THE NEW SOUTH WALES ARBOVIRUS SURVEILLANCE & MOSQUITO MONITORING PROGRAM

2019-2020 Annual Report

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EXECUTIVE OVERVIEW

- **For the 2019-2020 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 Sydney area, (ii) monitored flavivirus transmission through the testing of sentinel chickens across inland NSW. Surveillance operated from November to mid-May.
- **The climatic conditions** leading up to 2019-2020 was a spell of extremely dry weather over the entire year of 2019, with many periods of record low rainfall. In contrast to the dry weather, February 2020 experienced very much above average rainfall along the entire coast. Neither the Forbes' nor the Nicholls' hypotheses were suggestive of a potential MVEV epidemic for the 2019-2020 season.
- **For the inland**, only 7,382 mosquitoes were trapped, and this was one of the lowest collections for the history of the program. There was one Kokobera virus (KOKV) detection from Leeton. There were no seroconversions in the sentinel chickens.
- **Human notifications from the inland** of Ross River virus (RRV) and Barmah Forest virus (BFV) totalled 177 (174 RRV and 3 BFV), which was similar to that of the previous season and around half the long-term average over 2009-2019 of 326 (299 RRV and 27 BFV). There were no human cases of infection notified from Murray Valley encephalitis virus (MVEV) or Kunjin virus KUNV.
- **As of September 2020**, the Forbes' hypothesis is not suggestive of a possible MVEV epidemic for 2020-2021. However, the El Niño-Southern Oscillation is in a La Niña phase, with a more wet spring and summer forecasted. The last La Niña episode was associated with major arboviral activity including MVEV.
- **For the coast**, 163,952 mosquitoes were trapped, which was almost four times that of last season. There was extensive arboviral activity this season with 40 detections from the coast, including: 1 BFV and 1 RRV from Ballina; 1 BFV, 1 RRV, 3 Edge Hill virus (EHV) and 1 Stratford virus (STRV) from the Central Coast; 2 EHV from Coffs Harbour; 2 RRV, 1 EHV and 1 STRV from Kempsey; 1 STRV from Lake Macquarie; 3 BFV, 8 RRV, 2 EHV and 5 STRV from Port Macquarie; 1 BFV, 1 RRV, 1 KOKV and 2 STRV from Tweed; and 1 EHV and 1 STRV from Yamba.
- **Human notifications from the coast** (excluding Sydney) totalled 1,690 cases, including 1,528 RRV and 162 BFV. This was more than twice the previous ten season average of 712 (537 RRV and 175 BFV) and the largest ever recorded RRV outbreak.
- **Sydney** experienced dramatic increase in mosquito numbers with more than 250,000 trapped. A new record collection for the history of the arbovirus program was made this season with over 26,000 mosquitoes trapped on one night from Duck River. There were 21 arboviral detections, including: 1 EHV from Hawkesbury; 2 BFV from Liverpool; 3 BFV, 8 RRV, 1 EHV, and 5 STRV from the Northern Beaches; and 1 STRV from Sydney Olympic Park. Human notifications were more than double the average with a total of 251 (234 RRV and 17 BFV). Northern Sydney had unusually high number of RRV notifications of 74. **Exotic mosquito detections continue**. This included 8 detections of *Aedes aegypti* trapped at Sydney International Airport and 1 detection at an approved arrangement facility (that handles freight). The decline in international travel with the COVID-19 pandemic ensured that the risk of exotic mosquitoes was reduced.

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NSW ARBOVIRUS SURVEILLANCE AND MOSQUITO MONITORING PROGRAM 2019-2020

INTRODUCTION

The aim of the NSW Arbovirus Surveillance and Mosquito Monitoring program (Program) is to provide an early warning of the presence of MVEV and 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 RRV and 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 the creation of new ones.

METHODS

Background

Arbovirus activity within NSW has been defined by the geography of the state, and three broad viro-geographical 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). There are also 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 result, 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 enzootic virus activity in mosquito/host populations is high enough for transmission to occur, increasing the risk to humans.

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.

The Program uses the above methods of arbovirus surveillance and the results for the 2019-2020 season are detailed in this report.

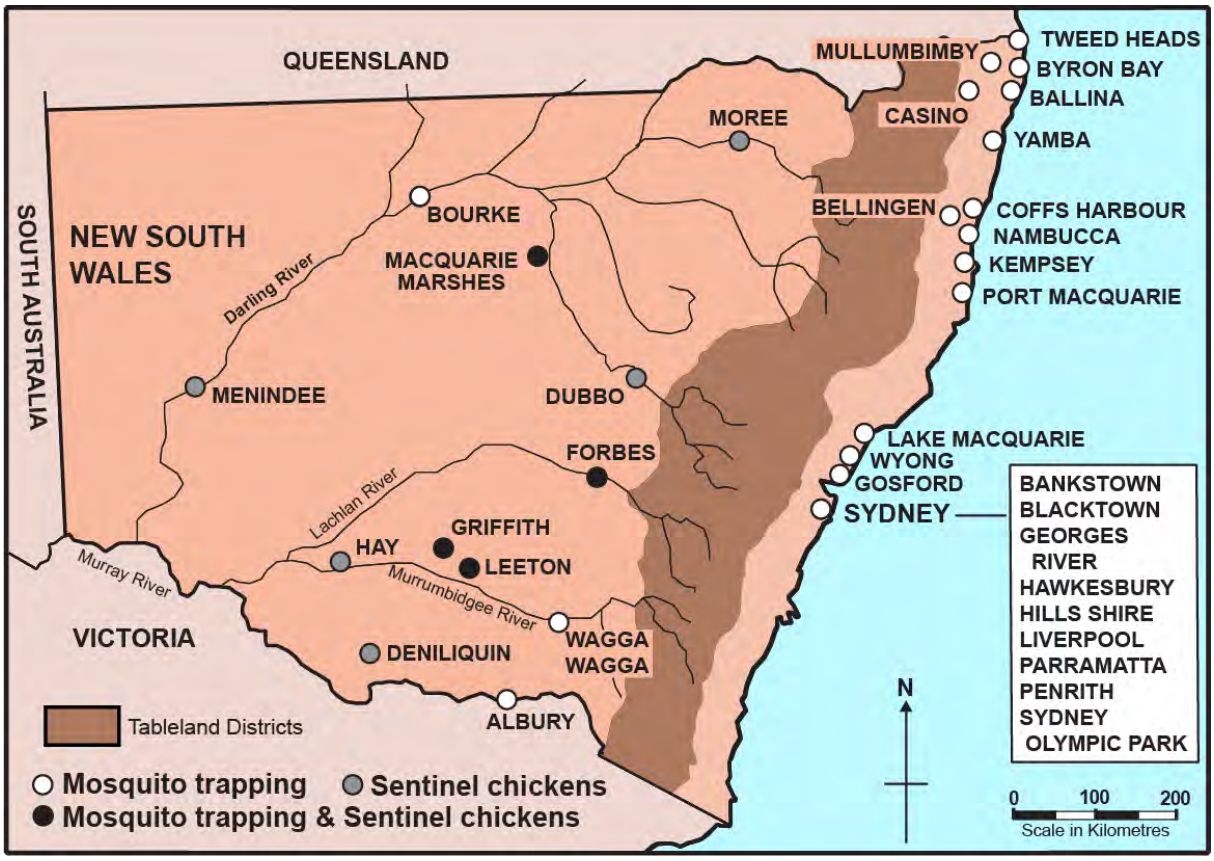


Fig 1. Mosquito trapping locations and Sentinel Chicken sites, 2019-2020.

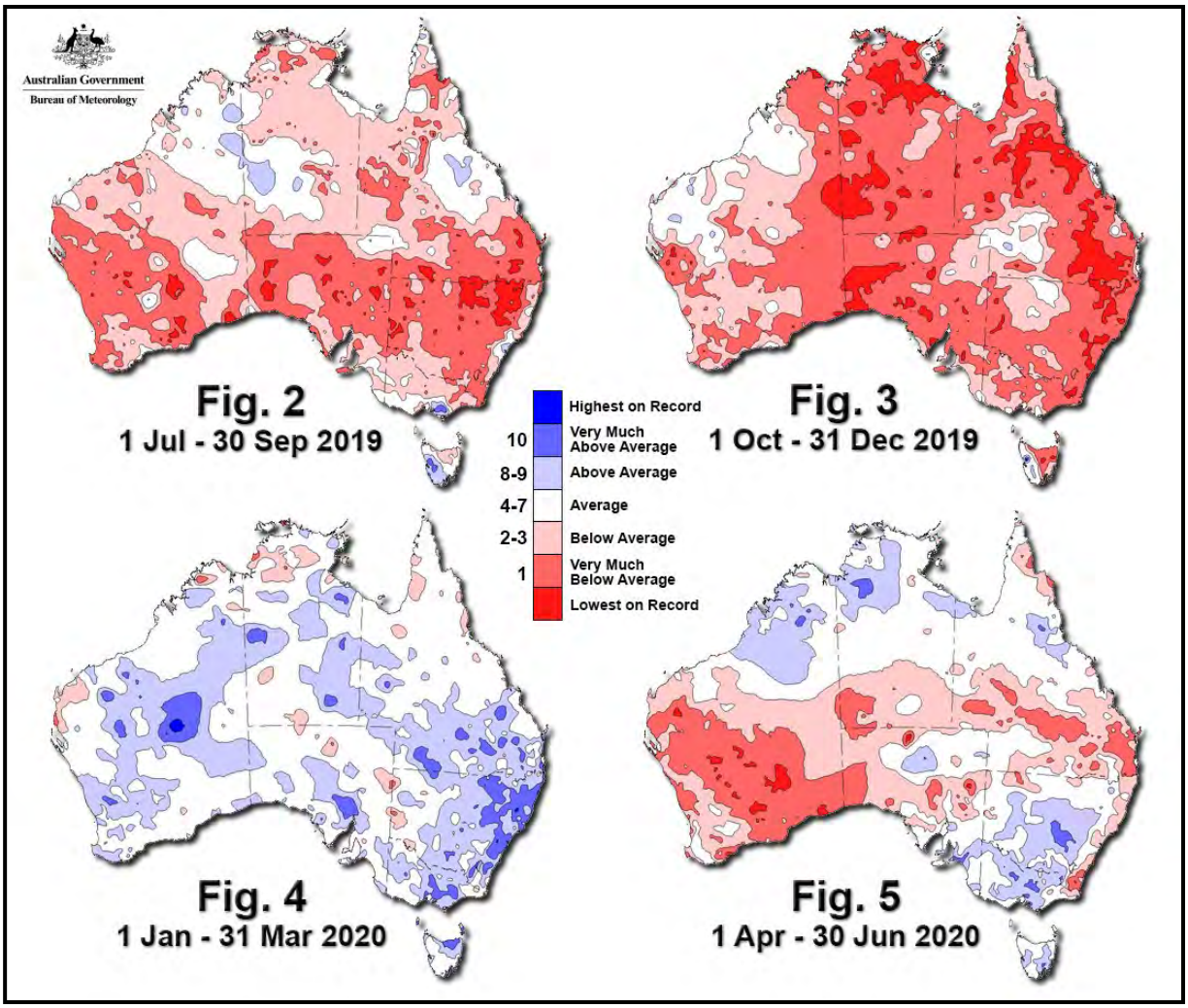
MONITORING LOCATIONS

For 2019-2020, mosquito-trapping sites were operated at 7 inland, 14 coastal and 9 Sydney locations. Chicken sentinel flocks were monitored at 9 locations (Fig 1).

WEATHER DATA

Mosquito abundance is dictated principally by rainfall patterns and irrigation practices in inland regions, while in coastal regions tidal inundation along with rainfall is important. Temperature and/or day-length are often critical in determining the initiation and duration of mosquito activity for species in temperate zones. Hence, the monitoring of environmental parameters, especially rainfall, is a crucial component of the Program.

The first quarter of 2019 (January to March, not shown) produced below average rainfall patterns for most of the state, with very much below average precipitation in the far north coast. The second quarter (also not shown) was similarly dry with very



Figures 2-5. Australian Rainfall deciles for the three month periods, Jul-Sep 2019, Oct-Dec 2019, Jan-Mar 2020 & Apr-Jun 2020. The stronger the red, the drier the conditions. Conversely, the stronger the blue, the wetter the conditions. *Modifed from the Australian Bureau of Meterology, 2020*.

much below average precipitation recorded for most of the Great Dividing Range and below average rainfall for the entire coast. Inland regions had more normal rainfall patterns. During the third quarter of 2019 (Figure 2) almost the entire state of NSW

experienced very much below average rainfall, with parts of northern NSW having record low rainfall levels. The final quarter of 2019 (Figure 3) was even drier, with virtually the entire country having very much below average rainfall. For NSW, this covered the vast majority of the state, with regions of the tableland having record low precipitation. The first quarter of 2020 (Figure 4) was in stark contrast to the extended dry period experienced during 2019. Most of NSW had above average rainfall, with most of the coast experiencing very much above average rainfall. Only the far west of the state had normal rainfall patterns. Most of the above average rainfall during this period occurred during February. January had average rainfall, while March had above average precipitation across the inland.

Maximum temperatures for the last half of 2019 were 2-3^{o} above average, with temperatures being higher than average closer to the coast. In the first six months of 2020, maximum temperatures were around normal.

MVEV Predictive Models

Two main models have been developed for the prediction of MVEV epidemic activity in south-eastern 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 2018) or the first quarter of the current year (January-March 2019) and the last quarter of the current year (October-December 2019), then a MVEV outbreak is probable. By comparing the relevant quarterly rainfall amounts with historical decile 7 years, it is possible to obtain a ratio: a figure of 1 or greater indicates that rainfall was above the historical decile 7 average (Table 1). Rainfall was below decile 7 in all of the catchment basins for the last quarter of 2018, the first quarter of 2019, and below decile 7 in all of the catchment basins for the last quarter of 2019, thus the Forbes' hypothesis was not fulfilled for 2019-2020 (Table 1). Additionally, decile 7 or above rainfall occurred in only one of the catchment basins during the first quarter of 2020. Therefore, according to Forbes', there should be a lower risk of an MVEV epidemic for the upcoming 2020-2021 season.

Table 1. Rainfall indices for the main catchment basins of eastern Australia as per Forbes' hypothesis, relevant to the 2018-2019 and 2019-2020 seasons.

Catchment Basin	Oct-Dec 2018	Jan-Mar 2019	Oct-Dec 2019	Jan-Mar 2020
Darling River	0.71	0.47	0.23	0.92
Lachlan/Murrumbidgee/ Murray Rivers	0.87	0.96	0.30	1.54
Northern Rivers	0.70	0.78	0.28	0.78
North Lake Eyre system	0.56	0.82	0.22	0.89

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 2019, the SO values were respectively: 1010.27mm, 1014.10mm and 1011.87mm (indicated on Figure 6 by the yellow arrows and Table 2). The graph on the right has been updated from the originally published figure to include those MVEV active years between 2000 and 2012 (added to the MVEV tallied black columns), and includes the values for the years 2000-2001, 2007-2008, 2010-2011 and 2011-2012. The SO values leading up to the 2003-2004 season were not included as there was only one detection of MVEV, which may have resulted from over-wintering mosquitoes.

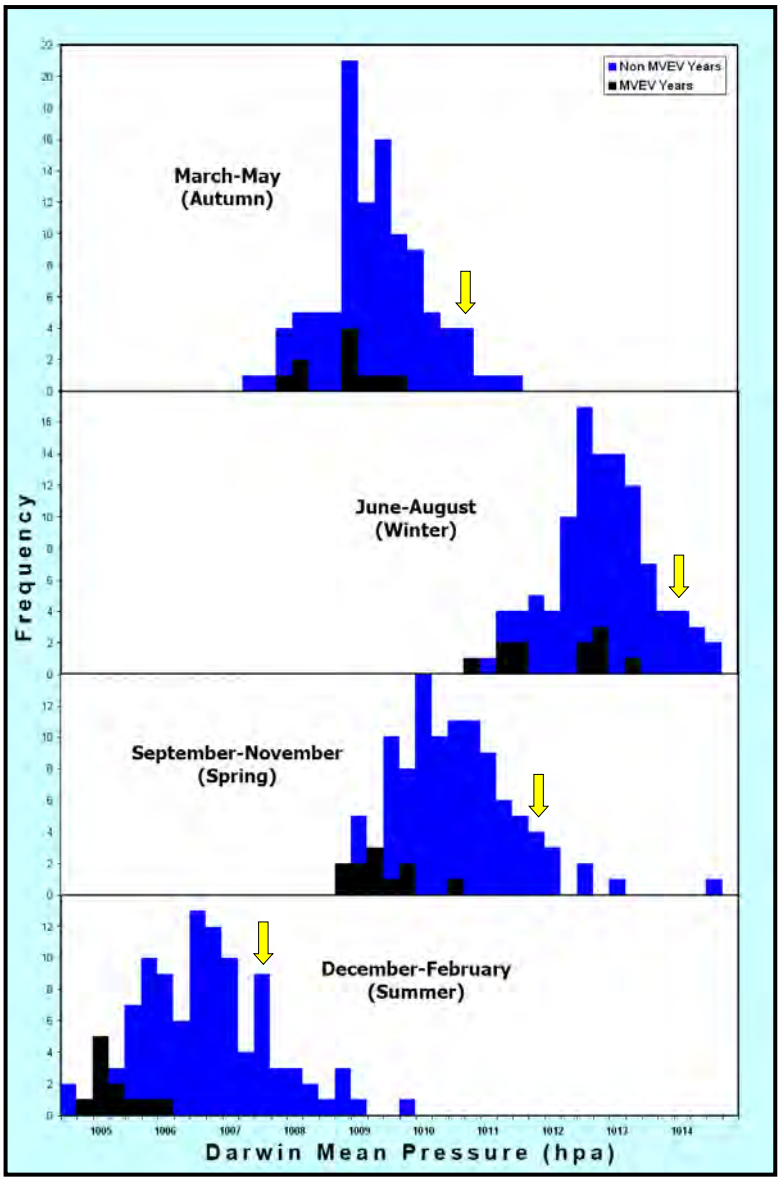


Figure 6. The SO by seasons prior to MVEV active years, according to Nicholls (1986), updated up to Spring 2019. The black bars represent the pre-MVEV active seasons. The yellow arrows indicate the respective SO values relevant to the 2019-2020 season.

Table 2. The seasonal atmospheric pressures (in mm) according to Nicholls' hypothesis, relevant to the 2019-2020 season.

	Autumn 2019	Winter 2019	Spring 2019
2019 Value	1010.27	1014.10	1011.87
Past MVEV seasons	<1009.74	<1012.99	<1009.99

As of December 2020, the autumn Nicholls' value is 1010.67mm, the winter value is 1012.70mm, and the spring value is 1010.20. The winter and spring figures are within the range of values for past MVEV outbreak years. As of September 2020, the El Niño–Southern Oscillation (ENSO) is in a La Niña phase, suggesting above average rainfall for spring and summer. Previous La Niña seasons have been associated with a dramatic increase in arboviral activity including MVEV.

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 as those experienced during the summer of 2008, when 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 occurred during the 2010-11 season, when 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). These models also do not take into account virus existing in cryptic foci in south-eastern Australia.

MOSQUITO MONITORING

Methods

Mosquitoes were collected overnight in dry-ice baited Encephalitis Virus Surveillance (EVS) type traps. They were then sent live in cool, humid Eskies via overnight couriers to the Department of Medical Entomology, Institute of Clinical Pathology and Medical Research (ICPMR), NSW Health Pathology, Westmead, for identification and processing for arbovirus isolation. The mosquitoes were identified via taxonomic keys and illustrations according to Russell (1993, 1996), Dobrotworsky (1965) and Lee *et al*. (1980 – 1989). A brief description of the main mosquito species for NSW appears in Appendix 2.

Mosquito abundances are best described in relative terms, and in keeping with the terminology from previous reports, mosquito numbers are depicted as:

- 'low' (<50 per trap),
- 'moderate' (50-100 per trap),
- 'high' (101-1,000 per trap),
- 'very high' (>1,000 per trap), and
- 'extreme' (>10,000 per trap).

All mosquito and arboviral monitoring results (with comments on the collections) were compiled into a weekly report, which was disseminated to stakeholders and included on the NSW Health website.

Results

Overall, 425,734 mosquitoes representing 58 species were collected in NSW during 2019-2020, with the total being more than twice the previous season (with a total of 187,397 mosquitoes). *Culex annulirostris* was the most abundant and most important of the inland mosquito species during the summer months, whereas *Aedes vigilax*, *Culex annulirostris, Culex sitiens,* and *Aedes notoscriptus* were the most numerous species on the coast. A full summary of the results on a location-by-location basis is included in Appendix 1. A brief description of the most important vectors is provided in Appendix 2.

Inland

The total of 7,382 mosquitoes comprising 21 species was one of the lowest from the inland area for the history of the program, and slightly lower than the previous season of 7,998. *Culex annulirostris* was the dominant species trapped at most sites and comprised 50.3% of the total inland collections. *Culex quinquefasciatus* (21.2%) was the next most common species followed by *Anopheles annulipes* (17.1%).

Coastal

In total, 163,952 mosquitoes comprising 49 species were collected from coastal NSW, and this was around 3.4 times that of the previous season's collection (48,214). The most common species collected were *Culex annulirostris* (41.9%), *Aedes vigilax* (18.9%), *Culex sitiens* (16.8%), *Aedes procax* (5.6%), *Aedes notoscriptus* (3.6%), *Aedes multiplex* (3.5%), and *Verrallina funerea* (3.1).

Metropolitan Sydney

A total of 254,401 mosquitoes, comprising 37 species, was collected from metropolitan Sydney and this was around 50% more than the previous season's total collection of 179,395. *Aedes vigilax* (78.7% of the total Sydney mosquitoes trapped) was the most common species, followed by *Culex annulirostris* (8.5%), *Culex quinquefasciatus* (2.8%), *Aedes notoscriptus* (2.7%), and *Aedes procax* (1.7%). This season's trapping during the week of 23 February 2020 at Duck River recorded a large collection of 26,000 mosquitoes. Almost all of these mosquitoes (26,253) were *Aedes vigilax*.

ARBOVIRUS DETECTIONS FROM MOSQUITOES

Methods

Viral detection involves modern molecular techniques for identifying viral nucleic acid. For viral nucleic acid detection through molecular analysis from the mosquito grinds, the homogenates were screened for alphaviruses (BFV, RRV, and Sindbis virus (SINV)), and flaviviruses (MVEV, KUNV, EHV, KOKV, and STRV) using a series of multiplexed fluorogenic Taqman real-time RT-PCR assays, with modifications (Pyke AT, *et al*. 2004, van den Hurk AF, *et al*. 2014). Viral RNA was extracted using the EZ1® Virus Mini Kit (Qiagen) and amplified on the Corbett™ Rotor-Gene 6000. In the case of identifying flavivirus 'unknowns', a general screen using a pan-flavivirus PCR

was performed (Moureau G*, et al.* 2007). For other unidentified virus from cell culture, a Pan-TBMV (Trubanaman, Buffalo Creek and Murrumbidgee virus), Pan Salt Ash and Gan Gan virus, and PCRs specific for Umatilla virus, Wongorr virus, Liao Ning virus, Wallal virus, Warrego virus, Beaumont virus, Whataroa, and North Creek virus were used. Positive amplification of any one of these viruses was confirmed by Sanger Sequencing at the Australian Genome Research Facility. The test sequence was compared by alignment against a database via the National Centre for Biotechnology information using the Basic Local Alignment Search Tool.

The 'whole trap grind' method validated in the previous season was used again as this method was found to be the most sensitive procedure for detecting arboviruses. The basic procedure was:

1. Tap mosquitoes into urine pot containing ± 35 sterile glass beads and add TE Buffer (pH 8.0) using the following volumes. (These volumes should be adjusted accordingly).

    a) 10 mL for 700 - 1000 mosquitoes
    b) 5 ml for 100 - 600 mosquitoes
    c) 3 ml for 40 - 100 mosquitoes
    d) 2 ml for 25 - 40 mosquitoes

2. Shake on MOSAVEX shaker for 30 minutes.

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

Results

From the mosquitoes processed, there were 62 detections, including 11 BFV, 20 RRV, 12 EHV, 2 KOKV and 17 STRV (Table 3). From inland areas, 1 KOKV was detected from Leeton. From the coast, Port Macquarie had the most detections with a total of 17 (3 BF, 7 RRV, 2 EHV, and 5 STRV. Tweed had five detections (1 BFV, 1 EHV, 1 KOKV, and 2 STRV). The Central Coast also had five detections (1 BFV, 1 RRV, 3 EHV, and 1 STRV). For the Sydney trapping locations, the Northern Beaches had 18 detections (4 BFV, 8 RRV, 1 EHV, and 5 STRV).

All arboviral detections are detailed Location-by Location in Appendix 1.

Table 3. Arboviral isolates from NSW, 2019-2020.

Location		Date	Number of Isolates					Total
Town	Site		BFV	RRV	EHV	KOKV	STRV	
PORT MACQUARIE	Partridge Creek	03-Feb-20					1	**1**
YAMBA	STP	18-Feb-20					1	**1**
PORT MACQUARIE	Stevens Street	24-Feb-20			1			**1**
SOPA	Has 2	27-Feb-20					1	**1**
COFFS HARBOUR	Christmas Bell Rd	02-Mar-20			1			**1**
KEMPSEY	North STP	02-Mar-20		1				**1**
PORT MACQUARIE	Stevens Street	02-Mar-20		1			1	**2**
LAKE MACQUARIE	Belmont	03-Mar-20					1	**1**
PORT MACQUARIE	Partridge Creek	09-Mar-20	1	1			1	**3**
HAWKESBURY	Wheeny Creek	09-Mar-20			1			**1**
LEETON	Almond Road	09-Mar-20				1		**1**
TWEED	Koala Beach	09-Mar-20					1	**1**
TWEED	Beltana Drive	09-Mar-20				1		**1**
PORT MACQUARIE	Stevens Street	16-Mar-20	1					**1**
PORT MACQUARIE	Wall Reserve	16-Mar-20		1				**1**
LIVERPOOL	Hammondville	18-Mar-20	1					**1**
NARRABBEN	Deep Creek	19-Mar-20					1	**1**
CENTRAL COAST	Empire Bay	23-Mar-20			1			**1**
KEMPSEY	North STP	23-Mar-20			1		1	**2**
PORT MACQUARIE	Partridge Creek	23-Mar-20			1		1	**2**
PORT MACQUARIE	Stevens Street	23-Mar-20	1				1	**2**
PORT MACQUARIE	Wall Reserve	23-Mar-20		1				**1**
TWEED	Beltana Drive	23-Mar-20					1	**1**
TWEED	Koala Beach	23-Mar-20			1			**1**
COFFS HARBOUR	Christmas Bell Rd	24-Mar-20			1			**1**
KEMPSEY	North STP	30-Mar-20		1				**1**
NARRABBEN	Deep Creek	30-Mar-20		1				**1**
PORT MACQUARIE	Stevens Street	30-Mar-20		1				**1**
PORT MACQUARIE	Wall Reserve	30-Mar-20		1				**1**
CENTRAL COAST	Empire Bay	01-Apr-20		1	1			**2**
CENTRAL COAST	North Avoca	01-Apr-20	1					**1**
BALLINA	Lindsay Ave	06-Apr-20		1				**1**
NARRABBEN	Deep Creek	06-Apr-20	1	1				**2**
WARRIEWOOD	Wetland	06-Apr-20		1				**1**
CENTRAL COAST	Empire Bay	07-Apr-20					1	**1**
TWEED	Beltana Drive	06-Apr-20	1					**1**
BALLINA	Lindsay Ave	15-Apr-20	1					**1**
NARRABBEN	Deep Creek	20-Apr-20	1	1			1	**3**
WARRIEWOOD	Wetland	20-Apr-20					1	**1**
PORT MACQUARIE	Stevens Street	20-Apr-20		1				**1**

Table 3 cont. Arboviral isolates from NSW, 2019-2020.

Location		Date	Number of Isolates					Total
Town	Site		BFV	RRV	EHV	KOKV	STRV	
NARRABBEN	Deep Creek	27-Apr-20	1	1	1		1	**4**
WARRIEWOOD	Wetland	27-Apr-20		1				**1**
LIVERPOOL	Hammondville	27-Apr-20	1					**1**
PORT MACQUARIE	Partridge Creek	27-Apr-20		1				**1**
CENTRAL COAST	Halekulani	28-Apr-20			1			**1**
CLARENCE VALLEY	Yamba	28-Apr-20			1			**1**
NARRABBEN	Deep Creek	04-May-20		1				**1**
WARRIEWOOD	Wetland	04-May-20		1				**1**
NORTHERN Beaches	Careel Bay	04-May-20					1	**1**
		Total:	**11**	**20**	**12**	**2**	**17**	**62**

SENTINEL CHICKEN PROGRAM

Location of flocks

The 2019-2020 season began on 24 November 2019 with the first bleed and ended on 20 April 2020 with the last. A total of nine flocks each containing up to 15 Isa Brown pullets were deployed, with one flock each at Deniliquin, Dubbo, Forbes, Griffith, Hay, Leeton, Macquarie Marshes, Menindee, and Moree (Figure 1).

Methods

The NSW Chicken Sentinel Surveillance 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 must inspect all new flock locations prior to deployment to ensure animal housing is adequate. 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 relevant government agencies as determined by NSW Health and are uploaded weekly to the NSW Arbovirus Surveillance website. Confirmed positives are notified by telephone to NSW Health and the Communicable Diseases Network Australia.

Results

The season began with 135 pullets. A total of 1,944 samples was received from the nine flocks in NSW over the five-month surveillance period in 2019-2020. This represented 3,888 ELISA tests (excluding controls and quality assurance samples), with each specimen being tested for MVEV and KUNV antibodies. There were no seroconversions in the sentinel chickens.

NOTIFICATIONS OF LOCALLY-ACQUIRED ARBOVIRUS INFECTIONS

All arboviral infections detected in humans are notifiable under the *NSW Public Health Act 2010*. When a person tests positive for an arboviral infection, pathology laboratories notify public health authorities who assess the notification against agreed surveillance case definitions and take appropriate actions using NSW Health disease control guidelines. Annual reports (by calendar year) of notifiable vector borne diseases (VBD), including locally acquired arbovirus infections, are available on the NSW Health VBD reports website.

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

- NSW Health vector-borne diseases reports
- NSW Health notifiable diseases data (and select the relevant disease).

The two most common locally-acquired arbovirus infections notified in NSW are RRV and BFV. This section presents notifications of RRV and BFV by place of residence of the case, local health district (LHD), geographic region (coastal, inland or Sydney metropolitan) and Australian Bureau of Statistics (ABS) statistical area level 2 (SA2). Note: the place of residence of a case may not be where the infection was acquired.

Population rates presented in this report are based on the Australian Bureau of Statistics estimated resident populations. Population projections for LHDs in 2019-2020 are based on data from the NSW Department of Planning, Industry and Environment.

Table 4 presents a summary of RRV and BFV. It shows that in the 2019-2020 financial year, there were 1,936 notifications of RRV infection in NSW residents, close to four times the previous season (585) and more than double the previous ten season average (924). There were 182 notifications of BFV infection, which is 160% higher than the previous year (70 notifications), but lower than the previous ten season average (211). There were no notifications of other locally-acquired arbovirus infections in NSW during 2019-2020.

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

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

Table 4. Barmah Forest virus and Ross River virus infections in NSW residents: notifications and population rates (notifications per 100,000 population) by local health district for the 2019-2020 mosquito season*.

Local Health District	Barmah Forest virus		Ross River virus	
	Notifications	Population Rate	Notifications	Population Rate
Central Coast	11	3.14	79	22.52
Far West	1	3.32	7	23.25
Hunter New England	37	3.91	626	66.0
Illawarra Shoalhaven	2	0.48	26	6.22
Mid North Coast	52	23.16	379	168.81
Murrumbidgee	0	0	61	20.50
Nepean Blue Mountains	8	2.1	80	20.61
Northern NSW	58	18.8	408	132.19
Northern Sydney	5	0.53	75	7.89
South Eastern Sydney	1	0.10	29	3.04
South Western Sydney	0	0	11	1.07
Southern NSW	2	0.93	10	4.64
Sydney	3	0.43	14	2.02
Western NSW	2	0.70	106	37.28
Western Sydney	0	0	25	2.40
Total	**182**	**2.24**	**1936**	**23.81**

Figures 7 and 8 present notifications of BFV and RRV infection by geographic region of residence (Coastal, Inland, and Sydney metropolitan) and by financial year of disease onset from 2010-2011 to 2019-2020. The Coastal region accounted for the vast majority of BFV notifications (n=161, 88.5%) followed by the Sydney region (n=6, 8.2%), with only 6 notifications reported in a resident of the Inland region (Figure 7).

Figure 8 shows the Coastal region again accounted for the majority of RRV notifications (n=1,458, 75.4%), similar to the previous financial year. In 2019-2020, the proportion of RRV notifications for residents in the Inland region (12.6%) and in Sydney (12.0%) were similar to the previous year (Figure 8).

Figure 9 shows notifications of RRV by month and Figure 10 shows notifications of BFV by month over 2019-2020. Both figures show that activity of both viruses was below the long-term average (LTA) until after the February 2020 rainfall, when case numbers quickly rose and exceeded the average.

Figures 11 and 12 present maps of notifications of BFV and RRV infection by ABS statistical area level 2 (SA2) of residence and maps of population notification rates for the 2019-2020 financial year. The SA2 areas with the highest total number of BFV notifications were Wauchope (n=8), Bangalow (n=7) and Mclean-Yamba-lluka (n=7) (Figure 11(a)). The two SA2 areas with the highest notification rates per 100,000 population were Jiliby – Yarramalong (123) and Bangalow (122) (Figure 11(b)).

The SA2 areas with the highest total number of RRV notifications were Taree Region (n=55) and Forster-Tuncurry Region (n=48) (Figure 12(a)). The SA2 areas with the highest notification rates per 100,000 population were Forster-Tuncurry Region (808) and Port Macquarie Region (688) (Figure 12(b)).

Figure 7: Barmah Forest virus infections in NSW residents: annual notifications by year of disease onset and geographical region for the past 10 years (2010-2011 to 2019-2020).

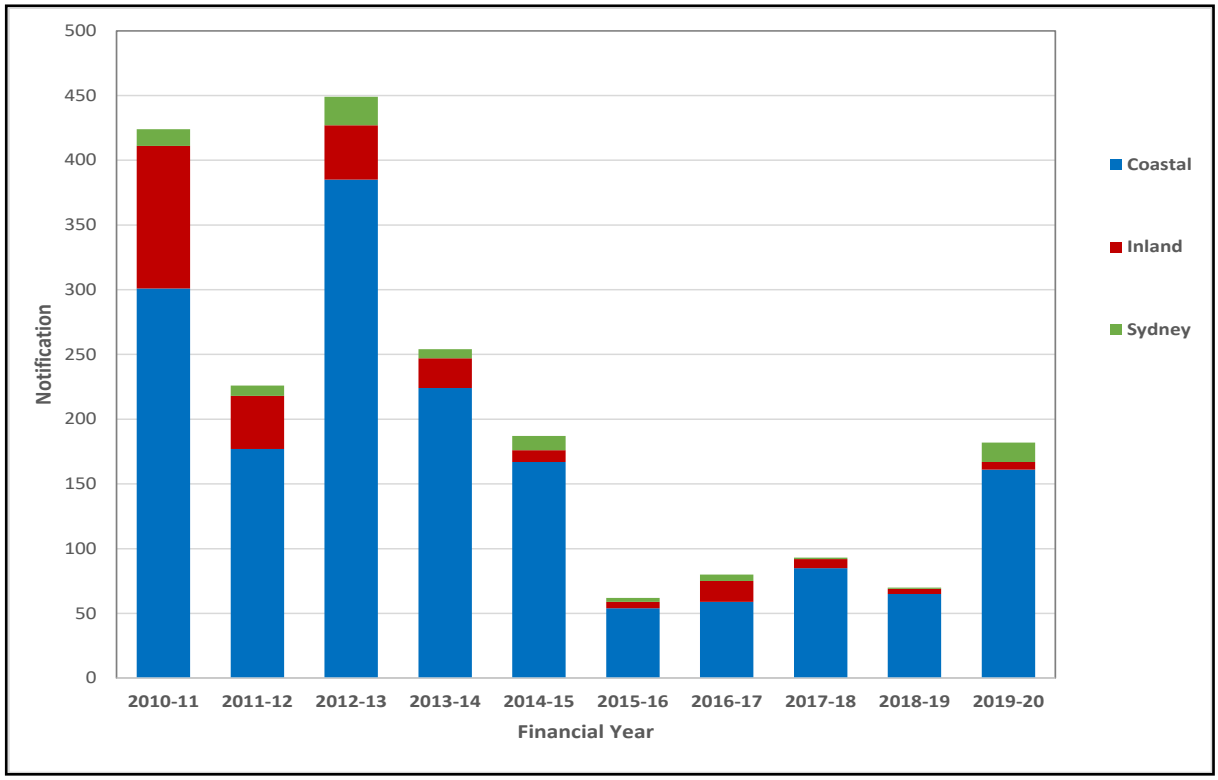


Figure 8: Ross River virus infections in NSW residents: annual notifications by year of disease onset and geographical region for the past 10 years (from 2010-2011 to 2019-2020).

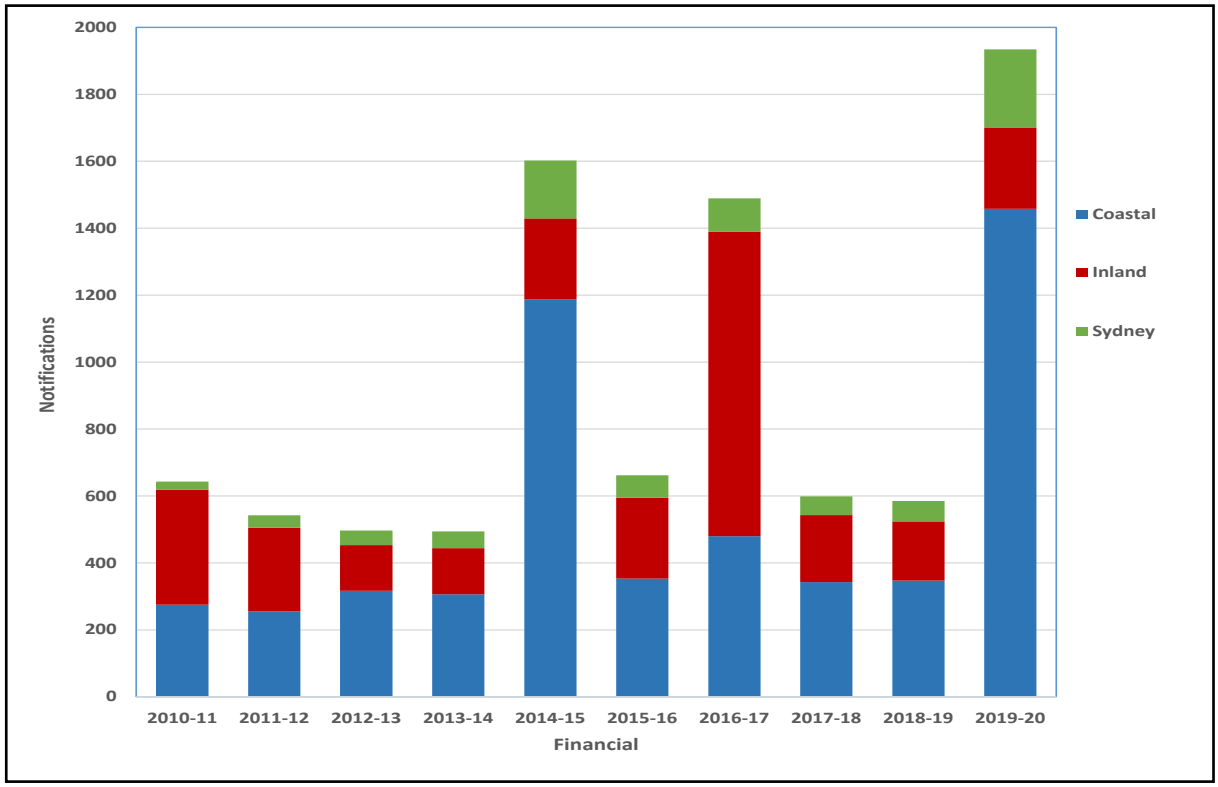


Figure 9. Monthly BFV notifications for NSW over 2019-2020, compared with the LTA.
Data source: NSW Health Infectious Diseases Data

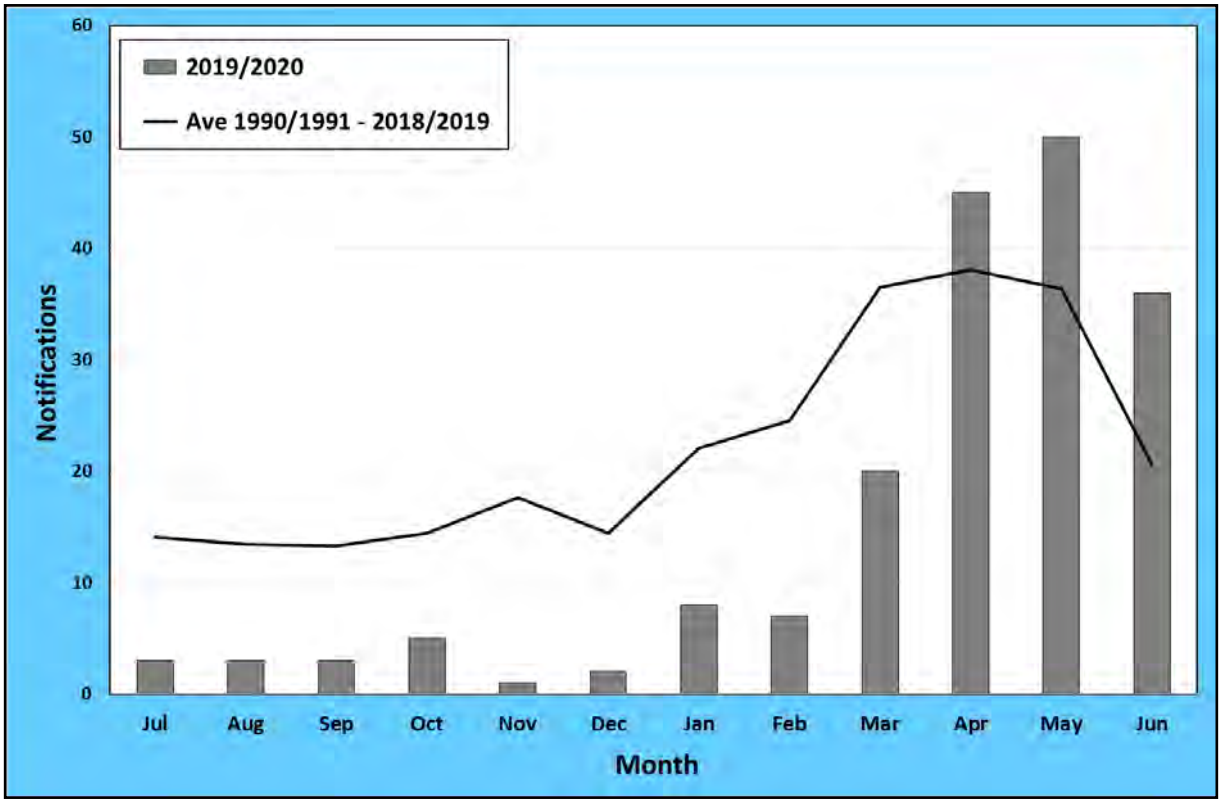


Figure 10. Monthly RRV notifications for NSW over 2019-2020, compared with the LTA.
Data source: NSW Health Infectious Diseases Data

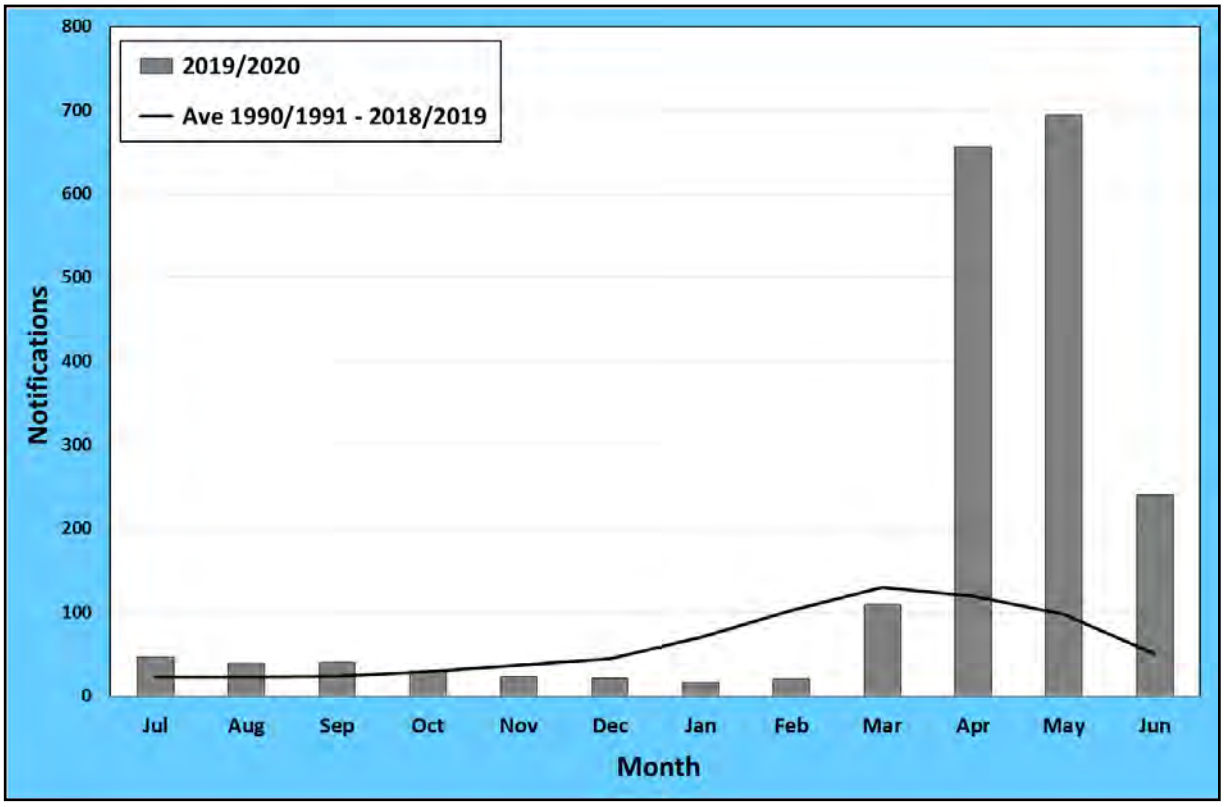
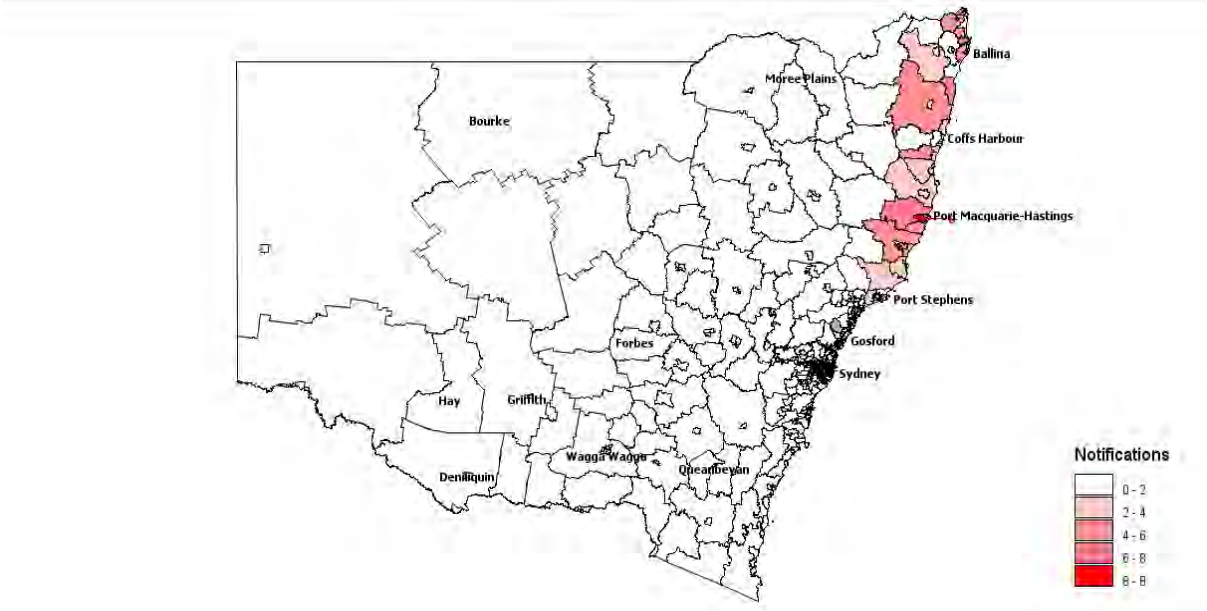
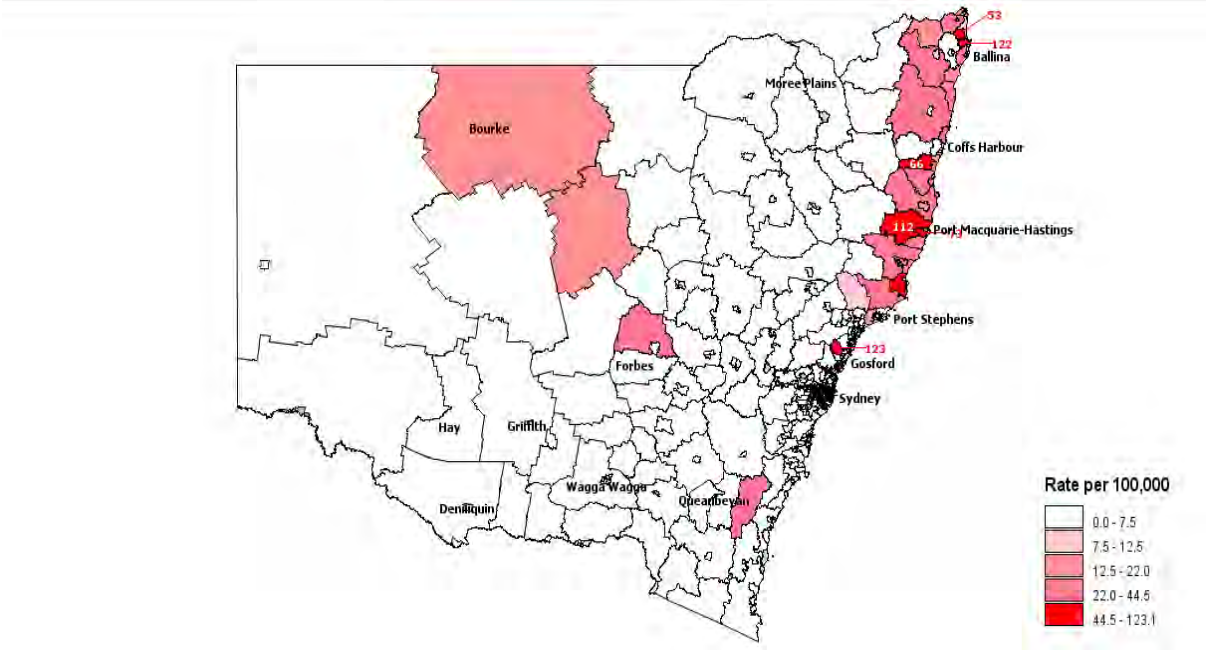


Figure 11: Barmah Forest virus infections in NSW residents.

(a) Notifications by statistical area level 2 (SA2), for 2019-2020.



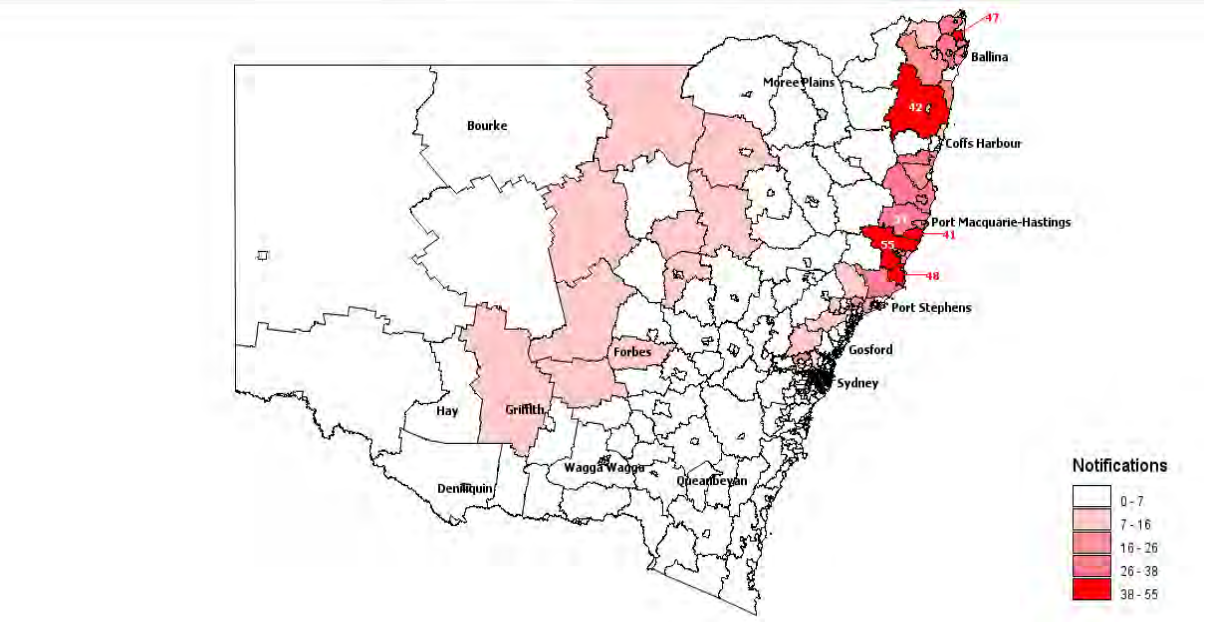
(b) Population notification rates* by statistical area level 2 (SA2), for 2019-2020.



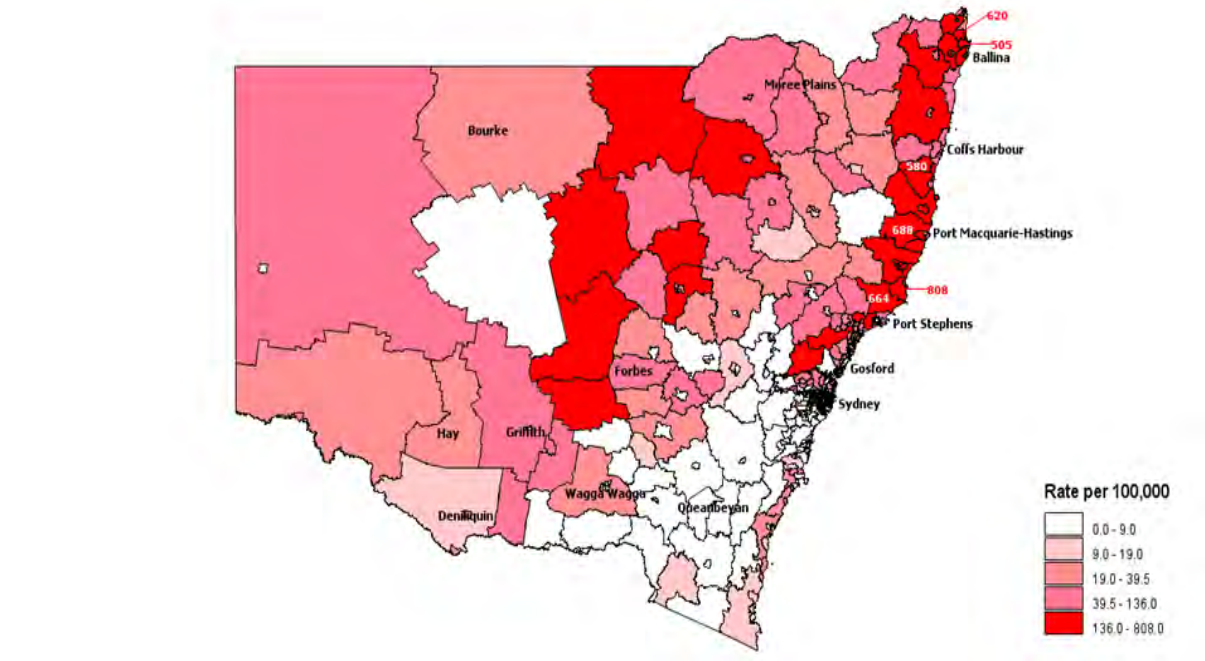
* Notifications per 100,000 estimated resident population based on ABS census data.

Figure 12: Ross River virus infections in NSW residents.

(a) Notifications by statistical area level 2 (SA2), for 2019-2020.



(b) Population notification rates* by statistical area level 2 (SA2), for 2019-2020.



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

DISCUSSION

The 2019-2020 mosquito season was a year of contrast between the two large viro-geographical regions of NSW: the inland and the coast.

For the inland, the continual dry weather patterns and extreme heat throughout the entire year of 2019 and early 2020 meant that mosquito numbers were very low. In fact, only 7,382 mosquitoes were trapped, representing one of the lowest collections for the history of the program. It is thus not unexpected that there was only one arboviral detection from the mosquitoes, no seroconversions in the sentinel chickens, and human notifications were half the long-term average. By the time the February rains came, mosquito populations were past their typical January peak, and collections failed to increase.

The dry 2019 also means that the Forbes' hypothesis will not be fulfilled for the 2020-2021 season. In contrast, the Nicholls' hypothesis is close to being fulfilled, suggesting potential risk of MVEV for the upcoming season. Furthermore, with the La Niña phase currently being experienced, rainfall patterns are set to increase. The last major La Niña events were in the summers of 2010-2011 and 2011-2012. In the latter season, there was widespread flooding across the inland and massive mosquito collections ensued, being around 25x the most recent season. In addition, there was intense arboviral activity, with a large number of isolates, widespread RRV activity, and a number of seroconversions in the sentinel flocks. La Niña phases are also associated with an increased cyclone risk. It was the low pressure cells that formed from tropical cyclones in 2008 and 2011 and subsequently moved south into NSW that brought MVEV into the region from the north (Finlaison *et al*. 2008). As there has been minimal arboviral activity across the inland for some years, it can be expected that immunity levels would be low in both the human population and reservoir hosts, thus increasing the risk of a major outbreak.

Furthermore, extensive bird breeding has been occurring in western NSW. Birds are the vertebrate hosts for most arboviruses including MVEV and can introduce the virus from northern endemic regions. According to an ABC media report (https://www.abc.net.au/news/2020-10-25/waterbirds-return-to-lake-cowal-wetlands-after-drought/12789226), Lake Cowal near West Wyalong has not only had a major increase in bird breeding but is also a site frequented by migratory birds from the north. Again, this increases the risk of possible MVEV introduction.

Therefore, it is predicted that 2020-21 season to be of highest risk periods for arboviral activity for the inland, including MVEV.

The mosquito and arboviral activity along the coast for 2019-2020 strongly contrasted to that of the inland. Not only were mosquito numbers extraordinarily high, especially with very large collections of *Culex annulirostris* and a greater number of arboviral detections than normal, there was an all-time record outbreak of RRV. The 1,941 notifications was the highest number of cases recorded since reporting began.

The events leading up to the massive RRV outbreak was almost a “Perfect Storm” of conditions. Many sites stopped mosquito trapping early as staff were redeployed to COVID-19 activities. People in the community were engaging in outdoor activities more with the COVID-19 lockdown, which exposed them to mosquitoes and the pathogens they carry. There was also the added challenge of getting mosquito-related health messages heard when the media was dominated by COVID-19. These conditions all coincided with the peak incidence of mosquito numbers and arbovirus activity.

In the past, major arboviral activity for the coast north of Sydney and into southeast Queensland has been associated with elevated *Culex annulirostris* numbers. This is in contrast to the south coast of NSW, where the saltmarsh mosquito *Aedes vigilax* has a greater role in arboviral transmission. The risk with *Culex annulirostris* is that following heavy rain, breeding tends to be much more widespread than *Aedes vigilax*, which is very coastal occurring only in saline habitats. As a consequence, a greater proportion of the population is exposed to adult *Culex annulirostris*.

With such an extraordinary year of arboviral activity, it is unlikely that such a large outbreak will again be experienced in the upcoming season. Furthermore, with native wildlife numbers heavily reduced due to the recent bushfires, there will be a smaller pool of potential vertebrate hosts to perpetuate the virus. The only unknown will be related to the greater rainfall forecasted with the La Niña and if this may lead to increased vector breeding and enhanced arboviral activity in coastal areas, such as the south coast, that have experienced little activity in recent years.

Sydney also experienced elevated arboviral activity, especially with RRV. The 234 RRV cases were around three times the long-term average of 88 (Table 4). It was not surprising that Northern Sydney had a large number of these (75 reports, Table 4) as trapping from the Northern Beaches area around the base of the Peninsula (Narrabeen and Warriewood), produced 17 arboviral detections including 3 BFV, 8 RRV, 1 EHV, and 5 STRV (Table 3). The Northern Beaches is now participating in the 2020-21 arbovirus surveillance season.

The total of 254,401 mosquitoes trapped from the Sydney locations was the highest to date. Many large collections followed the high tides and rainfall events. Thus, collections of over 10,000 (‘extreme’ numbers) were recorded from Picnic Point (>15,000 trapped) and Alfords Point (~10,000 trapped) along the Georges River in mid-January. However, these numbers pale in comparison with one collection from Duck River in late February when more than 26,500 mosquitoes were collected from one trap. This is the highest number ever collected from one trapping night for the history of the program. As usual, these Sydney locations are dominated by *Aedes vigilax*. Fortunately, there were few arboviral detections from these locations this season compared with the Northern Beaches.

EXOTIC MOSQUITO DETECTIONS AT SYDNEY INTERNATIONAL AIRPORT

Background. Over the last decade there has been an increasing number of detections of exotic mosquitoes at major Australian ports. The main species have been the Dengue/Yellow Fever mosquito, *Aedes aegypti*, and the Asian Tiger

Mosquito, *Aedes albopictus*. Both pose a serious biosecurity risk to Australia through being major vectors of serious arboviral diseases including Dengue, Yellow Fever, Zika, and Chikungunya viruses.

Aedes aegypti, being a tropical species, mainly poses a threat to the more northern regions of the nation, whereas *Aedes albopictus* is more cold-tolerant. This species therefore has the potential to become established along the eastern coast of Australia including the major population centre of Sydney. As such, *Aedes albopictus* has the potential to cost the national economy hundreds of millions of dollars, through the transmission of diseases and vector control costs. It is thus imperative that these mosquitoes are kept out of regions of the country where they presently do not exist. Furthermore, the mosquitoes that have been collected have undergone genetic analysis for the presence of insecticide resistant genes and most of the mosquitoes have these. In Australia, there is no evidence for insecticide resistance in our local mosquitoes, including invading species such as *Ae. aegypti*. The presence of resistant mosquitoes would make the mosquito much more difficult to control and limit management options.

Sydney has seen numerous detections of exotic mosquitoes in recent years. The majority have been *Ae. aegypti*, although specimens of *Ae. albopictus* and *Culex gelidus* have also been trapped. Most detections have occurred within the baggage handling area of Sydney International Airport. However, some detections have occurred at various freight handling facilities, both at Sydney Airport and in a facility close to the airport.

Table 5 details exotic mosquito detections for the period July 2019 to June 2020. All wereidentified as *Aedes aegypti*. A number of actions were implemented in response to these detections. NSW Health established regular teleconferences, the Department of Agriculture, Water and the Environment (DAWE) undertook enhanced surveillance (both increasing the number of traps used and the frequency of trap inspections), insecticidal treatment of the detection areas were undertaken, and vector surveys were conducted both within and around the sites with previously unrecorded detections, which included staff from the Department of Medical Entomology at Westmead Hospital.

It was quite evident that the number of exotic detections declined with the COVID-19 pandemic, which resulted in a dramatic decline in international flights and a reduced risk of the introduction of exotic mosquitoes.

Table 5. Detection of Exotic Mosquitoes in NSW, July 2019 – June 2020.

Date*	Mosquito Species & Sex	Location
30 Jul 2019	*Aedes aegypti* ♀	Sydney International Airport
3 Aug 2019	*Aedes aegypti* ♀	Sydney International Airport
24 Dec 2019	*Aedes aegypti* ♀	Sydney International Airport
30 Dec 2019	*Aedes aegypti* ♂	Sydney International Airport
1 Jan 2020	*Aedes aegypti* ♀	Sydney International Airport
13 Jan 2020	*Aedes aegypti* ♂	Sydney International Airport
28 Jan 2020	*Aedes aegypti* ♂	Approved Arrangement Facility, Sydney Airport
18 Feb 2020	*Aedes aegypti* ♀	Sydney International Airport
3 Mar 2020	*Aedes aegypti* ♀	Approved Arrangement Facility, Sydney Airport

*this represents the trap date.

Appendix 1. LOCATION-BY-LOCATION SUMMARY

Inland Locations

Albury: Mosquito numbers were 'low' for the entire season. There were no arboviral detections from the trapped mosquitoes. Sentinel chicken flocks did not operate at Albury.

Bourke: Mosquito collections were 'low' for the entire season. There were no arboviral isolates and sentinel chickens did not operate at this location.

Deniliquin: No mosquito collections were undertaken at this location. There were no seroconversions to MVEV or KUNV in the sentinel chickens.

Dubbo: No mosquito collections were undertaken at this location. There were no seroconversions to MVEV or KUNV in the sentinel chickens.

Forbes: For the early part of the season until January, collections were mainly 'low' from both sites. Through February, several 'high' collections were made, dominated by *Culex annulirostris*. There were no arboviral isolations nor any seroconversions to MVEV or KUNV in the sentinel chickens.

Griffith: For the entire season only 3,917 mosquitoes were trapped, which is around the number that is usually trapped during one night on a more regular year. Collections remained consistently 'high' during January to March but were never 'very high' as they tend to be most years through this period. There were no arboviral isolations nor any 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: Only 1,409 mosquitoes were trapped for the entire season – this is more akin to one night's collection during an average rainfall year. Collections were 'low' to 'medium' throughout the entire season. There was one Kokobera viral isolate from Almond Road from mosquitoes trapped on 9 March 2020. There were no seroconversions to MVEV or KUNV in the sentinel chickens.

Macquarie Marshes: No mosquito collections were made this year and there were no seroconversions to MVEV or KUNV in the sentinel chickens.

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

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

Wagga Wagga: Mosquito numbers were 'low' for the entire the season and there were no arboviral detections. Sentinel chickens did not operate at Wagga Wagga.

Coastal Locations

Ballina: Mosquito numbers were 'high' throughout the season with a number of 'very high' traps through early February and into mid-March. *Culex sitiens* comprised around one third of the collections (36.6%), with large numbers of freshwater breeding species also being trapped including *Aedes multiplex* (14.2%) and *Culex annulirostris* (12.9%). There were two arboviral detections, both from Lindsay Avenue. These included one Ross River virus detected on 6 April 2020 and one Barmah Forest virus detected on 15 April 2020.

Bellingen: This was one of several new sites where trapping was initiated this season. Mosquito numbers tended to be 'low' to 'medium' during January and February, and then rose to 'high' levels for the remainder of the season. There was one 'very high' collection in March from the Urunga treatment plant that was dominated by *Culex annulirostris*. No arboviruses were detected.

Byron Bay: Mosquito collections were 'high' for much of the season with similar numbers of *Aedes vigilax* and *Culex annulirostris*. No arboviruses were detected.

Casino: Only four collections were made for the season and mosquito numbers were mostly 'low'. No arboviruses were detected.

Coffs Harbour: Trapping was conducted at Moller Drive and Christmas Bells Road. This year produced the largest collections for the history of trapping at this location. While numbers were 'low' in early January, they quickly rose to become 'high' in late January and then 'very high' in February. Number peaked in the 'extreme' range during mid-March, with a trap yield of over 14,500 mosquitoes at Moller Drive. These big numbers were dominated by *Culex annulirostris*. There were two Edge Hill viral detections, both from Christmas Bell Road, on 2 March 2020 and the other on 24 March 2020.

Gosford: As usual, the Empire Bay site dominated the mosquito collections on the Central Coast, where numbers were 'high' for most of the season. *Aedes vigilax* dominated the collections. There were four arboviral detections from Empire Bay, these include one Edge Hill virus from 23 March 2020, one Ross River virus and one Edge Hill virus from 1 April 2020, and one Stratford virus from 7 April 2020.

Kempsey: One trapping site was operated at Kempsey at North Street. Mosquito collections were mainly 'low' until the first week of March, when they jumped to be 'very high' and continued to be so for the entire month. Thereafter, collections were mostly 'high' for the remainder of the trapping season. Collections were strongly dominated by *Culex annulirostris*. There were four arboviral detections. These include one Ross River virus from 2 March 2020, one each of Edge Hill virus and Stratford virus from 23 March 2020, and one Ross River virus from 30 March 2020.

Lake Macquarie: Collections were conducted at three sites: Belmont Lagoon, Teralba, and Dora Creek. Mosquito numbers started 'low' during January and early February and rose to be 'high' for the remainder of the season. The species composition was dominated by freshwater mosquitoes, notably *Aedes multiplex*. There was one detection of Stratford virus from a collection made on 3 March 2020 from Belmont Road.

Mullumbimby: Mosquito collections were 'medium' to 'high' for the entire season, peaking in early March with large numbers of *Culex annulirostris*. No arboviruses were detected.

Nambucca: Only one collection was made with 'medium' mosquito numbers ensuing. No arboviral isolates were detected.

Port Macquarie: Trapping was again conducted at three sites: Wall Reserve, Fernbank Creek Road, and Steven Street. Mosquito collections were consistently 'high' for much of the season, reaching 'very high' numbers during early March. *Culex annulirostris* was the most common species trapped, comprising some 31.6% of the collections. There were an extraordinary 18 arboviral detections and these are detailed in the Table below.

Table 6. Arboviral isolates from Port Macquarie, 2019-2020.

Site	Date	Number of Isolates				
		BFV	RRV	EHV	STRV	Total
Partridge Creek	03-Feb-20				1	**1**
Stevens Street	24-Feb-20			1		**1**
Stevens Street	02-Mar-20		1		1	**2**
Partridge Creek	09-Mar-20	1	1		1	**3**
Stevens Street	16-Mar-20	1				**1**
Wall Reserve	16-Mar-20		1			**1**
Partridge Creek	23-Mar-20			1	1	**2**
Stevens Street	23-Mar-20	1			1	**2**
Wall Reserve	23-Mar-20		1			**1**
Stevens Street	30-Mar-20		1			**1**
Wall Reserve	30-Mar-20		1			**1**
Stevens Street	20-Apr-20		1			**1**
Partridge Creek	27-Apr-20		1			**1**
	Total	**3**	**8**	**2**	**5**	**18**

Table 7. Arboviral isolates from Tweed, 2019-2020.

Site	Date	Number of Isolates				
		BFV	EHV	KOKV	STRV	Total
Koala Beach	09-Mar-20				1	**1**
Beltana Drive	09-Mar-20			1		**1**
Beltana Drive	23-Mar-20				1	**1**
Koala Beach	23-Mar-20		1			**1**
Beltana Drive	06-Apr-20	1				**1**
	Total	**1**	**1**	**1**	**2**	**5**

Tweed Heads: Trapping continued at the three sites of Koala Beach, Beltana Drive, and Piggabeen Road. Large mosquito numbers were trapped from the location - over 30,000 for the season. Collections were consistently 'high', becoming 'very high' during March. *Culex annulirostris* was the most frequent species trapped, comprising almost half (48.1%) of the collections. There were five arboviral detections and these are detailed in Table 7 above.

Wyong: Trapping was conducted at the sites of Ourimbah, Halekulani, North Avoca, Bateau Bay, and Pearl Beach. Mosquito numbers were 'low' for the early part of the season, with several 'high' collections during February from Halekulani. There were two arboviral detections, one Barmah Forest virus from North Avoca (1 April 2020) and one Edge Hill virus from Halekulani (28 April 2020).

Yamba: For most of the season, mosquito numbers were 'very high', particularly during February and into mid-March. *Aedes vigilax* was the predominant species, although there were large collections of *Culex annulirostris*. There was one Stratford virus detection at the Sewage Treatment Works from 18 February 2020.

Sydney Locations

Bankstown: Collections this season were at Deepwater and Picnic Point. These sites are known for intense local *Aedes vigilax* production, which again dominated the catches this year comprising over 94% of the collections. Of the two sites, Picnic Point was especially productive, trapping over 63,000 mosquitoes from this site alone. Mosquito numbers were consistently 'high' to 'very high' for the entire period from early December to mid-March. No arboviruses were detected.

Blacktown: Mosquito trapping was undertaken at Nurrangingy Reserve and Ropes Crossing. Numbers were 'low' for most of the season with the very occasional 'medium' collection. No arboviruses were detected.

Georges River: Trapping continued at Alfords Point and Illawong, with the former site producing the vast majority of the catch and almost 43,000 mosquitoes for the season. Collections were 'high' or greater for most of the season, with numbers peaking in late January. *Aedes vigilax* comprised more than 90% of the collections. No arboviruses were detected.

Hawkesbury: Trapping occurred at four main sites, including at Wheeny Creek, McGraths Hill, Yarramundi, and Richmond. Mosquito numbers tended to be 'low' through to mid-February and then consistently 'high' for the remainder of the trapping season. There were even two 'very high' collections made at Wheeny Creek, dominated by *Culex annulirostris* during March. There was one detection of Edge Hill virus from a collection made on 9 March 2020 from Wheeny Creek.

Hills Shire: Mosquito trapping occurred at Rouse Hill, Glenorie, and Baulkham Hills. Numbers were 'low' for most of the season, although collections did increase to become 'high' in early March. No arboviruses were detected.

Liverpool: Collections took place around Liverpool this year following complaints of large mosquito numbers in the area. Mosquito collections were consistently 'high, although 'very high' numbers were trapped in mid-February and again in mid-March. *Culex annulirostris* was the species most commonly trapped, comprising 30% of the collections. There were two Barmah Forest viral detections from the Hammondville site, one each on 18 Mar 2020 and 27 April 2020.

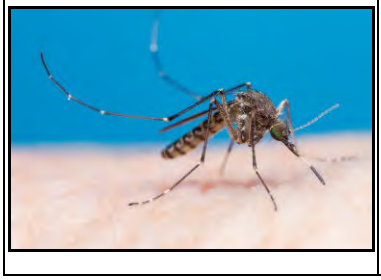
Parramatta: Collections occurred at the three sites of George Kendall Reserve, Eric Primrose Reserve and Duck River. The latter site yielded the largest collections, trapping over 65,000 mosquitoes during the season. Here, *Aedes vigilax* comprised almost 95% of the collections. For the season, numbers were consistently 'high' and 'very high' from late December through to late March. There was even on 'extreme' collection of over 26,000 mosquitoes in mid-February from Duck River and this was a new record for the history of the NSW Arbovirus Surveillance Program. No arboviruses were detected.

Penrith: Trapping occurred at the sites of Emu Plains, Muru Mittiger, Orchard Hills, and Werrington. Mosquito numbers averaged 'low' to 'medium' up until February and thereafter collections tended to be 'high' for the remainder of the season. No arboviruses were detected.

Sydney Olympic Park (SOP): Mosquito monitoring at this location included the long-term locations of Narawang and Haslams Creek, as well as Newington. Mosquito numbers were consistently 'high' for almost the entire season, with a series of 'very high' traps in late January and again in late February following the high tides. As usual, *Aedes vigilax* was the predominant species, however large numbers of *Culex annulirostris* were trapped late in the season. There was one Stratford virus detection on 27 February 2020 from Haslams Creek.

Appendix 2. THE MOSQUITOES

The following briefly details the main mosquito species collected in NSW.

	**The Common Domestic Mosquito**, ***Aedes notoscriptus***. A common species that breed in a variety of natural and artificial containers around the home. It is the main vector of dog heartworm and laboratory studies shows it be an excellent transmitter of both RRV and BFV.
	**The Bushland Mosquito**, ***Aedes procax***. Common throughout coastal NSW and breeds in bushland freshwater groundwater. Numerous isolates of BFV have been recovered from this species and it is probably involved in the transmission of the virus.
	**The Northern Saltmarsh Mosquito**, ***Aedes vigilax***. An important species along coastal NSW. This species breeds on the mud flats behind saltmarshes and can be extremely abundant and a serious nuisance biter. It is a major vector for RRV and BFV along the coast.
	**The Common Australian Anopheline,** ***Anopheles annulipes***. A mosquito from throughout NSW, but is most common in the irrigated region of the Murrumbidgee where it can be collected in the thousands. 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 and RRV have been isolated from this species and is probably involved in some transmission.
	**The Common Banded Mosquito**, ***Culex annulirostris***. The species is common in the NSW inland regions that have intense irrigation. This species is highly efficient at transmitting most viruses and is responsible for the spreading of most of the arboviruses to humans inland. It is also involved in coastal RRV transmission.

Appendix 3. THE VIRUSES

Alphaviruses

Barmah Forest virus (BFV): Disease from this virus is clinically similar to that of RRV disease, although BFV disease tends to be associated with a more florid rash and a shorter duration of clinical severity. Serological over diagnosis of this condition through the non-specificity of the commercial kit was a major issue, and the kit was withdrawn from the market. This led to a dramatic reduction in BFV notifications and the disease may now be under reported. Despite being first isolated from an inland region, cases of BFV disease tend to occur mostly in coastal regions in NSW. The main vector in NSW is *Aedes vigilax* although other species are involved, notably *Aedes procax*. In 2010-2011 for the inland, there was a small epidemic of BFV, but this was the largest outbreak to date for the region.

Ross River virus (RRV): This virus causes RRV disease and is the most common cause of human arboviral disease in Australia. In NSW, approximately 700 cases per season are usually 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 coast) 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 July 1995-June 2003 (Doggett 2004), with few cases reported since then. Symptoms of disease include fever and rash. Birds are the main host, although other animals can be infected, including macropods, cattle, dogs and humans. The virus has been isolated from many mosquito species, but most notably *Culex annulirostris* in south-eastern Australia. It is also no longer routinely tested and it is possible that this would cross react with RRV in commercial tests.

Flaviruses*

Alfuy virus (ALFV): No clinical disease has been associated with this virus and it has not been isolated from south-eastern Australia.

Edge Hill virus (EHV): A single case of presumptive infection with EHV has been described, with symptoms including myalgia, arthralgia, and muscle fatigue. *Aedes vigilax* has yielded most of the EHV isolates in southeast Australia, although it has been recovered from several other mosquito species. The virus is quite common, with isolates from most years. The vertebrate hosts may be wallabies and bandicoots, but studies are limited.

Kokobera virus (KOKV): Only three cases of illness associated with KOKV infection have been reported and all were from southeast Australia. Symptoms included mild fever, aches and pains in the joints, and severe headaches, and lethargy. Symptoms were still being reported by the patients five months after onset. This virus historically was only known from inland regions of NSW until it was detected in a mosquito trapped from the coastal region in 2009-2010. *Culex annulirostris* appears to be the principal vector.

Kunjin virus (KUNV): Disease from this virus is uncommon, with only two cases being notified from 1995-2003 (Doggett 2004), and one case in 2011 (Doggett *et al*. 2012). Historically, activity has been confined to the inland region of NSW where it is detected every few years. However, in the summer of 2010-2011, the virus was detected on the coast, which resulted in an outbreak amongst horses and a number of animal deaths. *Culex annulirostris* appears to be the main vector.

Murray Valley encephalitis (MVEV): Major activity of this virus is rare in south-eastern Australia and the last epidemic occurred in 1974. However, since 2000 there have been six seasons when MVEV activity has been detected within NSW: 2000-2001, 2003-2004, 2007-2008, 2010-2011, 2011-2012, and most recently, 2013-2014. There were 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.

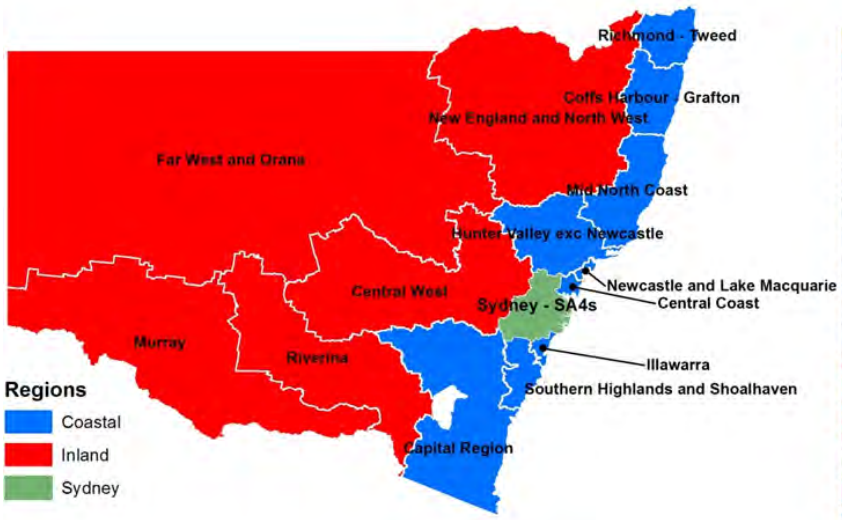
Stratford virus (STRV): There have been very few documented symptomatic patients, with only three described to date and symptoms including fever, arthritis, and lethargy. The virus has mostly been isolated from coastal NSW, particularly from the saltmarsh mosquito, *Aedes vigilax*, although recent isolates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

ALFV	Alfuy virus
BFV	Barmah Forest virus
BOM	Bureau of Meteorology
EHV	Edge Hill virus
IgG	Immunoglobin G (a type of antibody)
IgM	Immunoglobin M (a type of antibody)
ICPMR	Institute for Clinical Microbiology and Medical Research
LHD	Local Health District
MVEV	Murray Valley encephalitis virus
KOKV	Kokobera virus
KUNV	Kunjin virus
PHU	Public Health Unit
RRV	Ross River virus
SA2	Statistical area level 2
SINV	Sindbis virus
SLA	Statistical Local Area
SO	Southern Oscillation
STRV	Stratford virus

Appendix 5. NSW GEOGRAPHIC REGIONS - COASTAL, INLAND, AND SYDNEY METROPOLITAN – USING ABS STATISTICAL AREA LEVEL 4 (SA4) GROUPINGS.



SA4 Name	Region
Capital Region	Coastal
Coffs Harbour - Grafton	Coastal
Newcastle and Lake Macquarie	Coastal
Southern Highlands and Shoalhaven	Coastal
Illawarra	Coastal
Hunter Valley excluding Newcastle	Coastal
Central Coast	Coastal
Richmond - Tweed	Coastal
Mid North Coast	Coastal
Central West	Inland
Far West and Orana	Inland
New England and North West	Inland
Riverina	Inland
Murray	Inland
Sydney - all 14 Sydney SA4s	Sydney

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REFERENCES

Bureau of Meteorology, Australia. (2020). **Rainfall Maps**. http://www.bom.gov.au/cgi-bin/climate/rainmaps.cgi, *accessed 15/Aug/2020.*

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.L. (2018). **Your number's up!** *Mosquito Bites*, *13*(1): 24-27.

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.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.L., Webb C., Clancy J. and Haniotis J. (2017). **Mosquito survey of the QANTAS freight terminal, Sydney International Airport, 2017**. *Report for the NSW Ministry of Health*.

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.

Ibáñez-Justicia A., de Vossenberg B., van den Biggelaar R., Voogd J, Metx E, Jacobs F., Dik M. and Stroo A. (2019). **Detection of *Aedes flavopictus* (Yamada, 1921), Netherlands, June 2019**. *EuroSurveillance*, 24(30): 1900433.

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.

Knope K.E., Kurucz N., Doggett S.L. *et al*. (2016). **Arboviral diseases and malaria in Australia, 2012-23: annual report of the National Arbovirus and Malaria Advisory Committee**. *Communicable Diseases Intelligence*, 40(1): E17-E47.

Kurucz N., Markey P., Draper A. *et al.* (2016). **Investigation into High Barmah Forest Virus Disease Case Numbers Reported in the Northern Territory, Australia in 2012-2013**. *Vector-Borne and Zoonotic Diseases*, 16(2): 110-116.

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

Pyke, A. T., I. L. Smith, A. F. van den Hurk, J. A. Northill, T. F. Chuan, A. J. Westacott, and G. A. Smith. 2004. **Detection of Australasian Flavivirus encephalitic viruses using rapid fluorogenic TaqMan RT-PCR assays**. *Journal of Virological Methods*, 117: 161-167.

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.

van den Hurk, A. F., S. Hall-Mendelin, M. Townsend, N. Kurucz, J. Edwards, G. Ehlers, C. Rodwell, F. A. Moore, J. L. McMahon, J. A. Northill, R. J. Simmons, G. Cortis, L. Melville, P. I. Whelan, and S. A. Ritchie. 2014. **Applications of a sugar-based surveillance system to track arboviruses in wild mosquito populations.** *Vector Borne Zoonotic Diseases*, 14: 66-73.