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MONITORING THE SPREAD OF RABBIT HEMORRHAGIC DISEASE VIRUS AS A NEW BIOLOGICAL AGENT FOR CONTROL OF WILD EUROPEAN RABBITS IN AUSTRALIA

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ABSTRACT: Following the escape to the mainland of the rabbit hemorrhagic disease virus (RHDV) from Wardang Island off the coast of South Australia, a monitoring program was implemented over a 13 mo period, between October 1995 and October 1996 to determine the activity and rate of spread of the disease in the wild European rabbit (*Oryctolagus cuniculus*) population. All reports of dead rabbits were investigated. Whenever possible, liver and spleen tissue samples were collected from fresh carcasses and subsequently analysed for the presence of RHDV. Maximum rates of spread of rabbit hemorrhagic disease (RHD) in Australia ranged from 9 km/mo during summer to 414 km/mo in spring. New cases of RHD were moderate during late autumn and winter and peaked in spring. In summer the disease was rarely reported.

Key words: Epidemiology, European rabbit, monitoring, *Oryctolagus cuniculus*, rabbit hemorrhagic disease, transmission rate.

INTRODUCTION

Rabbit hemorrhagic disease (RHD) is a rapidly fatal disease of adult and sub-adult rabbits. The first clinical cases of RHD were reported during the spring of 1984 in Jiangsu Province, China (Xu, 1991); however, the virus possibly originated in Europe much earlier and was brought into China with European breeding stock (Xu, 1991; Morisse et al., 1991). Since the initial clinical cases, RHD has spread through Asia, Africa, the Americas, and Europe where it has reduced wild European rabbit (*Oryctolagus cuniculus*) populations during the initial outbreak of RHD. In Spain 55 to 75% of wild rabbits died (Villafuerte et al., 1995). However, mortalities as high as 90% have been measured in laboratory experiments (Morisse et al., 1991), and 80 to 100% mortality has been reported in small Italian rabbitries (Cancellotti and Renzi, 1991).

In 1989, government agencies in Australia and New Zealand began to investigate the potential benefits and risks of RHD as a biological agent for the control of the wild rabbit. In September 1991, a strain of Rabbit hemorrhagic disease virus (RHDV) (CAPM V-351) from the Czech Republic was imported into the quarantine facilities of the CSIRO Australian An-

imal Health Laboratory (AAHL; Geelong, Victoria, Australia) (Lenghaus et al., 1994). Following the successful demonstration of host-specificity (Lenghaus et al., 1994), the virus was released from quarantine in AAHL for assessment of field efficacy in a quarantined compound on Wardang Island (34°30'S, 137°22'E), a small island 4 km off the coast of South Australia (Cooke et al., 1996).

Despite all precautions, RHD crossed the quarantine barriers and was found in rabbits elsewhere on Wardang Island in late September 1995 (Cooke, 1997). It was found subsequently to have spread to Point Pearce on the mainland of South Australia, approximately 4 km from where the dead rabbits were found on Wardang Island and then, within 2 wk, to sites in the Flinders Ranges National Park (30°15'S, 139°05'E), more than 300 km away. This paper reports the rapid spread of RHD in Australia during the 13 mo period between its escape from Wardang Island in October 1995, and when official approval to make releases of RHDV was given in October 1996.

MATERIALS AND METHODS

The spread of RHD across Australia was monitored by the following Australian state government agencies: The Animal and Plant



Control Commission and Department of Environment and Natural Resources (Adelaide, South Australia), Northern Territory Parks and Wildlife Commission (Alice Springs, Northern Territory), New South Wales Agriculture (Dubbo, New South Wales), Australian Capital Territory Parks and Conservation Service (Tuggeranong, Australian Capital Territory), CSIRO Division of Wildlife and Ecology (Gunghalin, Australian Capital Territory), Department of Conservation and Natural Resources (East Melbourne, Victoria), Agriculture Western Australia (Forrestfield, Western Australia), Conservation and Land Management (Perth, Western Australia), Department of Natural Resources (Inglewood, Queensland) and Department of Primary Industries and Fisheries (Kings Meadows, Tasmania).

All reports of dead rabbits were investigated. Whenever possible, liver and spleen tissue samples were collected from fresh carcasses and subsequently were analysed for the presence of RHDV. Serum samples also were collected from surviving rabbits in populations where landholders reported decreasing rabbit numbers, but no carcasses were available for tissue collection due to predation and/or tissue decomposition. At some sites liver, spleen and serum samples were collected from rabbits ahead of the known distribution of the disease to confirm the reliability of carcass surveys for determining the extent of spread of the disease.

Map coordinates were obtained using Global Positioning System (GPS) recorders or from the 1975 Australian 1:250,000 map series Gazetteer (Lambert, 1975). All collected data were stored in Microsoft Access version 2.0 (Microsoft Corporation, Redmond, Washington, USA) at the Animal and Plant Control Commission (Adelaide, South Australia, Australia). Map coordinates were used to plot both confirmed and unconfirmed RHD sites using Mapinfo Professional version 4.0 (Mapinfo Corporation, Troy, New York, USA) while Statistix version 4.1 was used for any statistical analysis (Analytical Software, Tallahassee, Florida, USA).

A virus capture enzyme linked immunosorbent assay (ELISA) (Collins et al., 1995) was used to detect RHDV in liver and spleen tissue samples collected from rabbit carcasses. Initially, all test samples were sent to AAHL or Vetlab, Department of Primary Industries and Resources (Adelaide, South Australia, Australia) for analysis. However, as the disease spread beyond South Australia to other States and Territories, testing facilities were established in individual state veterinary laboratories.

A competition ELISA (Collins et al., 1995) was used to detect RHD antibodies in sera collected from rabbits. Rabbits were shot with a

22 calibre rifle. Approximately 2 to 3 ml of blood was collected from the heart using plain 5 ml vials or 10 ml vacutubes. The blood samples were stored in portable refrigerators at 4 C until centrifuged at $2,000 \times g$ for 10 min. Supernatant serum was then separated into aliquots and stored at -20 C for future analysis.

A confirmed RHD site was defined as a specific location (defined by longitude and latitude coordinates), property or town from which a rabbit had been collected that returned a positive result to either the antibody competition ELISA or the virus capture ELISA. Confirmed sites were collated into monthly records using the sample collection date or, if that was unavailable, the date when RHD was confirmed. These monthly records were used to determine the number of new sites for each season (spring data included October and November 1995 and September and October 1996, giving a sample interval of 4 mo).

Confirmed new RHD sites also were used to determine the monthly maximum rate of RHD spread, by measuring the monthly increase in the known geographic distribution of the disease. The extension of distribution was calculated by selecting the outer most northerly, westerly, southerly and easterly confirmed RHD sites at the end of each month. The distance RHD moved north for a given month was determined by extending a line directly east and west from that month's most northern site and the previous month's most northern site and measuring the north-south distance between the two lines. Distances between confirmed RHD sites were determined using Mapinfo version 4.0. A similar process was used to measure spread to the south, east and west. The seasonal values were calculated from the mean of the monthly maxima, in any direction, for the 3 mo in each season (spring sample size was 4 mo). Wardang Island was used as the point of origin when determining the rate of spread during October 1995.

Monthly records of confirmed new sites of RHD and logarithms of maximum rates of spread were analysed using a one-way analysis of variance, with season as the variable. If seasons differed significantly ($P \leq 0.05$), a subsequent analysis was performed using a Tukey multiple comparison test (Zar, 1974).

RESULTS

On 29 September 1995, a rabbit carcass found outside the quarantine pens on Wardang Island was confirmed as having died from RHD. Over the next 2 wk a further 12 dead rabbits, later confirmed as

having died from RHD were collected from outside the quarantine pens. On 12 October 1995 the first case of RHD was confirmed on the mainland of Australia at Point Pearce (South Australia), approximately 4 km away from Wardang Island. On 28 October 1995, RHD was confirmed at two further sites; one in the Flinders Ranges National Park and the other at Plumbago Station; both more than 300 km from Wardang Island and over 100 km apart (Fig. 1A). By the end of December 1995 the disease was well established in the arid and semi-arid region of South Australia and had moved into western New South Wales and south western Queensland (Fig. 1B, C). In the next 2 mo no new confirmed sites were reported from those states; however, the disease was still active in South Australia, but at a lower rate (Fig. 1D, E).

In March 1996, RHD was confirmed in central Victoria, (Fig. 1F) over 300 km southeast of the nearest previously known confirmed RHD site. Rabbit hemorrhagic disease was also present in a narrow band along the Murray River in South Australia; however, a broad survey of rabbit populations in the adjacent mallee region (Fig. 1A) of South Australia and Victoria, during May (Fig. 1H) provided no evidence of RHD. Rabbit hemorrhagic disease did not appear in the lower mallee (Fig. 1G, H) and southeast of South Australia until June 1996 (Fig. 1I). Confirmed sites in southeastern New South Wales also began to occur shortly after the Victorian outbreak (Fig. 1I).

By May 1996, RHD was confirmed in Western Australia and the Northern Territory at isolated sites over 300 km from the nearest previously confirmed sites thereby completing its spread to all states and territories on mainland Australia (Fig. 1H). From August to October 1996 (Fig. 1J–M), RHD had spread throughout southern Australia covering over 50% of mainland Australia. Antibody tests of sera from rabbits taken >100 km ahead of the known extent of spread were negative.

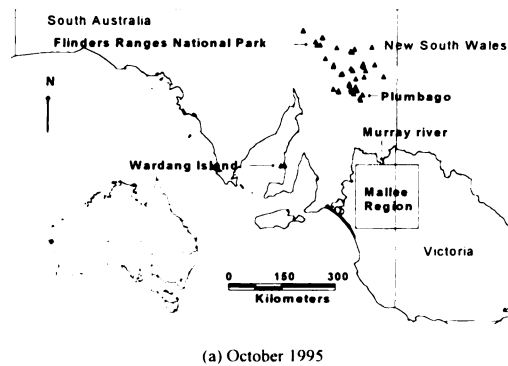


FIGURE 1. Distribution of confirmed rabbit hemorrhagic disease in European rabbits in Australia from October 1995 to October 1996. (▲) Confirmed RHD sites for that month. (○) Previously confirmed RHD sites.

During the monitoring period, no confirmed sites of RHD were recorded in Tasmania.

Monthly records of new RHD sites (Fig. 2) indicate that the majority of the new sites were confirmed in the months of August, September and October. The lowest number of new RHD sites were recorded during the summer months.

One-way analysis of variance of seasonal variations in the mean confirmed RHD sites (Table 1) showed that no significant differences occurred between autumn, winter and spring. However, there were significantly fewer confirmed sites in summer compared with spring. No significant differences were detected between autumn, winter and summer. These results indicate that after the initial outbreak of RHD in the spring of 1995, the number of new confirmed RHD sites significantly decreased in summer before gradually increasing again during autumn and winter, peaking again in the spring of 1996.

One-way analysis of variance on the logarithms of the maximum rate of RHD spread (Table 1) also showed significant differences between seasons, ($P < 0.05$). No significant differences were observed in the rates of spread between spring, autumn and winter, but the rate of spread in summer was lower than in all other

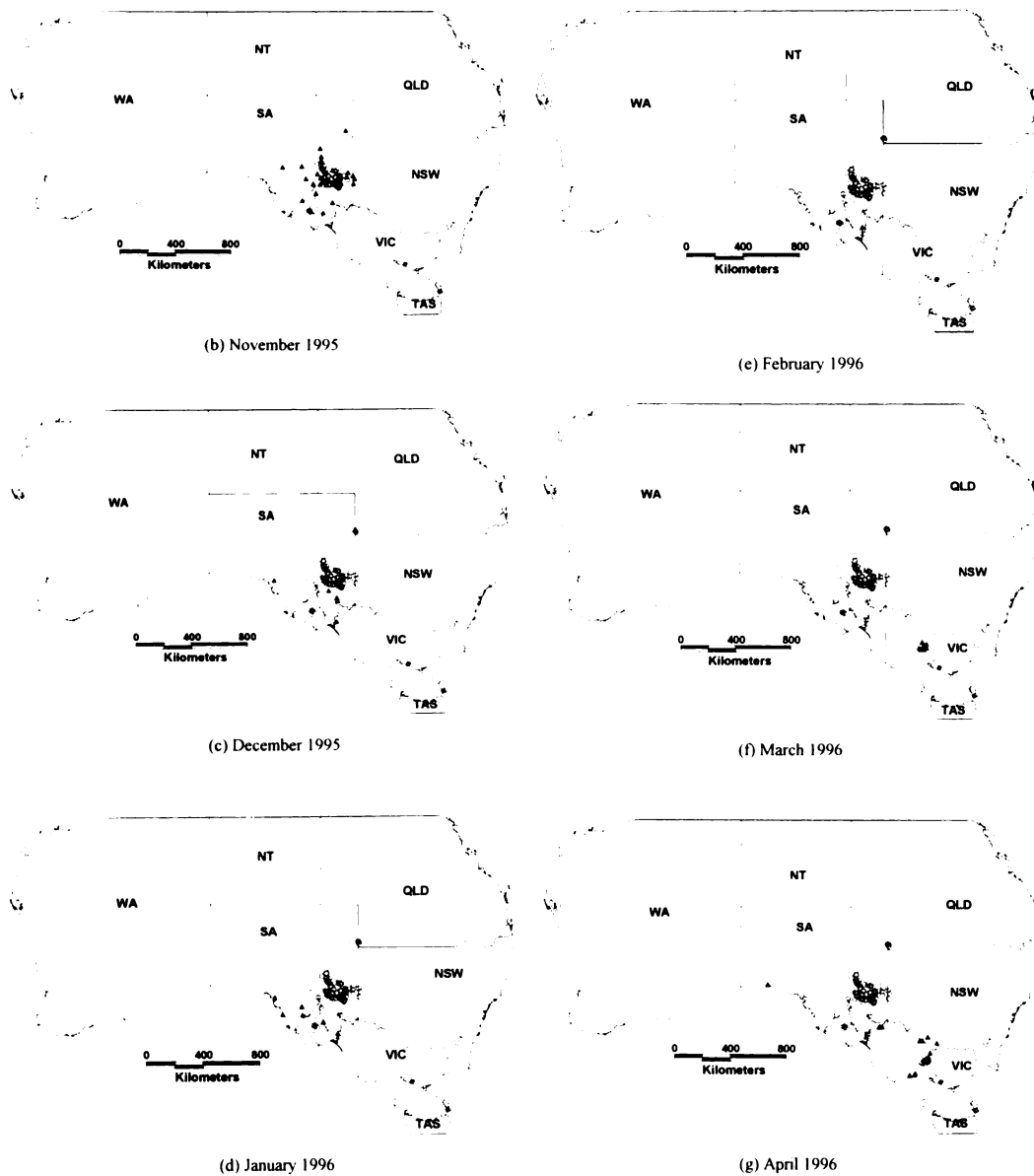


FIGURE 1. Continued.

seasons (all $P < 0.05$). In spring, RHD spread at an average rate of 414 km/month which corresponded with the high number of new RHD sites detected over the same period. In contrast, RHD spread at a rate of 9 km/month in summer, and corresponded with the low number of confirmed new RHD sites in the same period.

DISCUSSION

The procedures established to monitor the spread of RHD across Australia following its escape from Wardang Island relied primarily on the collection of rabbit carcasses soon after death. This may have resulted in the seasonal activity and rate of spread of the disease being underestimated, e.g., due to the low likelihood of rabbit

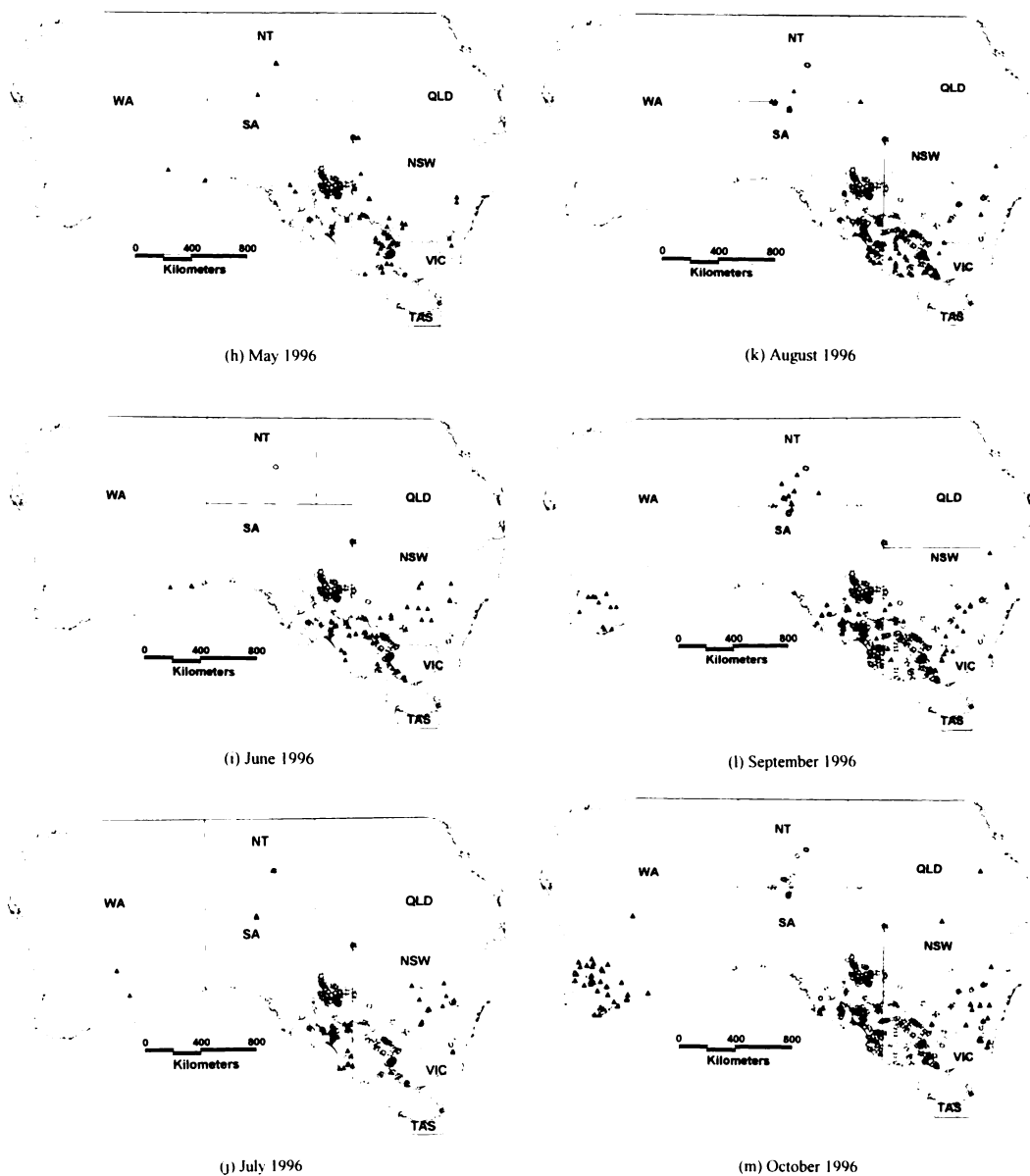


FIGURE 1. Continued.

carcasses being found in areas sparsely populated by humans. A high proportion of rabbits in Australia live in complex burrow systems underground and field observations indicated that most rabbits died from RHD inside the warren; only the occasional rabbit carcass was detected above ground (Cooke et al., 1996). Predators would have removed the latter within a short period of time. Nevertheless, the ex-

tensive media coverage of RHD overcame this problem to some extent, and encouraged property owners to remain vigilant. In addition, RHD appeared at a time when rabbit numbers in the Flinders Ranges were the highest recorded in 4 yr, and dead rabbits were easily detected in the area (Mutze et al., 1998).

It is evident that the rates of spread of RHD in Australia far exceeded those ob-

TABLE 1. Seasonal activity of rabbit hemorrhagic disease during its initial spread in European rabbits through southern Australia.

Season	Sample size (n) ^a	Mean number of confirmed new sites per month	Maximum rate of spread (km/mo)
Summer	3	5.7 (0.2–78.9) ^b	9 (0–2,318)
Autumn	3	27.0 (2.5–174.0)	364 (135–978)
Winter	3	59.7 (17.5–174.7)	176 (30–1,034)
Spring	4	80.0 (47.4–125.9)	414 (249–690)

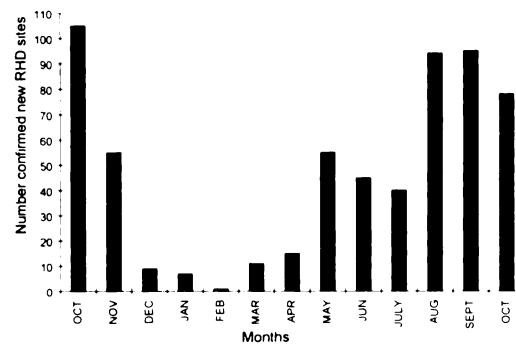
^a n = number of monthly values contributing to each mean.^b Mean confidence intervals were calculated from transformed data, so are asymmetrical when transformed back to km/month.

FIGURE 2. New rabbit hemorrhagic disease (RHD) sites confirmed on a monthly basis from October 1995 to October 1996.

TABLE 2. Confirmed new rabbit hemorrhagic disease sites used to determine the rate of spread of the disease in European rabbits through Australia.

Month	Site	Date	Longitude	Latitude	Distance spread (km) ^a				
					North	South	East	West	Maximum
October	Flinders Ranges	21/10/95	139°05'	30°15'	382				
	Tikalina	21/10/95	140°45'	32°13'			300		382
November	Merty Merty	10/11/95	140°26'	28°33'	273				
	Lewiston	14/11/95	138°36'	34°36'		15			
	Thackaringa	16/11/95	141°07'	32°04'			55		
	Kokatha	18/11/95	135°14'	31°16'				202	273
December	Tailem Bend	17/12/95	139°28'	35°15'		73			
	Kooma	21/12/95	134°21'	32°13'				82	82
January	Coolangatta	6/1/96	134°15'	32°30'				11	11
February		no extension of known range							
March	Newstead	6/3/96	144°04'	37°06'		209			
	Epsom	28/3/96	144°18'	36°43'			292		292
April	Head of Bight	9/4/96	131°13'	31°29'				286	
	Wooroondoo	16/4/96	142°47'	37°54'		88			
	Deniliquin	24/4/96	145°00'	35°35'			62		
	Blue Gate	29/4/96	144°15'	35°10'	155				286
May	Yambuck	17/5/96	142°04'	38°19'		50			
	Gunnadurah	23/5/96	125°51'	30°54'				510	
	Ambalindum	30/5/96	134°41'	23°23'	576				
	Mt Boiga	30/5/96	149°33'	32°47'			420		576
June	San Remo	20/6/96	145°23'	38°22'		40			
	Coonabarabran	24/6/96	149°17'	31°16'	139				139
July	Coonamble	1/7/96	148°24'	30°57'	22				
	Gindalbie	4/7/96	121°46'	30°17'				392	392
August	Narrabri Board	29/8/96	150°07'	30°23'	100				100
September	Morree	19/9/96	149°45'	29°22'	112				
	Scone	19/9/96	151°10'	31°49'			101		
	Bunbury	20/9/96	115°38'	33°20'				554	554
October	Pinjarra	4/10/96	115°54'	33°27'				1	
	Rolleston	9/10/96	148°01'	24°43'	510				510

^a Distances only given where range extension was recorded in that direction for that month.

served in Europe. In parts of Spain, RHD spread at an estimated rate of 2 to 30 km/mo, (Villafuerte et al., 1995) while in England the rate of spread was measured at a few hundred meters a month (Trout, 1996). This compares to maximum rates of spread in Australia ranging from 9 km/mo in summer to 414 km/mo in spring. There is also evidence of a clear seasonal pattern in the activity of RHD. During this 13 mo study in Australia, RHD was more active in the cooler months, peaking in spring while in summer the disease was rarely reported. This is comparable to Spain where the disease appears annually during spring and winter (Villafuerte et al., 1995).

There has been much speculation about the mechanisms involved in the spread of RHD in Australia. Human intervention may have been involved and the initial spread to western New South Wales, Queensland, Northern Territory and Western Australia appeared to correspond to major road links. However, it is unlikely given the strict protocols in place, the difficulty in identifying an RHD infected rabbit without laboratory testing, and the failure of known attempts by some farmers to spread the disease by transferring rabbit carcasses around properties (Cooke, 1997). There is no evidence to support or refute the claim that avian predators or scavengers are involved. However, this is unlikely since there are no known migratory routes over Point Pearce, Flinders Ranges and Plumbago, and the outbreaks occurred within 2 wk, over 300 km apart. The most probable mechanism involved in the spread of RHD is transfer between rabbits by insect vectors (Wardhaugh and Rochester, 1996). The presence of infected rabbits and increased insect activity probably facilitated the spread of RHD within Wardang Island and its subsequent escape to the mainland (Cooke, 1997). Rabbit hemorrhagic disease may have spread across the English channel via airborne insect vectors which moved on air currents (Chasey, 1994). Insects could explain the seasonal variations in RHD ac-

tivity, as climate and weather are the predominant environmental factors which influence the activity and migration of insects (Naumann et al., 1991). Evidence in Europe also suggests that foxes and domestic canines could act as vectors of the disease; feces of dogs, not clinically affected by RHD, were a source of virulent material which affected susceptible rabbits (Delcarmen et al., 1994).

The role of insect vectors in transmission of RHD at different geographical scales is being investigated currently and appears critical to an understanding of the epidemiology of RHD in Australia.

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