

# Ross River virus and its vectors in Sorell Municipal Area, south-eastern Tasmania, January to March 2002

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## Abstract

In 2002, Tasmania reported the largest number of Ross River virus (RRV) infections ever recorded for the state. Of the 117 cases, 37 lived in, or had visited, the Sorell Municipal Area, east of Hobart. In early 2002, a combination of spring tides and high summer rainfall produced extensive saltmarsh habitat in the Sorell region, resulting in unseasonably high densities of the mosquito *Ochlerotatus camptorhynchus*, recognised vector of RRV. Four isolates of RRV were identified from collections of adult mosquitoes. All four isolates were from *Oc. camptorhynchus*, collected near the Carlton River. This is the furthest south that RRV has been identified in Australia and the first identification from south-east Tasmania. The virus carriage rate in the mosquito vector populations were very high, with successive weekly minimum infection rates of 17.1, 3.0 and 11.1 per 1,000 *Oc. camptorhynchus* at Carlton River from mid-February to early March. The first isolation of RRV from mosquitoes coincided with the onset dates of the first human cases of RRV infection. *Commun Dis Intell* 2004;28:261–266.

Keywords: *Ochlerotatus camptorhynchus*, Ross River virus

## Introduction

The number of cases of Ross River (RRV) disease have fluctuated in Tasmania between 8 and 117 during the period 1995–2002. The highest notifications were reported in 1996, 1999 and 2002, with 74, 67 and 117 notifications, respectively.<sup>1</sup>

Investigations into the dynamics of arboviral diseases within Tasmania have been limited to two previous studies. Both were undertaken on the east coast, largely focusing on the Coles Bay to Scamander region, and identified *Ochlerotatus camptorhynchus* and *Oc. flavifrons* as vectors of RRV for that region.<sup>2,3</sup>

Despite the presence of human cases of arbovirus disease further south in the Hobart region, there have been no investigations of the mosquito vectors and the relative disease risks in these south-eastern communities.

The Sorell Municipal Area is 25 km east of Hobart, and its population of 10,800 is located mostly in the coastal townships of Midway Point, Sorell, Dodges Ferry and Primrose Sands. Sorell Council has received notifications of cases of RRV infection since 1995. In 1999, following 30 cases of RRV and complaints of excessive local mosquito activity, Sorell Council commenced surveys of mosquito larval habitats and began adult trapping in several areas.

This surveillance was subsequently expanded throughout the municipal area, and in the summer of 2002, the Council decided to test field-collected adults for the presence of arboviruses to determine which local mosquitoes were likely RRV vectors. In order to determine the origin of the RRV infections, all local human cases were interviewed to establish the location of their residence and if they had travelled to, or outside of, the Sorell area in the 2–3 weeks before the onset of symptoms.

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## Methods

### Case investigation

Notifications of RRV were included in this study if they satisfied the Tasmanian case definition of RRV which required notification to the Director of Public Health, i.e. laboratory analysis of a blood sample satisfying one of the following criteria:

1. isolation of RRV from a sample;
2. detection of RRV by nucleic acid testing;
3. IgG seroconversion or a significant increase in antibody level, or a fourfold or greater increase in titre to RRV; or
4. detection of RRV specific IgM antibody titre.

Notifications of RRV infection in residents of Sorell Municipal Area, and in individuals that had travelled to the area within two weeks before the onset of symptoms were interviewed. The possible site of infection was determined by comparing residential address with proximity to known larval habitats. Demographic details of local cases were examined to determine if any particular group was at a greater risk. Infections acquired on the western side of Pittwater were not included in this study as that area is within the City of Clarence (which reported 50 of the 117 cases notified state-wide). The City of Clarence is located between Hobart and Sorell. Pittwater is an estuary extending from Richmond (City of Clarence) to Lewisham (Sorell Municipal Area). Pittwater contains the coastal towns of Midway Point and Sorell (Figure 1).

### Habitat investigation and mosquito surveillance

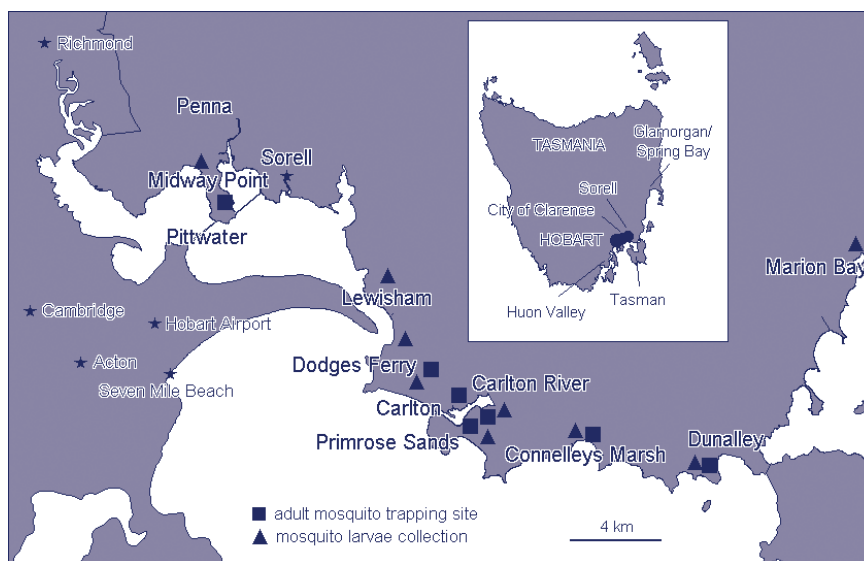
The purpose of the study was to determine which local mosquito species were the likely vectors of RRV.

Aquatic habitats were explored in the municipal area (west to east) at Penna, Midway Point, Lewisham, Dodges Ferry, Carlton, Primrose Sands, Connellys Marsh, Dunalley and Marion Bay (Figure 1). Mosquito larvae were collected and quantified by dip sampling techniques<sup>4</sup> and identified to species using larval identification keys.<sup>5</sup>

Adult mosquito populations were sampled with dry ice-baited EVS light traps<sup>6</sup> at seven sites; indicated in Figure 1. Traps were set late in the afternoon and collected early the following morning on 21 January, 20 February, 25 February, and 5 March 2002. Trapping days were selected considering climatic conditions and transport logistics for sending the mosquitoes to Sydney for virus isolation. All mosquitoes collected were sent for virus isolation testing.

Traps were set within coastal towns with the exception of the Carlton River site, which was set on the edge of the saltmarsh habitat. Areas where complaints about excessive numbers of mosquitoes had been received from local residents were also selected. Trapping focused around Carlton River where larval surveying revealed larger saltmarsh habitat areas. Trapping expanded to Dunalley, Connelly's Marsh, Dodges Ferry and Midway Point to establish the extent of distribution into residential areas.

**Figure 1. Adult and larval sampling locations**



## Arbovirus isolation

The mosquito collections were transported live to the Institute of Clinical Pathology and Medical Research, Sydney, for processing. Mosquitoes were sorted and identified<sup>4-8</sup> on a refrigerated table, into pools of up to 25, according to species, sex, bloodfed, date and site.<sup>9</sup> Each pool was placed into a sterile plastic 5 mL tube containing 5 x 5 mm glass beads and 5 mL of cell growth media, macerated in a grinder/shaker for 20 minutes and centrifuged at 4,000 rpm and 4° C for 20 minutes, and a 50 UL aliquot of supernatant from each tube was inoculated into the cell lines C6/36, BHK and PSEK, as previously described.<sup>9,10</sup>

The presence of viruses was indicated by cell death. Presumptive viral isolates were tested by Fixed Cell Enzyme-Linked Immunosorbent Assay (FCE)<sup>11</sup> using specific monoclonal antibodies to detect alphaviruses (Ross River, Sindbis, Barmah Forest viruses) and flaviviruses (Murray Valley encephalitis, Kunjin, Stratford, Alfuy, Edge Hill, Kokobera). As RRV was detected in the mosquito samples, a RRV FCE screen was performed on later samples to rapidly identify virus. Minimum infection rates (MIRs) per 1,000 were calculated<sup>12</sup> per trap for the species that yielded virus.

## Meteorological conditions

Rainfall data were recorded at the Park Beach Weather Station (Dodges Ferry), the closest weather station to Carlton River. Temperature data at Hobart Airport were obtained from the Bureau of Meteorology.

## Results

### Human cases

In 2002, a total of 117 cases of RRV were notified in Tasmania, with 19 of these resident in the Sorell Municipal Area.<sup>13</sup> Another 18 had travelled to the municipal area within two weeks prior to onset of symptoms and may have been infected during this period. The Department of Health and Human Services reported that more than 90 per cent of all cases notified were from single sample IgM results. Single sample IgM serology does not provide absolute certainty that infections occurred recently and cases notified should be regarded as presumptive.<sup>14</sup> However, in combination with clinical symptoms and RRV isolates from local mosquitoes, it is unlikely that a large number of previous infections are included within the 37 cases.

Of the 37 cases that had some association with the Sorell Municipal Area, 23 lived in, or had visited, the Carlton River, eight were from Pittwater, four were from Marion Bay and the other two cases were Sorell Municipal Area residents that had links with sites in the City of Clarence. RRV infection onset dates ranged from 22 February 2002 to 10 May 2002. Generally, infections linked to the Carlton River area occurred first and continued throughout the period, with cases from the Penna area occurring in late-March and those at Dunalley/Marion Bay in mid-April.

There were 20 males and 17 females with infections that could have been acquired locally. The average age of patients was 47 years (range 8 to 81); the majority (21) were in the 30-49 years age range, with three under 30 years and three over 70 years.

In addition to the cases reported in the Sorell Municipal Area another 50 RRV notifications were reported from the residents of the City of Clarence. Of these 50 cases, 22 lived in, or travelled to, the western Pittwater towns of Seven Mile Beach, Acton, Cambridge or Richmond.

Of the 117 cases in Tasmania, 105 lived in Southern Tasmania from the following Local Government Areas: Clarence (50), Sorell (19), Hobart (10), Glenorchy (7), Huon Valley (7), Kingborough (4), Tasman, (3), Brighton (3), and Glamorgan Spring Bay (2).<sup>13</sup> These areas are all situated on the south-east coast. Hobart, Glenorchy, Brighton, Clarence, and Kingborough are within 'Greater Hobart'. Huon Valley is 35 km south of Hobart, Glamorgan Spring Bay is on the east coast and Tasman is in the south east.

### Weather conditions

Coastal regions of the Sorell Municipal Area usually receive a low annual average rainfall of 560 mm, and have an average maximum summer temperature of between 20 and 22° C. However, the spring and early summer months of 2001/02 were very wet, with a total rainfall of 450 mm. Rainfall between August 2001 and April 2002 was compared with long-term (since 1962) averages (Figure 2). The above average rainfall coincided with high tides, resulting in the saltmarsh at Carlton River holding water from early spring until March. In contrast, the saltmarsh totally dried out for extended periods (4-6 weeks) in the previous two summers.<sup>15</sup>

The summer months had below average monthly temperatures, with December 2001, January 2002 and February 2002 being 1.9, 1.7 and 0.8° C below average, respectively, although March and April were 1.5 and 1° C above average, respectively.

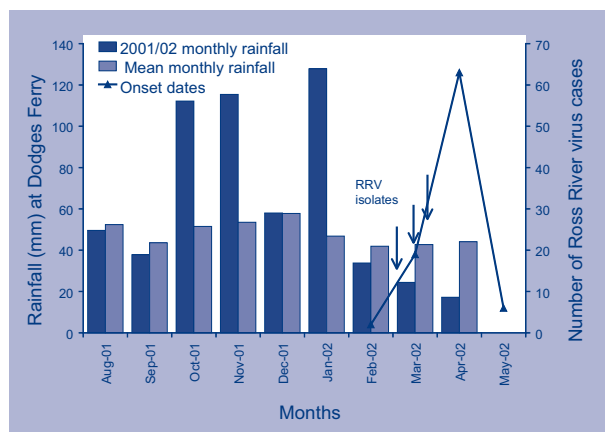
**Mosquito fauna**

Mosquito larvae identified included *Anopheles annulipes* s.l., *Culex australicus*, *Cx. fergusonii*, *Cx. globocoxitus*, *Cx. molestus*, *Cx. orbostiensis*, *Ochlerotatus alboannulatus*, and *Oc. camptorhynchus*.

Laval surveys found high numbers of *Oc. camptorhynchus* on the saltmarsh at Carlton River (n=257) at a density of >100 per larval dip on 21 February 2002. Surveys at other saltmarsh areas near Marion Bay, Penna, and Lewisham contained <5 per dip. Freshwater habitats had even lower numbers (<1 per dip).

Overall, there were 18 collections of adult mosquitoes. Four from the Carlton River (n=694) and Carlton (n=1,011), three from Dodges Ferry (n=336), two from Primrose Sands (n=143) and Midway Point (n=19), and one from Dunalley (n=8), Richmond (n=25) and Connelly's Marsh (n=11). A total of 2,247 adult mosquitoes were collected from five genera and 13 species (Table). *Ochlerotatus camptorhynchus* dominated the collections and comprised around 90 per cent of the total. Most other species appeared infrequently or rarely. Only two species collected as larvae were not trapped as adults; *Cx. fergusonii* and *Cx. orbostiensis*.

**Figure 2. Rainfall compared with onset dates of cases of Ross River virus, Sorell and Clarence municipal residents/visitors**



**Viruses isolated**

There were 153 mosquito pools processed and four RRV isolates obtained. All were from *Oc. camptorhynchus*, with three isolates coming from Carlton River at Primrose Sands and the other from Provence Drive at Carlton. Table 1 provides mosquito totals and details of the viral isolates. The MIRs varied from 3.0 to 17.1 per 1,000 and were highest at Carlton River, Primrose Sands.

**Table. Mosquito species collected and numbers processed for virus isolation with viruses recovered and minimum infection rates of the mosquito vectors**

Species	Collection dates with total adult numbers, virus isolations and minimum infection rates			
	21 January 2002	20 February 2002	25 February 2002	5 March 2002
<i>Anopheles annulipes</i> s.l.	3	0	4	1
<i>Coquillettidia</i> sp. nr. <i>linealis</i>	13	14	11	6
<i>Culex australicus</i>	6	0	3	0
<i>Cx. fergusonii</i>	0	0	0	0
<i>Cx. globocoxitus</i>	9	23	35	15
<i>Cx. molestus</i>	7	9	12	6
<i>Cx. orbostiensis</i>	0	0	0	0
<i>Culiseta inconspicua</i>	0	2	1	0
<i>Ochlerotatus australis</i>	0	1	3	0
<i>Oc. alboannulatus</i>	2	1	1	0
<i>Oc. camptorhynchus</i>	522	251	989	254
		1RRV* (MIR:17.1)	2RRV*,** (3.0, 7.3)	1RRV* (11.1)
<i>Oc. nigrithorax</i>	1	0	0	0
<i>Oc. notoscriptus</i>	0	3	18	8
<i>Oc. rubrithorax</i>	0	1	0	0
<i>Tripteroides tasmaniensis</i>	0	0	2	0

MIR = number of mosquitoes infected per 1,000 and based on pools of 25 (Chiang and Reeves 1962).

\* Ross River virus isolate from Carlton River, Primrose Sands, Tasmania.

† Ross River virus isolate from Provence Drive, Carlton, Tasmania.

## Discussion

The 117 RRV cases reported for Tasmania in 2002, exceeded the previous highest notification of RRV for Tasmania of 74 cases in 1996. The first cases of RRV reported for the Sorell Municipal Area were from the Carlton River locality (onset date 22 February) and coincided with the first collection of mosquitoes that yielded RRV (trapped 20 February 2002). Most human infections, based on onset date, occurred during March and April in the two months following the high rainfall and tides in January (Figure 2).

High tides and rainfall during spring and early summer provided extensive and sustained mosquito habitat, which allowed for the build-up of large vector populations compared to the previous year,<sup>15</sup> although previous preliminary studies focused on species identification and thus exact numerical comparisons of numbers of adults and larvae cannot be made. Carlton River was the most significant habitat area for the vector species *Oc. camptorhynchus*. Other larval populations of this species were at Marion Bay, Penna, Lewisham and Primrose Sands.

Several cases of RRV were linked to Penna and Marion Bay, which require further investigation. In addition, other saltmarsh areas in nearby Pittwater (not within Sorell Municipal Area), around Barilla Bay and the Coal River, may be significant habitat areas contributing to the 50 cases seen in the City of Clarence during the same period.

The Carlton River saltmarsh is, at its closest point, only 50–100 m from houses, and a large portion of residents of Primrose Sands, Carlton, Connelly's Marsh and Dodges Ferry live within 5 km of the saltmarsh. Carlton River is surrounded by pasture and forest, and these communities are habitats for macropods and other vertebrates<sup>16</sup> that may serve as vertebrate hosts for RRV and other arboviruses.<sup>17,18</sup>

Adult trapping in Primrose Sands, Carlton and Dodges Ferry revealed high numbers of the vector *Oc. camptorhynchus* compared to other species. This was consistent with the larval surveys, and reflected the large larval habitat at Carlton River (compared to freshwater habitats) and the lack of predators in the saltmarsh habitats. Other species from which RRV has been isolated elsewhere in Australia, such as *Coquillettidia* sp. nr. *linealis*, *Oc. notoscriptus*, *Oc. alboannulatus*, *Oc. rubrithorax*, *An. annulipes* s.l. and *Cx. australicus*<sup>17</sup> were present only in low numbers and did not yield any virus.

*Ochlerotatus camptorhynchus* is a major inhabitant of southern coastal Australia within saltmarsh habitats.<sup>17</sup> Adults can be active throughout the year, and may disperse widely from larval habitats.<sup>8</sup> They can be vicious biters, readily attacking humans and

other animals, and will feed during the day, at dusk and after sunset. RRV has been isolated from this mosquito in all southern states of Australia except South Australia.<sup>17,18,20</sup> This species is presumably responsible for the transmission of the majority of RRV infections in southern coastal Australia.

Other mosquito species also may be locally significant as vectors of RRV. *Coquillettidia* sp. nr. *linealis*, a mosquito associated with permanent vegetated freshwater, has been shown to be a putative vector for RRV and other viruses from elsewhere in Australia.<sup>17,18</sup> *Ochlerotatus flavifrons*, the only species that yielded RRV in the inaugural Tasmanian mosquito/arbovirus investigations<sup>2</sup> was not found in this study. Further collections for virus isolation may elucidate whether *Coquillettidia* sp. nr. *linealis* or *Oc. flavifrons*, or other local mosquitoes, are involved in the transmission of RRV in the Sorell region.

The infection rates observed were relatively high and comparable to epidemic situations investigated elsewhere. During the Barmah Forest virus outbreak along the south coast of New South Wales in 1995 where there were 135 human cases, MIRs over three successive weeks during the peak of activity were 15, 27 and 4 per 1,000 *Oc. vigilax*.<sup>19</sup> During the outbreak of RRV in Western Australia in 1995/96 MIRs per 1,000 *Oc. camptorhynchus* were calculated for three sites: Peel Inlet (0.2, 1.1, 0.4, 2.2 & 3.5), Leschenault Inlet (10.5) and Busselton wetland (18 & 5.5),<sup>20</sup> and compared with the weekly successive MIRs (17.1, 3.0 and 11.1/1,000) observed in the present study. In 1991, arbovirus investigations in the Scamander region (north east coast of Tasmania), during a period when several local human cases were identified, yielded RRV from *Oc. camptorhynchus* but with lower MIRs (approx. 3.5/1000) for *Oc. camptorhynchus*.<sup>3</sup>

As no longitudinal data on mosquito infection rates within the Sorell region were available, it was not possible to determine if the high MIRs in 2002 reflected typical arbovirus activity. However, the level of virus activity detected, especially from Carlton River, suggests that at least occasionally there can be a considerable arboviral disease threat to the nearby human community from *Oc. camptorhynchus*.

Carlton River was confirmed as a significant habitat for the vector *Oc. camptorhynchus*, and epidemiological follow-up established that most cases of RRV were associated with the Carlton River, although cases did occur in the far west of the municipal area at Penna and in the far east at Marion Bay. Strategies to reduce the risk of RRV in south-eastern Tasmania will therefore require further investigation and cooperation from neighbouring councils and the Tasmanian government.

As there is no coordinated mosquito vector monitoring conducted outside of the Sorell municipal area, the source of RRV infections from residents of 'Greater Hobart' Huon Valley, and East Coast (Glamorgan Spring Bay and Tasman) is not conclusive.

The results of the study will form the basis for more comprehensive vector monitoring and control programs. The saltmarshes at Pittwater are contained within a Ramsar site (wetland of international importance) and Carlton River is 15 km from the Ramsar site. In both situations regulatory approval will be required to conduct any form of mosquito control.

The study has enabled targeted public health education information to be developed and warnings issued to residents in high risk areas. The Tasmanian Director of Public Health issued a warning in early summer 2001/02 to all residents. Following this in March 2002, Sorell Council was the only council to issue a specific warning to residents of the risk of RRV infection and to take measures to avoid being bitten by mosquitoes.

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