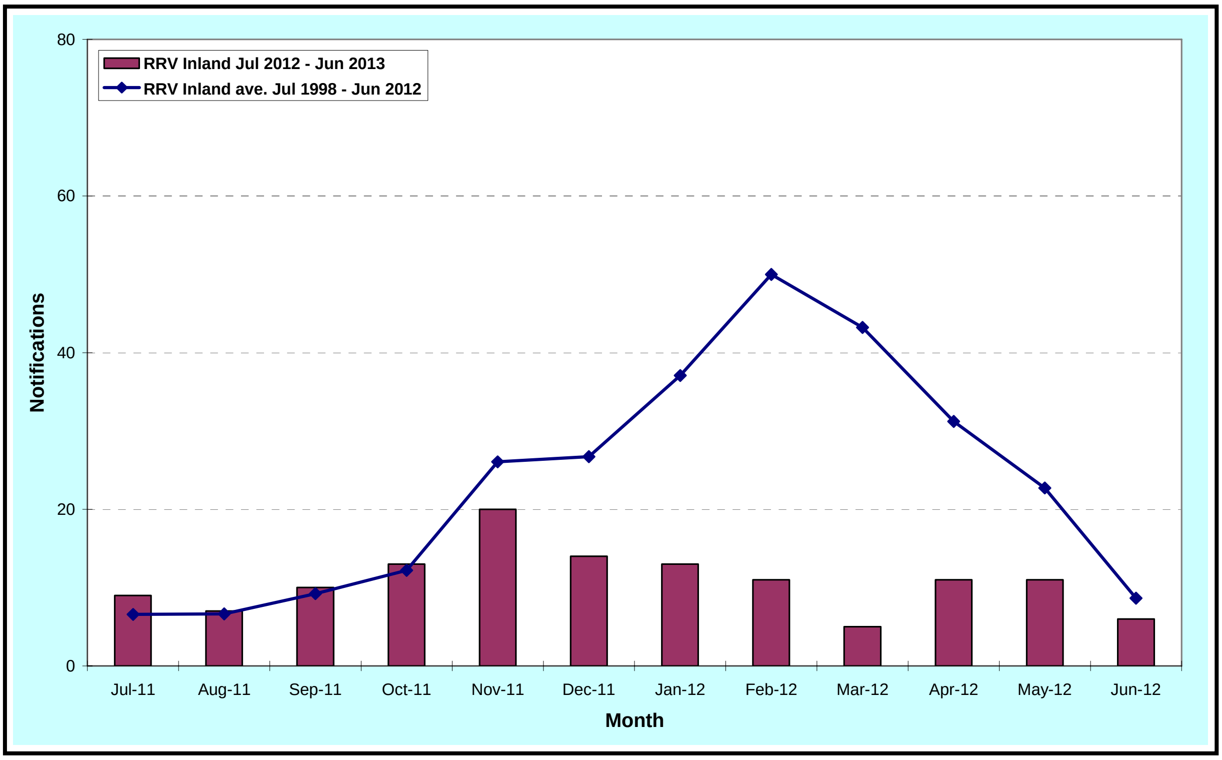


Figure 10. Notifications of RRV per month from coastal NSW. The bars are for 2012-2013 season and the line represents the long term average. Data from 'GODSEND'.

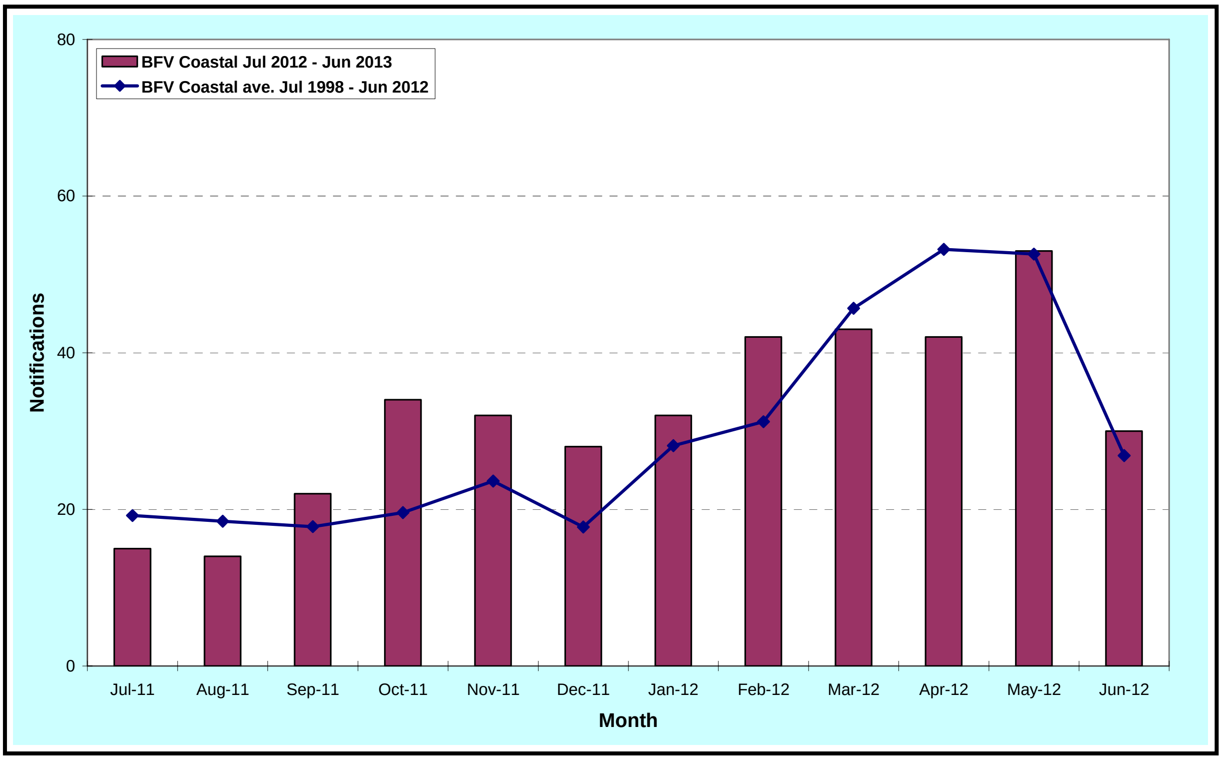


Figure 11. Notifications of BFV per month from coastal NSW. The bars are for 2012-2013 season and the line represents the long term average. Data from 'GODSEND'.

Sydney. For the Sydney region, seven trapping sites operated over 2012-2013. Total mosquitoes trapped were well above the previous season due largely to the highly productive Deepwater site at Bankstown, which collected many *Aedes vigilax*.

There were numerous arboviral isolates from with the Sydney region; in fact it was the most active season to date with 20 isolates (Table 7). Ten of the isolates were detected in the freshwater breeding mosquito, *Aedes procax*, while four were in the closely related *Aedes* Marks 51, and the remainder from *Aedes vigilax*. 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. The reservoirs of EHV and STRV are unknown (but probably avian) and the factors contributing to the increased flavivirus activity are unknown. There were no human cases of EHV or STRV notified.

Table 7. Arboviral Isolates from the Sydney region, 2012-2013.

LOCATION	Date Trapped	Virus			Total
		EHV	STRV	Virus?	
BANKSTOWN	20-Mar-13	1			**1**
BANKSTOWN	18-Apr-13	1	3		**4**
BLACKTOWN	26-Feb-13	1	2		**3**
BLACKTOWN	5-Mar-13		1		**1**
BLACKTOWN	12-Mar-13		1		**1**
GEORGES RIVER	2-Apr-13		1		**1**
GEORGES RIVER	14-Apr-13	1			**1**
HAWKESBURY	5-Mar-13			1	**1**
HAWKESBURY	20-Mar-13	1			**1**
HAWKESBURY	26-Mar-13		2		**2**
HAWKESBURY	2-Apr-13	1			**1**
HAWKESBURY	10-Apr-13	1			**1**
HOMEBUSH	8-Apr-13		1		**1**
PENRITH	8-Apr-13		1		**1**
	TOTAL	**7**	**12**	**1**	**20**

Despite the intense flavivirus activity there were no alphavirus isolates detected. This corresponds with human RRV notifications (43) with cases being well below the normal of 67 (Table 6). Reports of BFV were higher than average, however as mentioned above, there must be doubts about many of these. 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.

In 2010-2011, there was a major outbreak of KUNV with numerous cases in horses from western and south western Sydney. There was also an isolate made from the Baulkham Hills region of Lower Portland (Doggett *et al*. 2011). There was one case of KUNV in a horse from Richmond in Western Sydney during 2011-2012, but no activity was detected for the recent season of 2012-2013.

PASSIVE MOSQUITO TRAP FOR ARBOVIRUS SURVEILLANCE: PRELIMINARY INVESTIGATIONS IN NSW

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, animals may act as amplifying hosts for viruses, and cross reactions in serological assays means that it may be difficult to detect certain viruses with a high degree of accuracy (van den Hurk *et al*. 2012). Thus alternative technologies not employing animals would appear to have many 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).

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. Thus a 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.

Opportunistic testing was undertaken at Homebush and Georges River within Sydney, using dry ice as the CO_2 source for the PTs. A longer term comparison was initiated at Griffith and Leeton in the Riverina (locations of historical high arbovirus activity) and field operatives underwent training by John Haniotis and Stephen Doggett, from the Department of Medical Entomology. At these latter two locations, the CO_2 source for the PTs was bottle gas supplied via the ‘Giles’ regulator and two PTs traps were used for each trapping occasion, with one EVS. In all cases, the EVS used dry ice. Typically, traps were placed a few metres apart. For the traps set on 12/Mar & 19/Mar/13 the PTs were operated for seven days.

The honey-soaked FTA® cards (Flinders Technology Associates filter paper) from the PT were processed according to methods by Hall-Mendelin *et al.* (2010). Viral RNA was extracted from the FTA card eluates and tested by real-time RT-PCR using Pan-Flavivirus (Moureau G*, et al.* 2007) and Alphavirus primers. Amplified products were definitively identified by targeted multiplex RT-PCR.

Results. Total mosquito collections are presented in Table 8 of the PT vs EVS comparison. The results of the PT represent an average of the traps/trap night. Overall, the PT collected <10% of the EVS.

Initial testing at Homebush Bay resulted in very low catch numbers (<10%) with the

PT compared to the EVS traps and were not counted; only two collections were subsequently counted from the Sydney locations and these are presented in Table 4 below.

For the Riverina sites, a similar trend was observed with the PT collecting 7.8% of the totals compared with the EVS traps. With the species comparison (Table 9), very low numbers of *Anopheles* were collected with the PT.

Table 8. Total mosquitoes trapped in the PT v EVS comparisons.

Date	Location	PT	EVS
4-Feb-13	Griffith	1,024	9,770
11-Feb-13	Homebush	92	416
28-Feb-13	Georges River	168	953
5-Mar-13	Leeton	4	758
5-Mar-13	Griffith	6	2,000
12-Mar-13	Griffith	12	1,067
19-Mar-13	Griffith	1	552
19-Mar-13	Leeton	76	303
	Total	**1,382**	**15,819**

Table 9. Mosquito species % comparison between the PT and EVS for mosquito collections at the two Riverina locations.

Mosquito	PT	EVS
Anopheles annulipes	7.2	29.9
Culex annulirostris	92.1	69.2
Culex quinquefasciatus	0.6	0.6

Discussion. It must be stressed that these are preliminary results only, however the results were very consistent, with the PT collecting fewer than 10% of the EVS, and the former as well as having a distinct trapping bias against the *Anopheles* (Table 9). It appears that the mosquitoes in the regions of NSW where the traps were set do not readily fly into the PT (in contrast to published results from FNQ, van den Hurk *et al*. 2012) and a fan motor will probably need to be incorporated into the trap to increase catch numbers.

Unfortunately, the Giles regulators proved not to be robust, both failing within three weeks of initial setting. An alternative from another company will be sourced.

PCR of the honey treated papers were undertaken for the presence of arboviral RNA and one EHV was detected in the last Leeton collection. No viruses were isolated via processing of the mosquitoes through cell culture in those trapped via the EVS. The lack of arboviral activity this season from inland regions meant that an adequate comparison could not be undertaken.

Despite the less than promising preliminary mosquito trapping results, the PT system offers potentially tremendous savings in labour savings and arbovirus detection sensitivity. Thus the intention will be to continue the comparison into the next season, albeit with modified traps.

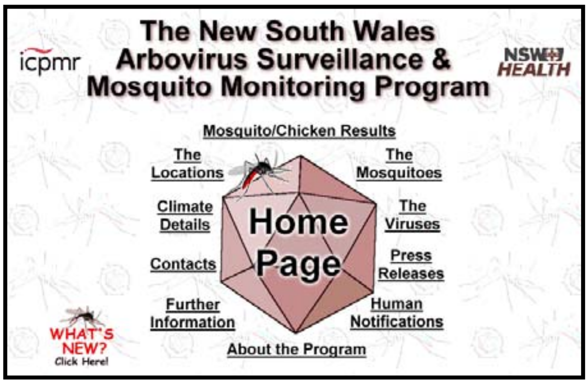
IDENTIFICATION OF NOVEL VIRUSES

During the history of viral isolation with the NSW Arbovirus Surveillance Program there have been a number of isolates that could not be readily identified with the panel of specific monoclonal antibodies presently employed in the immunoassays.

These ‘unknowns’ were sent to Dr Lark Coffey of the Bloods Systems Research Institute, University of California, for testing via ‘deep sequencing’, a form of modern molecular analysis. Several new viruses were identified, including Liao Ning virus (previously only detected in China) and several viruses new to science including Beaumont, North Creek, Murrumbidgee and Salt Ash viruses (Coffey *et al*. 2013), so named after the location of isolation. At present, it is unknown if any of these viruses have human or animal health implications.

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 293MB, and has 2,265+ pages of information.

Added to the site since the last annual report includes:

- Archived data for the 2012-2013 season,
- Weekly rainfall summaries,
- 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' to 'medium', with 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, and there were no seroconversions to MVEV or KUNV in the sentinel chickens.

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

Griffith: very large collections of mosquitoes continue to be trapped from Griffith, with a total for the season of 64,541. Collections at Hanwood were consistently 'very high' from mid December until mid April, with two notable peaks and one 'extreme' collection of over 10,000 mosquitoes in early February. Barren Box generally had lower mosquito numbers, although there was one 'extreme' collection of over 11,000 mosquitoes in mid-January. There was one unknown isolate from *Anopheles annulipes* trapped at Barren Box on 12/Mar/13. 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 Farm 347 were well above average for the entire season and mostly 'very high' from mid-December onwards. Almond Rd had two 'very high' collections in mid-February, but were mostly 'high'. There was one arboviral detection of EHV in the honey baited passive traps at Farm 347 from 19/Mar/13. There were no seroconversions to MVEV or KUNV in the sentinel chickens.

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', with only one 'medium' yield. There were no arboviral isolates nor any seroconversions to MVEV or KUNV in the sentinel chickens.

Menindee: Mosquito collections were undertaken at one site which produced consistently 'low' results, except for one 'medium' collection in early December. There were no arboviral isolates nor any seroconversions to MVEV or KUNV in the sentinel chickens.

Wagga Wagga: trapping was undertaken at two sites and mainly 'low' collections were made. There were no arboviral isolates nor any 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 consistently produced greater mosquito numbers and was 'high' for all but one week of the season. The dominant mosquito species at this site was *Verrallina funerea*. Pacific Pines also mainly trapped 'high' mosquito collections, and again *Verrallina funerea* was the main species captured. There were two arboviral isolates, one each of EHV and STRV from *Aedes multiplex* trapped at Pacific Pines on 25/Mar/13.

Batemans Bay: no mosquito trapping was undertaken this season.

Byron Bay: traps operated at Luan Court, Ocean Shores and Wirree Drive. Luan Court tended to produce 'medium' mosquito numbers for most of the season, with the occasional 'high' collection. *Aedes notoscriptus* contributed to around half of the mosquitoes trapped. Ocean Shores consistently produced 'high' yields, and again *Aedes notoscriptus* was the main species collected. Wirree Drive also produced mostly 'high' numbers, and similarly, *Aedes notoscriptus* was the predominant species. There were four arboviral isolates; two STRV from *Aedes procax* trapped on 25/Mar/13, one each from Laun Court and Wirree Drive, one RRV from *Culex annulirostris* trapped at Wirree Drive on 8/Apr/13, and one unknown isolate from *Aedes procax* trapped at Wirree Drive on 15/Apr/13.

Gosford: two sites at Gosford were again monitored this year: Empire Bay and Killcare Heights. For Empire Bay, collections were mainly 'high' during the early part of the season from December to mid-January and thereafter 'medium' to 'low'. *Aedes notoscriptus* contributed to more than half of the collections at this site. Mosquito collections at Killcare were more consistently 'high' and over 90% were *Aedes vigilax*. There was one EHV virus from *Aedes procax* trapped at Empire Bay on 15/Apr/13.

Lake Macquarie: collections were undertaken from three sites: Belmont Lagoon, Teralba and Dora Creek. The three sites mostly trapped 'low' mosquito numbers although several 'high' collections were yielded during March from Dora Creek. At this time, collections were dominated by *Aedes multiplex*. There were two isolates of STRV both from *Aedes procax* trapped at Dora Creek, one from collections on 26/Mar/13 and the other from 2/Apr/13.

Port Macquarie: Trapping was again undertaken at the two sites of Lord St and Partridge Creek. Collections tended to be greater at the latter site and were consistently 'high' from late January onwards. A range of mosquito species were trapped including those that breed in both fresh and saltwater, including *Aedes* *vigilax*, *Culex annulirostris*, *Aedes procax*, *and Verrallina* Marks no. 52. There were two isolates of EHV from Partridge Creek on 25/Mar/13; one each from *Aedes procax* and *Verrallina* Marks no. 52.

Port Stephens: no mosquito trapping was undertaken this season.

Tweed Heads: trapping was undertaken at Koala Beach in addition to the long standing sites of Beltana Drive and Piggabeen Road. This year mosquito collections were very consistent being ‘high’ through most of December to April, there were even two ‘very high’ collections during summer, one each from Koala Beach and Beltana Drive. The large collection at Koala Beach was dominated by *Aedes* Marks 51, a close relative of *Aedes procax*, while *Culex sitiens* predominated at Beltana. *Verrallina funerea* was the main species trapped at Piggabeen Road. There was one isolate of STRV from *Aedes vigilax* trapped at Beltana Drive on 25/Mar/13.

Wyong: trapping was undertaken at three sites: Ourimbah, Halekalani and Charmhaven. Collections were consistently ‘low’ to ‘medium’, with some ‘high’ numbers from Ourimbah. Most sites were dominated by *Aedes notoscriptus*, although large collections of *Coquillettidia linealis* were made from Halekalani. There were three arboviral isolates, all from Halekulani. One STRV was isolated from *Aedes vigilax* trapped on 11/Mar/13, one STRV from *Aedes procax* trapped on 26/Mar/13 and one EHV from *Aedes vigilax* trapped on 10/Apr/13.

Sydney Locations

Bankstown: Seven sites undertook trapping at Bankstown, the most productive by far was the Deepwater site, which produced two ‘very high’ collections over consecutive weeks in February, and numbers were otherwise ‘high’. *Aedes vigilax* constituted 70% of the mosquitoes at this site, while for the other sites (which produced from ‘low’ to ‘high’ mosquito numbers) then *Aedes notoscriptus* predominated. There were five arboviral isolates, all from Deepwater. One EHV was isolated from *Aedes procax* trapped on 20/Mar/13, two STRV and one EHV from *Aedes vigilax* trapped on 18/Apr/13, and one STRV from *Aedes procax* trapped on 18/Apr/13.

Blacktown: Collections were made at two sites; Nurrangingy and Ropers Creek. It was the former site that produced the greatest collections and a ‘very high’ yield of almost 2,000 mosquitoes was made in mid-February, dominated by *Aedes procax*. There were five arboviral isolates, all from *Aedes procax* trapped at Nurrangingy Reserve. There was one EHV and one STRV from collections made on 26/Feb/13, and a further three STRV, one each from 26/Feb/13, 5/Mar/13 and 12/Mar/13.

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 were two EHV isolates, both from *Aedes vigilax* trapped at Alfords Point, one each from 2/Apr/13 and 14/Apr/13.

Hawkesbury: trapping was undertaken four sites on various weeks, including at Wheeney Creek, Yarramundi, Sackville and McGraths Hill. Wheeney Creek consistently trapped the greatest number, with mainly 'high' collections through February and March. *Aedes* Marks no. 51 (a close relative of *Aedes procax*) was the main species trapped and constituted around 75% of the collections. The other sites generally trapped 'low' to 'medium' mosquito numbers. There were six arboviral isolates, all from Wheeney Creek. There was one isolate of EHV from *Aedes procax* trapped on 20/Mar/13. All of the remaining isolates were from *Aedes* Marks no. 51, this included two STRV from 26/Mar/13, two EHV, one each from 2/Apr/13 and 10/Apr/13, and one unknown from 5/Mar/13.

Penrith: trapping was undertaken at the three sites of Glenmore Park, Muru Mittigar in Castlereagh, and Palomino Reserve in Emu Heights. Collections from the first and third site were almost always 'low', whereas with Muru Mittigar mosquito numbers were 'high' to 'very high' during February. *Coquillettidia linealis* was the most common mosquito trapped at this site. There was one isolate of STRV from *Aedes procax* trapped at Muru Mittigar on 8/Apr/13.

Ryde: as per every season Wharf Road trapped the most mosquitoes for any of the sites at Ryde as it is closer to the *Aedes vigilax* breeding ground in Homebush Bay. Collections at Wharf Road tended to be 'high' from January through to late March and were dominated by *Aedes vigilax*. Most other sites trapped mainly 'low' mosquito numbers. 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 higher at Haslams Creek, with 'high' numbers for most of the season. *Aedes vigilax* constituted around 60% of the total mosquitoes trapped. There was one isolate of STRV from *Aedes procax* trapped at Narawang Wetlands on 8/Apr/13.

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 and there are doubts about many of the reported cases from the winter months. 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) from BFV across inland NSW.

Ross River virus (RRV): this virus causes RRV disease and is the most common arbovirus affecting humans in NSW and Australia. For the state, there are around 700 cases per season. A wide variety of symptoms may occur from rashes with mild fever, to arthritis that can last from months to occasionally 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 southeastern Australia.

Flaviruses

Alfuy virus (ALFV): no clinical disease has been associated with this virus and it has not been isolated from southeastern 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). 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 made it to the coast, which resulted in an outbreak amongst horses with a number of animal deaths resulting. *Culex annulirostris* appears to be the main vector.

Murray Valley Encephalitis (MVEV): 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 five seasons when MVEV activity has been detected within the state: 2000-2001, 2003-2004, 2007-2008, 2010-2011 and 2011-2012. With the latest two seasons of activity, there were two human cases. 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.

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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