# Analysis of the Spread of Rabbit Calicivirus from Wardang Island through Mainland Australia

B. D. Cooke,
CSIRO, Division of Wildlife and Ecology
PO Box 84, Lyneham, ACT 2602

**A report prepared for the Meat Research Corporation, October 1996, as part of Project CS.236 "Field Evaluation of RCD under Quarantine"**

STOCK CODE: LPI080
ISBN#: 1740367767
1996.

## Analysis of the Spread of Rabbit Calicivirus from Wardang Island through Mainland Australia

*B. D. Cooke,*
*CSIRO, Division of Wildlife and Ecology*

### Executive Summary

The escape of RCD from Wardang Island was associated with an experiment which began on 13th September 1995. The presence of infected rabbits and increased insect activity, associated with warm weather from 18th - 20th September, probably facilitated the spread of RCD from experimental sites to sentinel sites within the quarantine facility on Wardang Island. The light westerly winds at the time were consistent with the easterly direction of spread. Accidental spread into sentinel sites by personnel involved in the project can be ruled out. Scavenging birds also seemed unimportant.

An infected rabbit was collected on the north-east coast of Wardang Island, outside the quarantine area, on 29th September 1995. A similar focus was detected on the north coast a short while later. The disease spread rapidly; dead rabbits were found up to 300 metres from the foci within a few days.

It was subsequently shown that blowflies, *Calliphora stygia,* collected on 24th September 1995 from traps placed immediately outside the quarantine area on Wardang Island, were positive for rabbit calicivirus. The testing was done at AAHL using PCR techniques. No rabbit calicivirus was detected on *C. nociva* or bushflies, *Musca vetustissima,* collected at the same time.

A source of virus was available on Wardang Island from 13th September until at least the 19th October 1995 when the last active case of RCD was recorded on the island. However, because rabbits in the experimental sites within quarantine were quickly killed and removed, infected rabbit carcases would have been most common after 29th September 1995 when the first infected rabbits were discovered outside the quarantine area. Rabbit carcases may have provided an increasing source of virus at that time but this would have rapidly declined once the majority of rabbits were killed by poisoning, ripping and fumigation as part of the emergency protocol.

Rabbits infected with rabbit calicivirus were found on Point Pearce, on the mainland adjacent to Wardang Island, from 12th October onwards, but were first recorded at Yunta and Gum Creek in north-eastern South Australia on 28th October.

Nucleotide sequences of virus samples show that virus recovered from Yunta was identical to those recovered from Wardang Island but differed slightly from the strain recovered from Point Pearce. While such information must be treated carefully in view of natural variation in populations of viruses, the simplest explanation is that virus was transferred directly from Wardang Island to Yunta.

Working on the basis of the rate of spread of the virus from the initial focus at Yunta and from the age-structure of surviving rabbits at Gum Creek (assuming rabbits less than 6 weeks old suffered lower mortality than older rabbits) it seems likely that the disease arrived in north-eastern South Australia in the first two weeks of October 1995.

On this basis, the projections of insect movements from Wardang Island (Wardhaugh and Rochester 1996) are extremely interesting, indicating that movement of day-flying insects on 12th, 13th and 14th October 1995, could account for the spread of virus to Yunta and indeed the broad distribution of virus eventually recorded in South Australia during late 1995.

Despite reasonable evidence that insects are involved in the transmission of RCD, Wardhaugh and Rochester (1996) state that the slowing in the rate of spread and the small number of new cases during the summer of 1995 - 96 could not be explained by a lack of vectors. Furthermore, field samples collected at sites where RCD had been widespread confirmed that substantial numbers of flies and mosquitoes persisted throughout the summer. Factors other than the presence of vectors must affect the spread of the virus.

Temperature is likely to be the most important factor. Laboratory tests at AAHL show that survival of the virus declines rapidly with increasing temperature, and normal summer-time temperatures in inland Australia would be expected to reduce potential for the virus to survive. In the 1995-96 summer, new foci of RCD were mainly observed in coastal areas of South Australia where mean daily temperatures were generally below 27°C. Continuing, slow spread of the virus during the winter suggests that low temperature might be important, possibly by reducing the activity of flying insects.

A simple model is being developed to explore the seasonal behaviour of RCD in Australia. This can be tested against the seasonal timing of disease outbreaks in other countries. It predicts that within southern Australia outbreaks of RCD should occur mostly in spring and autumn whereas in inland Australia winter outbreaks would be more common.

## Introduction

In March 1995, tests were commenced within a quarantine compound on Wardang Island, South Australia, to determine the efficacy of Rabbit Calicivirus Disease (RCD) as a potential biological control for wild rabbits. Previously, testing of the virus within the quarantine facilities of the Australian Animal Health Laboratory at Geelong had shown that the virus was safe for use within Australia. It did not replicate in any of twenty-eight native and domestic species tested, further strengthening evidence from overseas studies that the virus was specific to the rabbit, *Oryctolagus cuniculus* (L).

The island trials were intended to find out whether the virus could spread among Australian wild rabbits living in natural warrens and to evaluate (a) the immediate impact of the disease in terms of humaneness of death and rates of mortality, (b) rates of transmission of the virus, (c) effect of season on rates of transmission, and (d) persistence of the virus.

It was considered at the time that transmission of the virus mainly occurred through the faecal-oral route or through contact between rabbits (Cancellotti & Renzi 1991) and there was some scepticism that the virus would be able to persist on a wide scale in Australia given the short time for which infected rabbits survived. Nevertheless, given some suggestion of insect transmission (Rosell *et al* 1989, Gehrmann & Kretzschmar 1991) elaborate quarantine protocols were set up to minimise spread by fomites or transmission by insect vectors. Staff carrying out experiments washed boots in footbaths containing gluteraldehyde and changed clothing and footwear four times on crossing barrier fences between the outer predator-proof perimeter and the inner sites where experimental rabbits were kept. In addition, the experimental burrows were sprayed with residual insecticide (Deltamethrin), saline swamps were regularly treated with *Bacillus thuringiensis israeli* (Bti) to remove mosquito larvae and fly traps used to trap large numbers of flies. A rabbit-free zone 300m wide was maintained around the perimeter of the quarantine site to further reduce risk of free-living rabbits approaching the quarantine site too closely.

However, despite all precautions, the virus crossed the barriers and in late September 1995 was found in rabbits elsewhere on Wardang Island then subsequently on the mainland, initially at Point Pearce, the closest point to Wardang Island.

At the time of the initial escape, when the virus appeared to be confined to a limited area, all efforts were made to contain it so that a properly controlled release could be made following general public consultation. However, it shortly became apparent that the virus had spread far beyond Point Pearce and at that stage all attempts to contain it were abandoned. Within three months the virus had spread to a large part of north-eastern South Australia and adjoining areas of New South Wales and Queensland.

Despite the fact that RCD is now well established and clearly causing heavy mortality of wild rabbits, it was nevertheless considered important to explain its spread from the experimental sites on Wardang Island to the Australian mainland. This would help to unravel the cause of the quarantine failure and give further insight into the epidemiology of the disease.

The initial step of this review was to obtain an entomological perspective on the possibility that insects were involved in the transmission of the disease. In this context climatic data have been collated and analysed by Wardhaugh and Rochester (1996) and subsequently used to predict likely trajectories of insects which might have carried rabbit calicivirus from Wardang Island to other parts of South Australia. These authors also provided comment on other aspects of insect behaviour which they considered useful in evaluating the potential role of insects found on the island.

A second major source of information has been the report on project CS.236 "Field evaluation of RCD" submitted to the Meat Research Corporation on 9th September 1996. This includes information from both the Australian Animal Health Laboratory in Geelong and information collected in South Australia, western New South Wales and central Victoria by staff from CSIRO, Division of Wildlife and Ecology, Canberra.

In attempting to bring the many pieces of information together, the ideas put forward in this review begin with the simplest models which encompass all the presently known facts and deliberately avoid more complicated proposals (Ockham's razor). Where possible, support from published information is used to test whether the ideas presented are realistic.

## The spread of the virus

### *Spread of the virus in quarantine sites*

Before RCD appeared on mainland Australia, there were two occasions when virus spread beyond experimental sites to adjacent 'sentinel' sites within the quarantine compound. In the first instance, virus was spreading in infected experimental sites D and E but suddenly appeared in site H on 2nd July 1995 (see Figure 1 for locations of experimental sites). The incident was considered serious and appropriate quarantine authorities were notified. Rabbits from site H and experimental sites D and E were trapped and killed as specified in protocols for such contingencies and all further experiments were delayed until it was confirmed that the virus had not spread to other sentinel sites or outside the quarantine area. At the time the spread was considered to have been caused by ravens, *Corvus mellori*, which had partly eaten a cadaver in site D. As a consequence, tighter precautions to guard against such scavenging were put in place and work was started again, this time on trials to determine the duration of persistence of virus. One of these involved 5 rabbits which were inoculated and died in site D between 17th and 19th August. The trial was completed, with no evidence of persistence beyond a week, on 31st August.

The second occasion, which culminated in the escape of the virus, was associated with an experiment beginning on 13th September 1995 with the inoculation of rabbits in experimental sites G and I. Sites A, B, C, and J were being prepared for later experiments and were regarded as 'sentinel' sites in case of spread of the disease. The inoculated rabbits died two days later, one of the two rabbits from site G died above ground and was removed immediately; the remaining cadavers were left in the warrens for a further day before being dug out for autopsy. There was no further spread of virus between rabbits in site G but in site I two more rabbits died from the disease on 18th and 19th September respectively. A dead rabbit was found in sentinel site B on 22nd September and five more dead rabbits were recovered there the following day as depopulation of the sites began. The virus also appeared in sites adjacent to site B and was detected in sites F and J on 25th September and in site A on 29th September. Assuming a two day incubation of the virus, the spread into sites F and J occurred on 23rd September. It probably spread into site A little later, but it can only be stated with certainty that all rabbits in that site appeared healthy on 24th September.

People involved in the original experiments in sites H and I did not enter sentinel sites, consequently accidental spread by research staff can be ruled out. Scavenging birds were also considered to be unlikely suspects. Ravens scavenged some carcases within quarantine as rabbits began dying in sentinel pens, however, the initial disease foci outside quarantine were not associated with areas of high raven activity such as the grove of trees in the village where the ravens were nesting and feeding young.

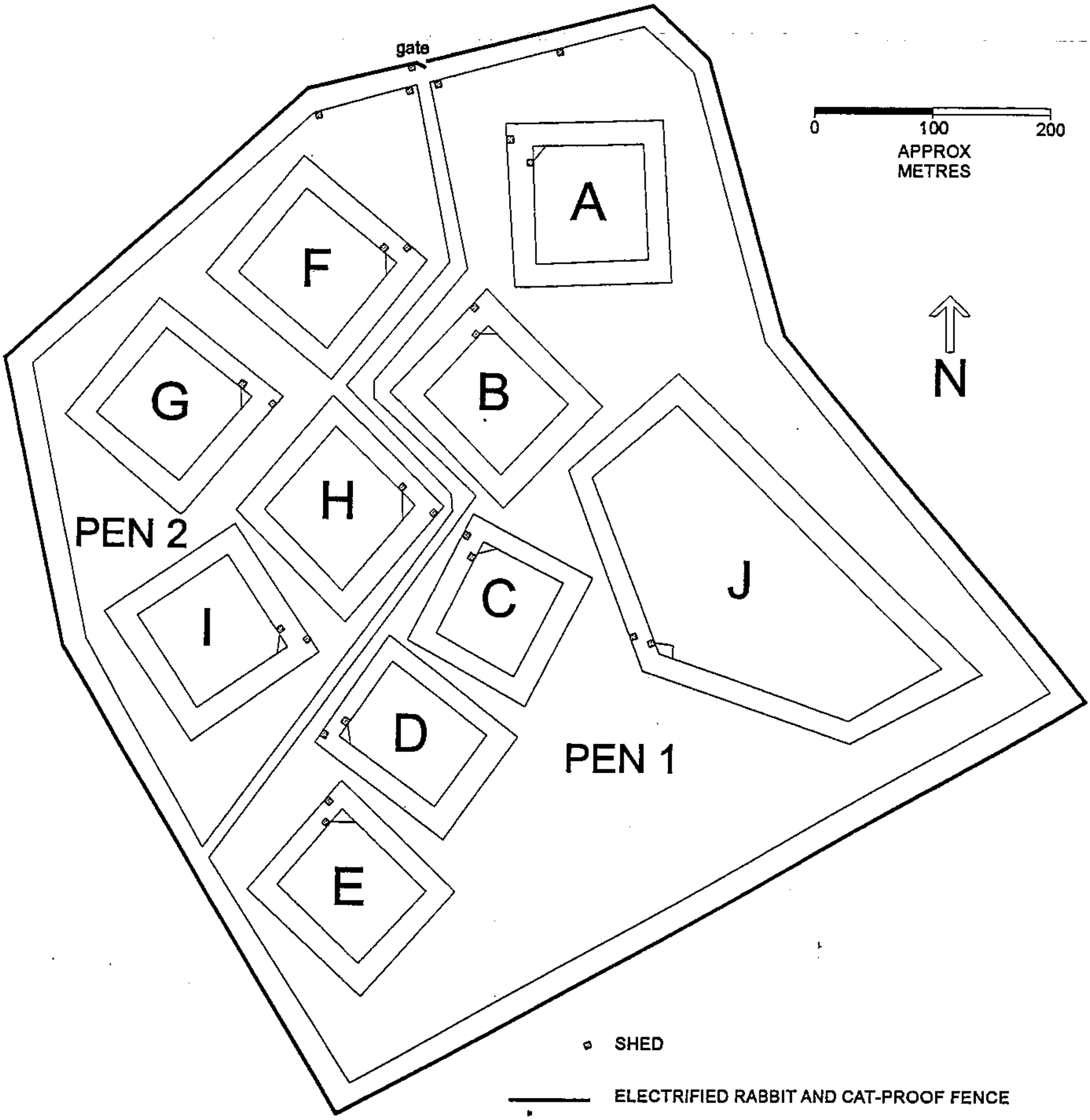
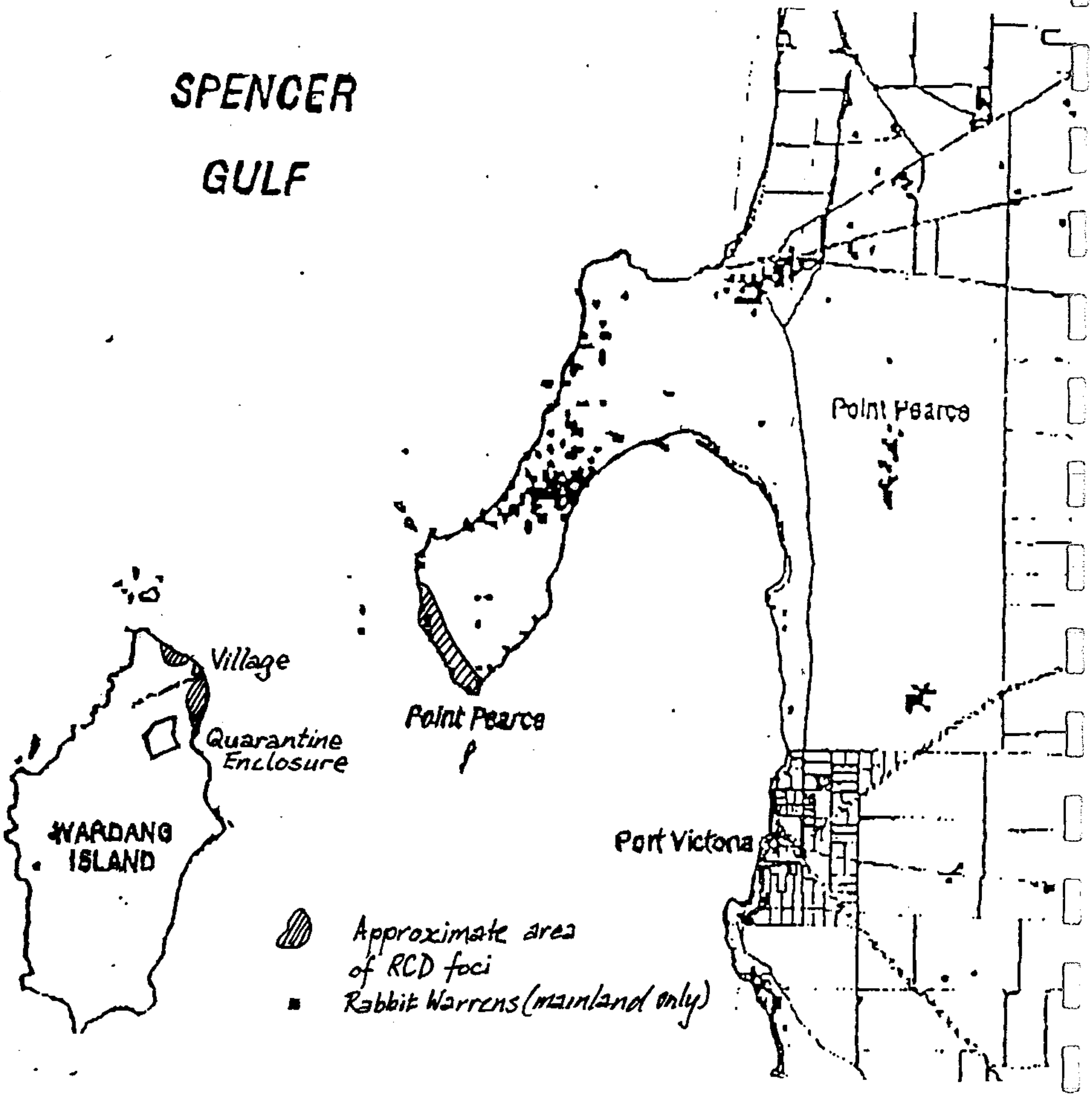



FIGURE 1. QUARANTINE ENCLOSURE ON WARDANG ISLAND
Showing relative locations of experimental sites.

Figure 2. Map of Wardang Island and Point Pearce showing the distribution of the initial disease foci following the escape.



*Incidence of the virus elsewhere on Wardang Island*

With the apparent spread of the virus between sites I and B, surveillance of the island outside the quarantine area was increased, particularly from September 24th onwards, with a team of four walkers. However it was not until 29th September that the first rabbit carcase positive to RCD was found outside and some 900 m to the north-east of the quarantine compound. Two days after this discovery, on 1st October, the surveillance team reported seeing rabbits which behaved oddly in the same vicinity; they could be approached very closely before they ran away. The following morning the team was asked to search this area again and subsequently found freshly dead rabbits which also proved positive for RCD. Given that many RCD infected rabbits die below ground, it is likely that while the disease was confined to a few rabbits it would have been difficult to detect. The rabbit found on September 29th had been dead for 2 or 3 days but given an incubation period of the disease of 2 days it can be argued that the virus must have escaped from quarantine on September 24th or 25th at the latest.

At this first uncontained disease focus, rabbits which died from the disease were soon found at distances of up to 300 m to the north and south of the location at which the initial rabbits were found. This observation is consistent with contact transmission of the disease as rabbits have a home range diameter of about 600 m, but it does not rule out insect transmission on a local scale.

A second focus of RCD was subsequently found on the northern coast of the island and a single rabbit which died from RCD was found in the village area on the north-eastern edge of the island (see Figure 2).

As part of the contingency plans for an outbreak from quarantine, the rabbits on Wardang Island were poisoned using "One-shot" 1080 oats, and their numbers were reduced by about 90%. The rabbit warrens in the area surrounding the foci were destroyed using disc harrows on a tractor, this being quite efficient given the small residual rabbit population and the shallow loamy soils. This treatment contained the spread of the disease to the north-eastern corner of the island but some rabbits, which avoided poisoning, nevertheless became infected and cases of RCD continued to appear on Wardang Island until 19th October. Most of the noticeable spread of RCD occurred before poisoning, fumigation, shooting and trapping had reduced size of the rabbit population to a low level.

*First mainland records of RCD*

Extensive searches were made of rabbit infested areas on the mainland adjacent to Wardang Island from 3rd October onwards. However, it was not until 12th October that a rabbit infected with RCD was collected at Point Pearce, which lies about 4 km from Wardang Island and about 5 km from the quarantine area. This new disease focus shared some interesting similarities with those on Wardang Island in that all three sites contained stands of large African Boxthorn bushes, *Lycium ferrocissimum*, which provided considerable shelter in an otherwise bare and windswept landscape. Even on cooler, windy days these sites were buzzing with flies and other insects.

Action was immediately taken to contain the Point Pearce outbreak with poisoning and destruction of warrens similar to that carried out on Wardang Island. Intense surveillance by a large team of people determined the extent of spread and the effectiveness of the efforts to contain the disease. Surveys across Point Pearce and all the land within a radius of 10 km around Port Victoria, revealed no further cases of RCD.

At the time of its initial discovery on the mainland the virus was spread over a radius of about 500 m suggesting that its spread was about the same as that seen around the two disease foci on Wardang Island before rabbit control was effective. It therefore seemed likely that this mainland focus had been initiated somewhat later than those on Wardang Island, ie several days after the 24th September.

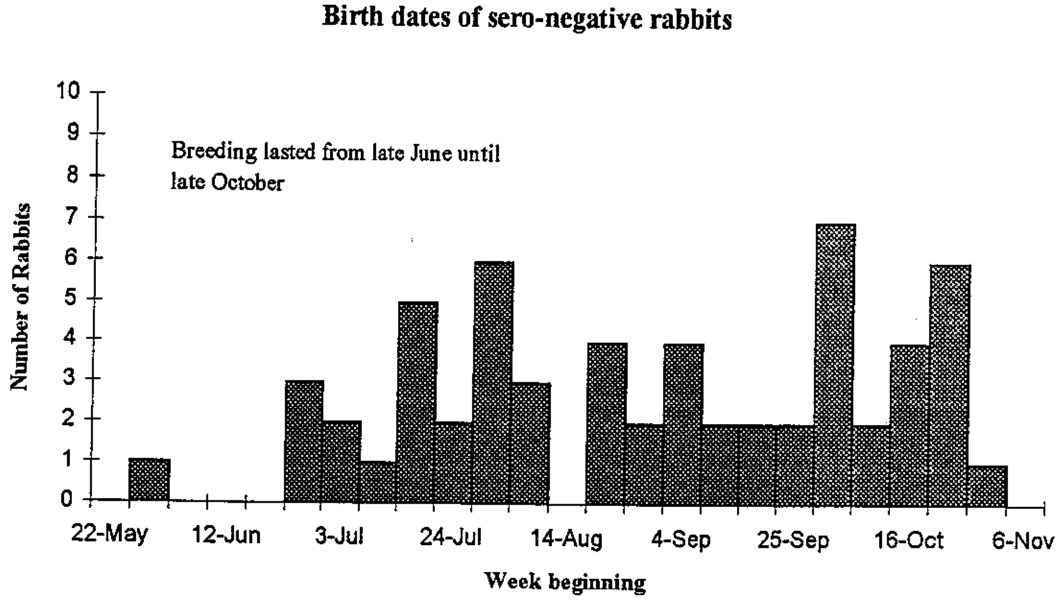
Rabbit control procedures implemented to reduce the spread of RCD from Point Pearce began on 17th October and rabbit numbers were reduced dramatically within the defined outbreak area by 22nd October. Nevertheless, free-living rabbits infected with RCD virus would have been present on either Wardang Island or Point Pearce from 24th September until at least 22nd October.

On 28th October, a major focus of RCD was found at Yunta in the north-eastern pastoral country of South Australia, some 380 km from Wardang Island, and a second was described soon afterwards at Gum Creek Station in the northern Flinders Ranges near Blinman. (A summary of major events is given in Appendix 1.)

**Estimating the time of arrival of RCD in north-eastern South Australia**

The foci at Yunta and Blinman were of similar size, about 100 km in diameter, and, on the basis of their extent and apparent rate of spread of the disease, the virus had probably been spreading out from these centres for some time. Information from the affected area not only suggests a rate of spread of the virus of about 5 - 8 km $day^{-1}$ but also suggests that there was a delay of about 2 weeks before significant numbers of dead rabbits were observed at any given locality (D Lord pers comm). This meant that at Yunta and Blinman the virus may have been spreading for 6 to 14 days when first discovered. In other words, the disease may have reached the area between the 14th and 22nd October.

This possibility was further tested by considering the ages of rabbits which survived the epizootic. Figure 3(a) shows that, among sero-negative rabbits taken in samples collected in the area where RCD was active, there was a fairly even distribution of rabbits born between late June and early November. This would be expected given the normal winter breeding pattern of rabbits in north-eastern South Australia (Cooke, 1970, 1974) and the fact that the opening rains of the pasture growing season fell in late April 1995. Given a period of 2 - 3 weeks for female rabbits to respond to the new growth and a gestation of 28 days, the first births would have been expected in late June. By contrast, sero-positive rabbits from Gum Creek had a completely different age-distribution (Figure 3 (b)). In this group of rabbits there was a preponderance of rabbits born in late August and September 1995. This can be explained on the basis that rabbits up to 6 weeks of age are less susceptible to RCD than older rabbits (Morrisse *et al.* 1991), and that these young, less susceptible rabbits made up the majority of survivors.



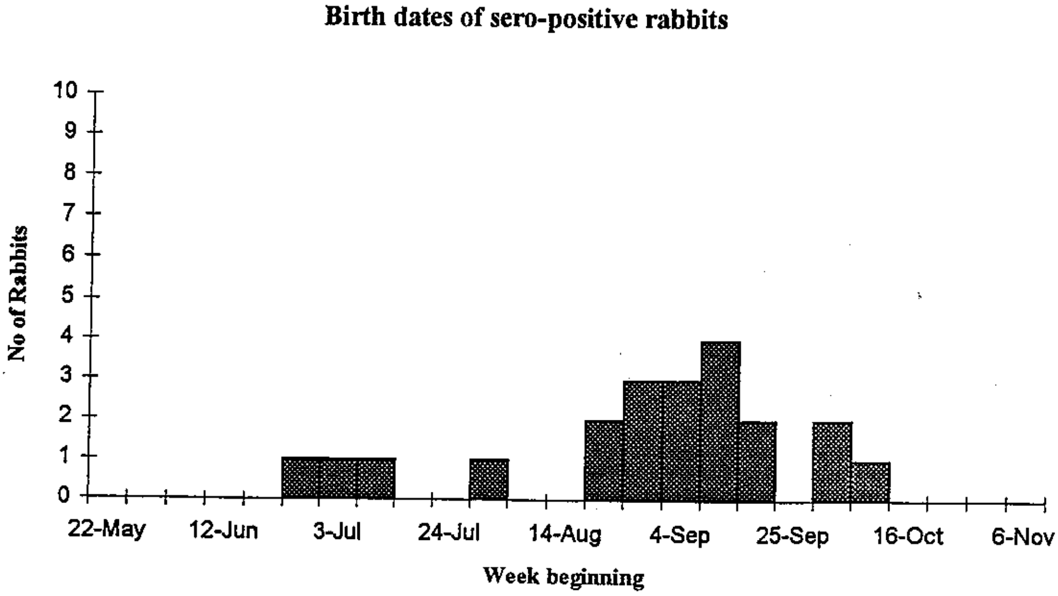




**Figure 3** (a) Sero-negative rabbits grouped according to date of birth estimated from dried eyelens weights. The data show a fairly even distribution of rabbits with birth dates between late June 1995 to mid- October 1995. (b) Sero-positive rabbits from Gum Creek showing how the majority of survivors were rabbits born from late August 1995 onwards. Assuming these surviving rabbits were less than 6 weeks old when challenged, RCD apparently broke out at Gum Creek in the early part of October 1995.

Using data from Gum Creek it was possible to carry out an iterative analysis of the numbers of sero-negative and sero-positive rabbits in different age classes and to show that the proportion of sero-negative to sero-positive rabbits differed most significantly for rabbits born either side of the 21st August. If the oldest rabbits in this group of young sero-positive survivors were under 6 weeks old at the time of the initial onset of RCD, then the 1st October is suggested as the most likely time of arrival of RCD. However, given the errors stemming from estimating age from eye-lens weights (Mykytowycz and Dudzinski 1961) and without knowing the precise age when rabbits become fully susceptible, it is possible that the disease might have been initiated a week or two either side of this date.

**Additional epidemiological information from AAHL**

The report of project CS.236 (Westbury *et al.*, 1996) discusses the nucleotide sequencing of rabbit calicivirus samples collected following the escape from Wardang Island. Most significantly, the data show that the sample from Site J (inside the quarantine area) was identical to the sample taken outside the quarantine area on Wardang Island and also the same as the virus sample collected at Yunta. However, the sample taken from Point Pearce (on the mainland adjacent to Wardang Island) differed from the others.

Although it is unwise to speculate too much on the basis of single samples taken from virus populations at each site, the evidence favours a transfer of the virus directly from Wardang Island to Yunta rather than a transfer via Point Pearce.

Other information of relevance concerns the experimental work with insect vectors carried out at AAHL. Recently, it has been demonstrated that bush flies, *Musca vetustissima*, can transfer rabbit calicivirus from sick or dead rabbits to susceptible rabbits under laboratory conditions. It has also been shown from tests of field-collected samples that insects can become contaminated with rabbit calicivirus. Sixteen pools of separate insect species collected in areas where RCD was active have been tested using PCR. No less than thirteen of these pools have been shown to be positive. The main species so far identified include blowflies *Chrysomya rufifascies, C. varipes, C. nociva/augur* and *Calliphora stygia*, bush flies, *Musca vetustissima*, and mosquitoes, *Aedes postspiraculosus* and *A. notoscriptus*.

A sample of *Calliphora stygia*, collected on 24th September, outside the quarantine area on Wardang Island, proved to be positive to rabbit calicivirus when tested using PCR.

Research at AAHL has shown that rabbit calicivirus can survive for up to three weeks in rabbit carcases at 22°C. This is the approximate temperature, at one metre depth, in rabbit burrows in inland South Australia during the late spring months (Cooke 1990). Clearly, it would be possible that some virus would be present in the carcases when flies emerged from pupation. If flies which developed in carcases of rabbits were involved in disseminating the virus from Wardang Island, then it might be expected that major spread of the virus would have occurred when flies emerged about 10 to 14 days after the virus became established outside the quarantine area. In such a case there may have been large numbers of infective blowflies present during the second week of October 1995. Bushflies have also been recorded as breeding in rabbit carcases (Anderson *et al.*, 1988).

**Entomological data collected on Wardang Island**

Despite the fact that insects were not considered to be vectors of RCD in Europe (Cancellotti and Renzi 1991) and are still considered to be unimportant (Villafuerte *et al* 1995) insects on Wardang Island were collected for identification and weather (maximum and minimum temperature, rainfall and wind speed) was monitored daily with the aim of having background information available if insect vectors proved to be important. This work was initiated by Dr K. Wardhaugh, CSIRO Division of Entomology and maintained throughout the project by Mr J. Hardy and Mr R. Wallis.

Regular trapping to monitor the numbers of flies was carried out for a week each month using four large wind-orienting traps baited with a mixture of chopped liver, cowdung and sodium sulphide. Mosquitos were monitored monthly using battery operated traps which used carbon dioxide (dry-ice) and a light source as attractants.

The species collected in fly traps included bushflies, *Musca vetustissima,* which were at times very noticeable owing to their persistence around staff monitoring the virus outbreak. No bush flies were caught in June 1995 and only three were caught in four traps during late July. August samples also yielded no bushflies but by late September hundreds were caught. Four traps operated during late September, at the time the virus escaped, yielded 283, 408, 1002 and 580 flies respectively when they were emptied on 24th September. This suggests a correlation between the arrival of bush flies on Wardang Island and the outbreak of RCD. Bushflies may become contaminated by feeding on secretions from rabbits as field sampling and experiments in AAHL have subsequently shown, however, it must be remembered that PCR testing of a pool of bushflies collected at the time of the virus escape was negative.

Blowflies, mainly *Calliphora augur* and *C. nociva* were also caught in the wind-orienting fly traps. Blowflies were present on Wardang Island throughout the year but were at their lowest abundance during late June and early July and only increased noticeably in late July, reaching a peak of abundance in October. They certainly laid eggs on rabbits deep within warrens although this only happened when rabbits began to decompose. However, it remains difficult to imagine how blowflies make contact with live rabbits in such a way as to transfer the virus from a carcase to a live rabbit.

Mosquitoes were never caught in high numbers and catches were erratic, reflecting weather conditions rather than the time of year. Species such as *Aedes camptorhynchus* were caught only on still nights. The highest catch was twenty-two mosquitoes caught in five traps on the night of 19th July and the next highest was 13 mosquitoes caught in five traps on the 27th September. Generally, very few mosquitoes were caught, suggesting that numbers were generally low throughout the winter and spring. However, mosquitoes were not entirely eradicated by the heavy treatment of tidal pools with Bti. Seven species of mosquito were collected from the island during the winter and spring. They were *Aedes australis, A. camptorhynchus, A. notoscriptus, A. vigilax, Culex australiacus, C. globicoxitus* and *C. quinquefasciatus.*

Of the potential vectors identified on Wardang Island at the time when the virus escaped, mosquitoes were the least numerous because they had been heavily controlled by application of Bti granules to tidal marshes. However, some mosquitoes escaped this treatment, possibly because they bred in water-filled holes of burrowing mud crabs on the edges of the marshes or were new migrants. Mosquitoes were caught in $CO_2$/light traps on the perimeter of the quarantine site on still, warm evenings. Observers working in the hides at sites G and I also reported being bitten by mosquitoes on at least one evening. The mosquitoes involved, *Aedes camptorhyncus*, are known to bite rabbits but generally have a coastal distribution because of their breeding habits. It is also probable that, having fed, most female *A. camptorhyncus* would seek sites for egg-laying before feeding again. Under these circumstances, transmission of a virus is most likely to occur where interrupted feeding has occurred.

**Climatic data associated with the escape of the virus**

Wardhaugh and Rochester (1996) have analysed climatic data from Wardang Island and associated sites in South Australia for the months of September and October 1995. From the known activity (entry into traps) of flies and mosquitoes at given temperatures, wind speeds and rainfall, they derived an activity index which indicated when insects could have been active vectors. This was combined with an insect migration model to indicate the most likely displacement of the potential insect vectors from Wardang Island to other parts of South Australia. The simulations are presented in the form of a series of daily maps showing the areas to which insects might have moved. There are separate simulations for flies, night-flying mosquitoes and day-flying mosquitoes. Simulations were also carried out assuming that movement occurred in (a) surface air flows and (b) upper air (100 - 500 metres above sea level).

Observations made on Wardang Island show that the initial spread of the virus, apparently between experimental sites I and B on 19th September, was associated with unseasonably warm calm weather with minimum overnight temperature of 12.5°C and daytime maximum of 21°C. Similar calm conditions occurred on 22nd September, when the overnight minimum was 11°C and the maximum 18°C. At that time, there were 5 infectious rabbits in site B, based on the carcases found early the next morning, so there was an opportunity for insect vectors to become contaminated with virus. This is supported by the fact that rabbit calicivirus was subsequently detected on blowflies, *Calliphora stygia*, collected from the fly traps on 24th September.

The simulations carried out by Wardhaugh and Rochester (1996) show that 19th September was the first day permitting significant activity of day-flying mosquitoes following the commencement of the experiment and there was likely to have been smaller increases in expected activity among night-flying mosquitoes and bushflies at the same time. It is also important to note that displacement of insects would have been towards the east, ie from site I towards site B and from Wardang Island towards Point Pearce.

While the initial documented spread of RCD seems to fit expectations based on insect behaviour, it seems odd that on later occasions, when insect activity should have been high, that RCD was not spread to other parts of the island. For example, it did not spread to the western edge of the island where the presence of Tammar wallabies, *Macropus eugenii*, precluded rabbit poisoning and the presence of Fairy Penguins, *Eudyptula minor*, in rabbit warrens prevented extensive fumigation to kill all rabbits. RCD remained confined to the north-eastern corner of Wardang Island despite winds on 26th, 27th, and 28th September which should have moved insects in a south-westerly direction.

It is not known precisely when RCD arrived on Point Pearce. The first rabbit found on 12th October had died a day or two previously, and, given a two-day incubation of the disease, the latest possible date of arrival was 9th October. In addition, the size of the disease focus in comparison to those on Wardang Island suggested that it might have been spreading for about 2 weeks. There were four or five times within the period 24th September to 9th October inclusive when mosquitoes or flies could have flown from Wardang Island to Point Pearce. However, the most likely time was 1st October when high insect activity and a westerly wind (see Wardhaugh and Rochester 1996) would have coincided with the presence of significant numbers of RCD infected rabbits on that part of Wardang Island which is closest to Point Pearce.

**Long distance movements of insects**

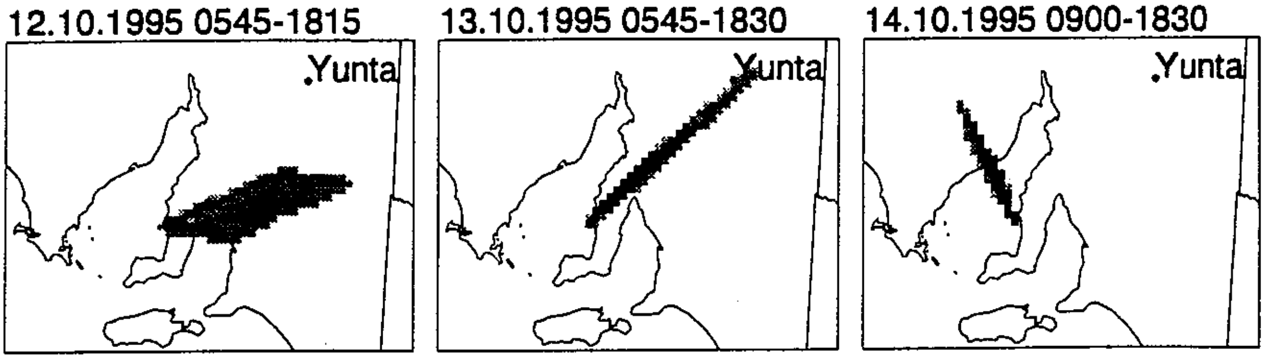
If RCD only became established at Point Pearce on 1st October, yet reached the Flinders Ranges area by mid-October, then northward movement must have been rapid, and likely to have been in one or two rapid jumps rather than a series of steps.

For the period in question, the simulations of Wardhaugh and Rochester (1996) suggest that insect activity, which would allow feeding on sick or dead rabbits was most likely on 11th October for night flying mosquitoes and between 9 - 11th October for flies. There was little activity of day-flying mosquitoes at that time.

Wind direction over this period was predominantly from the north and east, ie away from the Flinders Ranges, however, in the days which immediately followed, wind was from the west, then south-west and the south-east. The simulated trajectories for insects on those days, although flight activity was estimated to be low, would have taken flying insects from Wardang Island or Point Pearce across the agricultural areas north of Adelaide on 12th October, north-east to the Yunta region on 13th October and finally north-west across Eyre Peninsula to the Gawler Ranges on 14th October.

A similar weather pattern, beginning with high insect activity on 17th and 18th October was followed by winds which would have taken insects north along St Vincent's Gulf and over Eyre Peninsula from 19th October until 25th October.

Only rarely during October did the simulated trajectories of insects in surface air indicate that transfer of virus to the Adelaide region or south-eastern South Australia was likely. However, trajectories based on the movement of upper level air indicated that bushflies might have been able to reach the Adelaide region and the south-east on 17th October and that night flying mosquitoes might have reached Kangaroo Island and the Fleurieu Peninsula on the 16th and 18th October. Kangaroo Island is rabbit free but the Adelaide region and the south-east of South Australia must have had



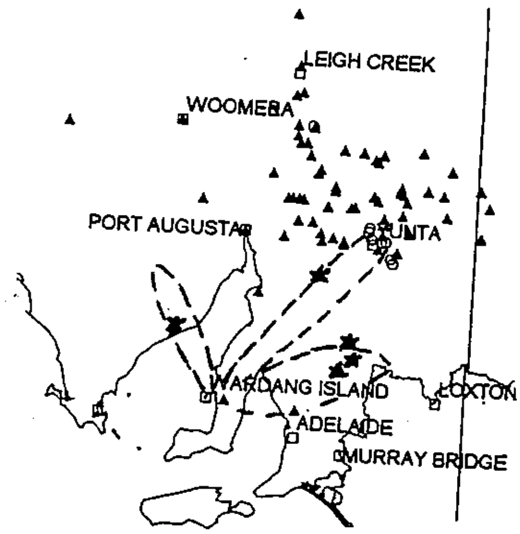




Figure 4. Predicted trajectories of insects from Wardang Island in relation to the distribution of foci of RCD identified in north-eastern South Australia from October to December 1995. O October, ▲ November, ✱ December. Details on insect flights from Wardhaugh and Rochester (1996).

sufficient rabbits to enable RCD to become established. This is borne out by the fact that RCD spread through both areas in the winter of 1996.

From this picture of climatic events, it might be predicted that insects carrying the virus could have spread from Wardang Island or possibly Point Pearce across a wide sector reaching from a little north of Adelaide, through the Flinders Ranges to the Gawler Ranges and central Eyre Peninsula. Areas south of Adelaide would have had a much lower chance of being affected.

It is significant that the likely trajectories of flies, generated for 12th - 14th October, accurately describe the sector over which new foci of disease were found during late-October, November and December. The areas where RCD was first noticed, Yunta and Gum Creek, were in a region where rabbits were abundant and were therefore sites at which the virus had a high chance of becoming established, spreading and subsequently being detected within a short period. By comparison, the farming areas north of Adelaide and on Yorke Peninsula and Eyre Peninsula have relatively few, patchily distributed rabbits and it may have taken longer for the disease to be noticed.

The eventual detection of infected rabbits at Quorn and Port Pirie on 9th November, and its confirmation at Port Augusta on 13th November, is not entirely consistent with the wind trajectories on 12th - 14th October but could be explained by movement of RCD contaminated insects from Wardang Island or Point Pearce as late as 22nd October. Beyond that time, there would have been lessening chance of further spread because rabbit poisoning and ripping of warrens was all but completed. Alternatively, some of the later discoveries might have been the result of the explosive spread from Yunta and Gum Creek.

It should be pointed out that, according to the models developed by Wardhaugh and Rochester (1996), the northward movement of mosquitoes and flies is likely to be minimal because winds from the south are usually too cool for major activity. However, it is also worth noting that, because the model is based on the trappability of insects, it tends to bias the measurement of fly activity. In the case of bush flies for example, the fly activity index suggests that on some days flies were barely active. However, when working in the field on Wardang Island and at Point Pearce there was quite clearly no such thing as a 'fly-free day'. Flies were simply more persistent and troublesome on some days than on others. If blow flies were involved in transmission they should have been active on most days.

**Subsequent Spread of RCD**

After spreading explosively through much of north-eastern South Australia and reaching western New South Wales and south-west Queensland, the rate of spread of the virus slowed during the summer months. All new foci were confined to coastal areas and along the lower Murray River during January and February 1996. However, in March 1996 a new outbreak was identified in central Victoria. The area involved had experienced above average rains for most of the summer.

From central Victoria, the virus spread north into the Riverina and beyond, into areas where rainfall had also been high, then across north-western Victoria and into the

South Australian mallee farmlands with the first winter rains. It has since spread south into south-eastern South Australia while at the same time becoming established in parts of western Victoria which had previously missed involvement. The delay in RCD reaching the south-east of South Australia may be related to abnormally low rainfall in that area during 1995. However, there were some exceptions to this pattern such as the outbreak of RCD north-east of Alice Springs and the Cooma district in southern New South Wales where rainfall had been less than average.

The virus also spread west, crossing the Nullarbor plain and reaching Kalgoorlie in winter 1996. In the early spring it began spreading rapidly through rabbit populations in the south-west of Western Australia.

**Seasonal abundance and movement of potential vectors**

If the escape of the RCD virus from Wardang Island involved some kind of biting or protein feeding insect such as a mosquito, midge or fly, then it is reasonable to expect that further spread of the virus might depend on the vector's population size and behaviour and the climatic variables likely to influence them.

It is not possible to review data on all insects in the initial outbreak area, however the bushfly, which has been widely studied, was chosen to provide a model for the purpose of further debate. The use of this species is not intended to imply that it is considered to be the major vector of RCD.

Data on bush fly abundance have been kindly supplied by Lynette McLeod, NSW Agriculture, Orange. These show that at Fowler's Gap (Figure 5) peaks in numbers of flies generally occur in October and again in April (Spring and Autumn). As Yunta and Fowler's Gap are similar sites, only 200 km apart, with similar average annual rainfall and shrub-steppe vegetation, it seems reasonable to assume that the behaviour of flies at both sites would be similar.

The data not only show that a spring peak in abundance of vectors might be anticipated but also suggest a sudden fall off in activity which, superficially at least, seems to be consistent with the observed slower rate of spread of the RCD virus in summer when the disease appeared to stop abruptly in December 1995 on crossing into western NSW and western Queensland.

The data from Fowlers Gap also suggest that in some years at least, bushflies should peak in activity in March, April and May. This raises the possibility that, if vectors of RCD showed similar patterns of abundance to bushflies, there could be renewed spread of RCD in the autumn. Such a prediction now seems all the more interesting following the widespread re-appearance of the disease in southern South Australia, central Victoria and inland New South Wales in April 1996. However, it should be noted that although large numbers of blowflies and mosquitoes were trapped in central Victoria at the time of the RCD outbreak very few bushflies were caught (J Hardy pers comm).

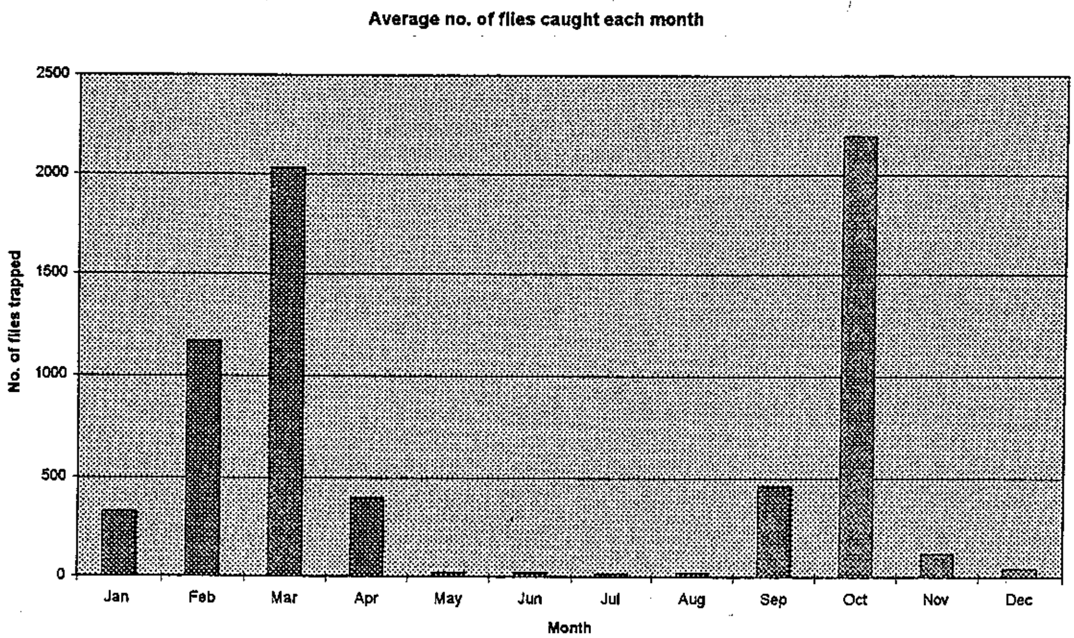



Figure 5. Mean number of bushflies trapped at Fowler's Gap in each month. Average of 3 years data. Data from McLeod and Anderson (unpubl).

Matthiessen (1983) states that bushflies migrate from pastoral areas into the wheat belt of Western Australia in early October each year. They reach a peak in late October but fall rapidly in summer and stabilise at moderate levels. Further south-west, breeding is highly successful in October and November and the adult population is highest in November and December.

To some extent the spread of RCD to the Kalgoorlie region of Western Australia during the winter provided a 'natural experiment' for testing the hypothesis of bushfly transmission. It would be expected that RCD should spread rapidly in the wheat belt areas of Western Australia in early October if flies were important vectors.

As it turned out, the explosive spread of RCD through the wheatbelt area was not consistent with Matthiessen's ideas on bushfly movements. It began in early September, some weeks before the expected spread of bushflies in early October. However, in view of the September 1995 arrival of bushflies on Wardang Island, within the cereal growing areas of South Australia, and the fact that the first bushflies may also arrive in cooler climatic regions such as Canberra in September (Hughes and Nicholas 1984) data on the exact time of arrival of bushflies in the wheatbelt of Western Australia are clearly needed for a final test of the hypothesis.

Although many of the observations correlate quite well with what is known of insect abundance and movement in general, there is nevertheless a major problem which remains. Wardhaugh and Rochester (1996) noted the slow rate of spread of RCD in summer and used this to argue against the idea that bushflies and other insects could be major vectors of RCD. They argue that, if RCD arrived in north-eastern South Australia and killed an estimated 20 million rabbits, why did the rate of spread of the disease slow noticeably despite there being a source of virus far, far greater than that provided by the few rabbits on Wardang Island and at Point Pearce. Warm winds, predominantly from the north-west would be expected to have carried infective flies into south-western New South Wales and Victoria in early summer. Quite clearly a simple model of insect vectors is insufficient on its own. Some other factor, possibly related to the survival of the virus, must also be important.

**Climatic Factors influencing the Survival and Transmission of the virus**

In view of the argument put forward by Wardhaugh and Rochester (1996) it is important to consider an associated counter argument that some other factor such as elevated temperatures of summer might have been important in stalling the initial rapid spread of the disease.

In this sense it is significant that work in AAHL (Report on Project CS.236) showed that purified virus survived between 28 and 35 days at 22°C but only between 3 to 7 days at 37°C and between 1 minute and 2 hours at 56°C. The survival of virus in rabbit carcases is likely to be similar given that at 22°C viable virus persisted somewhere between 20 and 26 days.

In north-eastern South Australia during summer air temperature commonly reaches 45°C and soil surface temperatures can be 60°C or more for up to six hours each day (Rogers 1970). Consequently, survival of the virus in carcases on the soil surface or on day flying insects is likely to be extremely short. Temperatures in rabbit burrows during mid-summer are considerably lower than ambient surface temperatures, nevertheless, they are unlikely to be less than 27°C in elevated locations such as Gum Creek and may exceed 32°C on the plains (Cooke 1990).

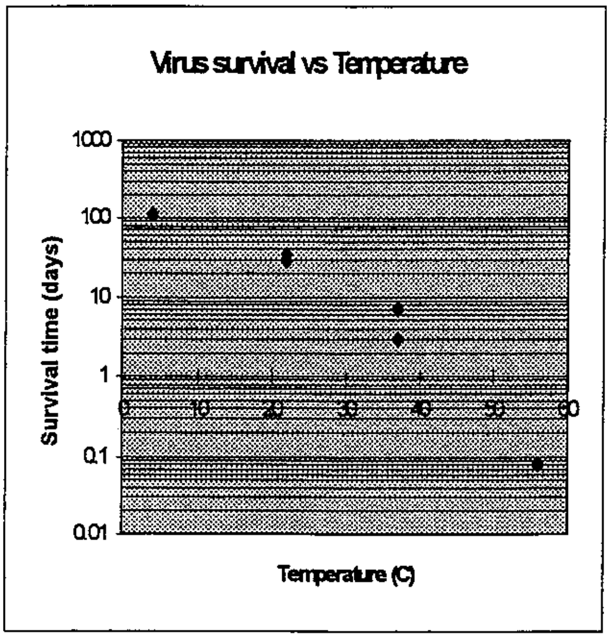



Figure 6. Summary of survival times of purified RCD virus in solution (semi-log scale). The virus might be expected to survive for about 14 days at 28°C.

Using the data available (Figure 6) it seems that the survival time of the virus would be substantially reduced by normal summer temperatures, and there may be as much as a rather than insects may have had a role in spreading the disease over long distances. It is interesting that the unusually warm English summer of 1995 was associated with greater spread of RCD than in previous summers.

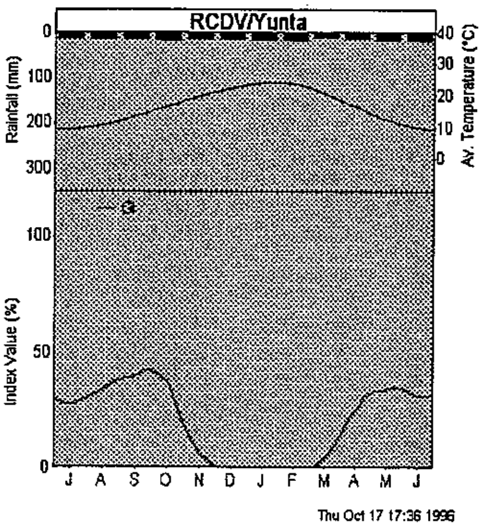



Figure 7. Seasonal abundance of RCD at Yunta in relation to average rainfall and temperature at the site.

Elsewhere in Europe the seasonal pattern of spread is quite different. In the low rainfall areas around Zaragoza in Spain, the model predicts that RCD should spread best in spring and autumn (Figure 8 b). This is an extremely interesting prediction in the light of recent correspondence from Dr Carlos Calvete, University of Zaragoza, who states (my translation):

*"....I have always had the impression that the appearance of rabbits killed by RCD in the field coincided (with a delay of about a week) with sharp declines in temperature and increases in the velocity of the winds. This impression was strengthened during the winter-spring of 1991, when there were very large changes in the weather each week, and curiously, after each week in which the weather was really cold, rabbits which had died from RCD were found. This occurred up until June, a time when the weather became more stable, and no more cadavers were found until the months of September and October (Autumn) when the weather again became unstable...."*

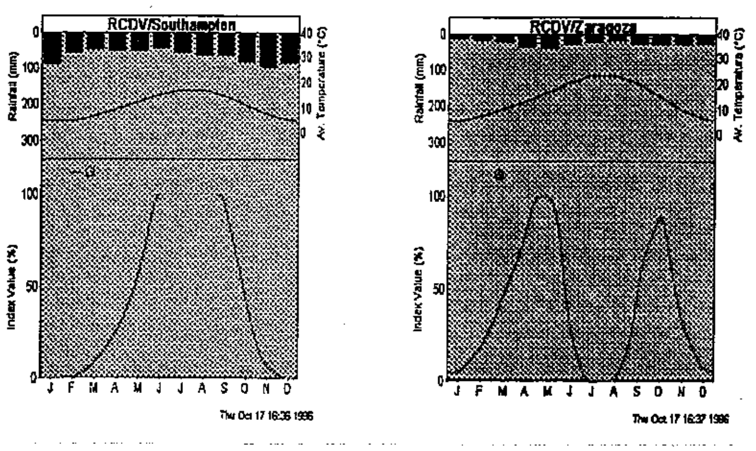



Figure 8 Predicted seasonal pattern of RCD outbreaks at
(a) Southampton, UK
(b) Zaragoza, Spain

At this stage, the agreement with predicted patterns is mainly appropriate in terms of confirming the low incidence of RCD in summer owing to high ambient temperatures. They do not necessarily indicate that insect vectors are of major importance.

**General conclusions regarding the escape of the disease**

The results of trials on Wardang Island show that RCD spread rather poorly among wild rabbits living in natural warrens when the mechanism of spread was confined to contact between rabbits. Only 13 rabbits became infected by contact despite 14 being inoculated. On this basis, it is arguable that the disease might barely maintain itself by contact transmission alone.

The escape of the virus strongly suggests that other agents, particularly flying insect vectors, transferred virus from one or two rabbits in experimental site I to affect millions of other rabbits across thousands of square kilometres within a few weeks. This spread was apparently facilitated by the vectors' ability to take advantage of climatic factors such as temperature, humidity and wind for long distance dispersal.

It is not possible to define exactly how or when RCD spread from Wardang Island to inland South Australia. Nevertheless, some progress has been made in narrowing down the likely timing. For example, it can be said that spread from the island must have occurred between the 24th September and 22nd October when there was a substantial pool of virus present outside the quarantine area. Working back from the approximate rate of spread of the virus and the age structure of surviving rabbits near Yunta, it can be further argued that the most likely time of arrival in north-eastern South Australia was in the first 2 weeks of October. There is obviously room for error in such calculations with the estimate based on the survival of young rabbits suggesting an earlier arrival than that based on the apparent rate of spread of the disease. However, both estimates might be reconciled if the virus arrived in the Flinders Ranges by mid-October.

As the virus crossed several fences, 4 km of sea and appeared about 360 km inland it is highly probable that flying vectors were involved. Given the extent of subsequent spread, insects such as flies or mosquitos were more likely vectors than birds. The possibility of human involvement seems unlikely given the relative remoteness of many of the sites and the tight security measures for staff and personnel visiting Wardang Island. Nevertheless, it is impossible to completely eliminate movement of the virus from Point Pearce due to human agency. There is, for example, the well documented case of two journalists who visited Point Pearce on 17th October then subsequently interviewed rabbit shooters at Yunta in north-eastern South Australia. It can only be said that any involvement of the journalists would not explain the two apparently simultaneous outbreaks of RCD at Yunta and Gum Creek, nor could it explain the extent of the spread at these foci at the time of their discovery. The apparent differences in nucleotide sequences between virus samples from Yunta and Point Pearce would also difficult to reconcile on this basis.

From the field data available it is likely that the spread of the virus over long distances is a relatively uncommon event. Furthermore, once the virus arrived in a given locality, mechanisms which enabled local spread were very important. This would explain why the initial outbreaks of RCD in South Australia appeared to occur in distinct foci. Nevertheless, where rabbit populations were relatively dense and continuous, the spread from these foci was rapid and the foci merged within a few weeks. It is unlikely that local spread of the virus could have been by contact transmission alone. Similarly, spread by predators or fomites is unlikely to explain the rate of spread of about 5 to 8 km/day or explain how the disease affected over 95% of rabbits in many populations.

On the weight of evidence, based on the appearance of the disease and preceding weather patterns, it seems most probable that the virus first became established on the mainland at Point Pearce on the 1st October. It is most likely to have been spread into northern South Australia in the period 12 - 14th October 1995.

Despite evidence that insects may be vectors of the disease there is no clear evidence to suggest that particular species are of singular importance. Although bushflies have been shown to transmit the virus in the laboratory and virus was detected on samples of bushflies collected in the field, the outbreak of RCD in central Victoria was not associated with large numbers of bushflies and the spread of the disease into the wheat-belt of Western Australia will remain equivocal unless adequate field data on bushfly movements are forthcoming. Mosquitoes or possibly blowflies as well as bushflies remain candidates for further work.

The likely involvement of winged insect vectors also implies that the virus will spread more readily in some seasons than in others. Vector transmission can be expected to add to underlying contact transmission within social groups of rabbits to ensure high morbidity rates in the field. However, in the absence of vectors, and with high summer temperatures the numbers of diseased rabbits may decline, and the disease will become extremely hard to detect, or even die out locally because it kills its host so rapidly. Nevertheless, the demonstrated ability of the virus to become established over wide areas from a small source demonstrates how it is likely to survive on a global scale in the face of local extinction.

The ability to observe the spread of a new virus in a widely distributed host animal gives an insight into epidemiology not available for long established diseases. The establishment of RCD in Australia has provided an opportunity to observe how a highly lethal virus can become established across a continent from a remarkably small source and maintain itself in the field.

Initial modelling using Climex suggests that RCD will occur across southern Australia in regular spring or autumn outbreaks. In inland Australia outbreaks will be less common, depending on rainfall, and will occur mainly in winter. In cool temperate areas, such as Tasmania, summer outbreaks might be expected.

## References

Anderson, P. J., Shipp, E., Anderson, M. J. E., and Dobbie, W. (1988). Population maintenance of *Lucilia cuprina* (Weid.) in the arid zone. *Australian Journal of Zoology*, **36**: 241 - 249.

Barlow, N. D. (1995) *"Modelling RCD in Rabbits"* Report to Landcare Research, July 1995, AgResearch, Canterbury Agriculture & Science Centre, Lincoln, NZ. 44 pp.

Cancellotti, F. M. & Renzi, M. (1991). Epidemiology and the current situation and the European brown hare syndrome in Italy. *Rev. sci. tech. Off. int. Epiz.* **10**: 409 - 422.

Cooke, B. D. (1990). Rabbit burrows as environments for European Rabbit Fleas, *Spilopsyllus cuniculi* (Dale) in arid South Australia. *Australian Journal of Zoology* **38**: 317 - 325.

Dudzinski, M. L. and Mykytowycz, R. (1961) The eyelens as an indicator of age in the wild rabbit in Australia. *CSIRO Wildlife Research*, **6**: 156 - 159.

Gehrmann, B. and Kretzschmar, C. (1991). Experimental investigation of rabbit haemorrhagic disease (RHD) - transmission by flies. (In German) *Berliner und Munchener Tierartzliche Wochenschrift* **104**: 192 - 194.

Matheissen, J. N. (1983). Seasonal distribution and characteristics of bush fly *Musca vetustissima* populations in south-western Australia. *Journal of Ecology*, **8**: 383 - 394.

Morrisse, J.-P., Le Gall, G. and Boilletot, E. (1991). Hepatitis of viral origin in Leporidae: introduction and aetiological hypothesis. *Rev. sci. tech. Off. int. Epiz.* **10**: 283 - 295.

Rodak, L. Smid, B. and Valicek, L. (1991) Application of control measures against rabbit haemorrhagic disease in the Czech and Slovak Republics. *Rev. sci. tech. Off. int. Epiz.* **10**: 513 - 524.

Rogers, R. W. (1970). *"Ecology of soil surface lichens in south-eastern Australia."* PhD Thesis, University of Adelaide.

Rossell, J. M., Badiola, J. I., Pujols, J., Perez de Rozas, A., Badiola, J. J., Garcia de Jalon, J. A. & Vargas, M. A. (1989). Enfermedad virica hemorragica del conejo. Epizootiologica y clinica. *Medicina Veterinaria* **6**: 275 - 284.

Villafuerte, R., Calvete, C., Blanco, J.-C., and Lucientes, J. (1995). Incidence of viral haemorrhagic disease in wild rabbit populations in Spain. *Mammalia* **59**: 651 - 659.

10 fold difference in survival time at different times of the year. The potential impact of these changes is best illustrated in terms of attempts at modelling RCD by Barlow (1995). He concludes that RCD would only persist if there is a "free-living virus reservoir in the environment in which the viral particles have a half-life of at least 2 weeks". Clearly, an environmental temperature of about 22°C might permit the disease to persist but a temperature of 28°C would be likely to inhibit it completely.

During the summer of 1995 - 96 new foci of disease were generally found in coastal areas of South Australia where average daily temperatures were less than 27°C and more commonly in the order of 22°C.

More work needs to be done on the survival of the virus at different temperatures. For example, the survival of the virus may be longer when adsorbed onto a dry surface than in solution (Rodak et al 1991). However, on considering all available data, it is clear that environmental temperatures are in the range where they may strongly affect survival of the virus and consequently the epidemiology of RCD.

Apart from temperature, rainfall also appears to be associated with the spread of RCD. The first major outbreaks in 1996 were in central and southern Victoria and on the Nullarbor Plain in Western Australia. Both areas had recorded rainfall well above average in the summer of 1995 - 96.

**CLIMEX model of RCD**

As the environmental constraints on the activity of vectors and the RCD virus are becoming broadly known, these can be used in conjunction with climatic data, such as that contained in CSIRO's Climex software, to test hypotheses and explore the likely seasonal changes in the behaviour of RCD at different sites across Australia.

An initial model has been set up assuming that the spread of the virus is limited at low temperatures because vector activity is low. Some mosquitoes have a flight threshold of 8°C but other potential vectors such as flies do not fly much below 12°C. Conditions for spread of the virus should be adequate until ambient temperatures become too high for the virus to survive for long. It is assumed that the disease persists at 27°C but not beyond 30°C. The Climex model also requires parameters for soil-moisture and so can be adapted to take into account the influence of rainfall on the spread of the disease. However, at this stage the parameters used are simply those levels known to limit rabbit populations (Cooke unpubl), the assumption being that the virus should be able to live wherever rabbits can survive.

Figure 7 shows the potential predicted patterns of outbreaks of RCD at Yunta, the site of one of the initial RCD outbreaks. There is a higher possibility of outbreaks occurring in October and April (Spring and Autumn) than in winter and little chance of spread in summer.

The model can be assessed further, using data from Europe. One result is the prediction that around Southampton, U.K. (Figure 8 a) RCD would be most likely in mid-summer. Such a pattern of seasonal incidence has been observed in the summers of 1993, 1994 and 1995 by R. Trout (pers comm) although he considers that birds

Wardhaugh, K. and Rochester, W. (1996) *"Wardang Island. A retrospective analysis of weather conditions in respect to insect displacement."* CSIRO Report to MRC.

Westbury, H. (1996) Unpublished preliminary report on Project CS.236 *"Field evaluation of RCD under Quarantine"*

**Appendix I**

Chronology of main events associated with the escape of the virus.

| [illegible] | [illegible] |
|---|---|
| 13 Sept | Rabbits inoculated with RCV sites G and I |
| 15 Sept | Initially inoculated rabbits died sites G and I |
| 18 Sept | Secondarily infected rabbit died in site I |
| 19 Sept | Secondarily infected rabbit died in site I |
| 22 Sept | Rabbit in site B dead from RCD |
| 23 Sept | 5 more rabbits dead in site B; remainder killed |
| 24 Sept | Virus present on blowflies collected outside quarantine |
| 25 Sept | Dead rabbits found in sites F and J, as sites were being closed |
| 29 Sept | Dead rabbits found in site A; first infected rabbit found outside |
| 30 Sept | Rabbit control started on Wardang Island |
| 1 Oct | Probable spread of RCD to Point Pearce |
| 12 Oct | First infected rabbit found on Point Pearce |
| 12-14 Oct | Likely spread of RCD to north-east South Australia |
| 17 Oct | Rabbit control started on Point Pearce |
| 19 Oct | Last known case of RCD on Wardang Island |
| 28 Oct | RCD identified at Yunta: further rabbit control abandoned. |