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Rabbit haemorrhagic disease and the biological control of wild rabbits, *Oryctolagus cuniculus*, in Australia and New Zealand

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Abstract. This review considers the history of the discovery of the rabbit haemorrhagic disease virus (RHDV) and its spread throughout the world in domestic and wild rabbits, which led eventually to its deliberate release into Australia and New Zealand for the control of a major pest, the introduced wild rabbit. The physical and genetic structure of RHDV is now well understood, and its pathogenic effects are also well known. The epidemiology of rabbit haemorrhagic disease (RHD) has been clearly documented in the field in European countries, Australia and New Zealand. Since its initial spread through largely naïve populations of wild rabbits it has established a pattern of mainly annual epizootics in most areas. The timing of epizootics is dependent on climatic variables that determine when rabbits reproduce and the appearance of new, susceptible rabbits in the population. The activity of RHDV is also influenced by climatic extremes that presumably affect its persistence and the behaviour of insect vectors, and evidence is growing that pre-existing RHDV-like viruses in some parts of Australia may interact with RHDV, reducing its effectiveness. The timing of epizootics is further modified by the resistance to RHD shown by young rabbits below 5 weeks of age and the presence of protective maternal antibodies that also protect against fatal RHD. RHD has reduced rabbit abundance, particularly in dry regions, but rabbits in cooler, high-rainfall areas have been able to maintain their populations. In Australia and New Zealand, RHD has raised the prospects for managing rabbits in low rainfall areas and brought substantial economic and environmental benefits.

Discovery and spread of rabbit haemorrhagic disease virus

The People's Republic of China is the world's largest exporter of domestic rabbit meat, with exports rising from 308 tonnes in 1975 to 53 200 tonnes in 1983 (Feng-Yi 1990). Adding to this the large internal consumption of rabbit meat produced by both commercial and domestic rabbitries, there was clearly a very large population of domestic rabbits in China by the 1980s. In 1984 a hitherto-unknown disease was seen in Angora rabbits that had been imported a few days earlier from the German Democratic Republic into China (Liu *et al.* 1984; Xu 1991). In less than nine months it had spread through rabbitries within an area of about 50 000 square kilometres (Xu 1991), and soon spread to Korea (Park *et al.* 1991). Chinese investigators initially suspected the agent to be a parvovirus, and an inactivated vaccine was developed using livers of infected rabbits (Huang 1991). By the end of 1986 the disease had been generally brought under control (Xu 1991).

Nevertheless, a new disease – 'Malattia-X' – was recognised among domestic rabbits in Italy in 1986 (Cancellotti and Renzi 1991) and soon appeared in other countries in Europe (Morisse *et al.* 1991). This new disease was at first regarded as being due to a toxin, or to fallout

from the Chernobyl disaster. However, in 1987–88 the link was established with the 'haemorrhagic pneumonia' of rabbits in China, and vigorous research was initiated on this new viral disease of an animal of significant commercial value for both meat production and hunting.

By 1988, rabbit haemorrhagic disease, or RHD as it eventually became known, occurred not only in Europe but had also spread to domestic rabbits in the Russian Federation, the Middle East and parts of Africa, Cuba, Mexico, the USA, India and Reunion Island (Morisse *et al.* 1991). It was probably spread by trade in rabbit meat, or by shipment of rabbits infected before dispatch. Mortality rates in many commercial rabbitries in Europe were exceedingly high until an inactivated vaccine was introduced. The virus was imported into Mexico with a shipment of 18 tonnes of rabbit meat exported from China to a supermarket outside Mexico City in December 1988. This outbreak was successfully eradicated (Gregg *et al.* 1991), an accomplishment made possible by the absence of wild European rabbits in Mexico. By contrast, once the virus spread into wild rabbits in Europe, it became firmly established in this natural reservoir population.

Rabbit haemorrhagic disease was found to spread by oral, nasal or parenteral transmission. Virus was also excreted in

the urine and faeces, making rabbits infectious for up to 4 weeks after infection if they survived that long (Gregg *et al.* 1991). As the virus is moderately resistant to environmental temperature, transmission of infection in commercial rabbitries in temperate climates readily occurred via contaminated foodstuffs or other fomites. The disposal of waste from small rabbitries and the custom of feeding domestic rabbits supplementary green food gathered from the field enabled the interchange of RHD between wild and domestic rabbits.

Although the causative agent of RHD was initially suspected to be a parvovirus (Xu 1991; Gregg *et al.* 1991), virologists in Germany, Italy and Spain demonstrated that the causative virus was a calicivirus (Ohlinger and Thiel 1991), and soon afterwards its genome was completely sequenced (Meyers *et al.* 1991).

In a remarkable coincidence, a very similar disease of hares, also caused by a calicivirus, had been causing significant losses in farmed and wild hares in northern Europe since 1980 (Gavier-Widén and Mörner 1991). Retrospective testing showed the existence of hares seropositive for the causative virus since 1971 (Moussa *et al.* 1992). Although related (see below), the two viruses were distinct; they do not cross-protect (Chasey *et al.* 1992; Lavazza *et al.* 1996), and the hare disease occurred in countries such as the UK before RHD became established there (Chasey and Duff 1990).

The Office International des Épizooties designated these diseases of lagomorphs as 'viral haemorrhagic diseases'. The disease of rabbits was called RHD and the disease of hares the European brown hare syndrome (EBHS).

Origin of the virus

Three possible sources of the rabbit haemorrhagic disease virus (RHDV) were considered: transfer of the European brown hare syndrome virus (EBHSV) to rabbits (which leaves unsolved the origin of that virus), change in the properties of a pre-existing non-pathogenic virus of rabbits, or transfer of a hitherto-unknown virus from another animal species (as was documented for myxomatosis). The first possibility was eliminated when it was shown that the viruses causing disease in rabbits and hares were related but distinctly different caliciviruses. The second possibility seemed more likely because antibodies that cross-reacted with RHDV were detected in rabbit sera collected in the Czech Republic 12 years before the first outbreaks (Rodak *et al.* 1990), and Capucci *et al.* (1994, 1997) showed that seroconversion occurred in asymptomatic rabbits in some rabbitries where RHD had never been seen.

On further investigation in a commercial rabbitry, Capucci *et al.* (1996) recovered a calicivirus that caused no symptoms in rabbits but produced seroconversion and protected the rabbits against infection with RHDV. A wider serological survey of rabbits in the rabbitry showed that infection occurred immediately after weaning (Capucci *et al.* 1997). The non-pathogenic virus, for which they proposed the name rabbit calicivirus (RCV), was more closely related to RHDV than to EBHSV when tested by serology and sequence comparisons of the capsid proteins (Capucci *et al.* 1996). The occurrence of the inapparent infection in some rabbitries but not in others explained the patchy nature of outbreaks of RHD in commercial rabbitries in Europe. Its discovery dispenses with the need to consider the third alternative, namely that RHDV came from some host other than the European rabbit. Given that EBHS is also apparently a 'new' disease, three intriguing questions remain unanswered:

(1) How long ago did the separation of the rabbit and hare caliciviruses occur?
(2) What were the genomic changes responsible for the transition from viruses like RCV to RHDV?
(3) Did EBHSV also originate from a non-pathogenic hare calicivirus?

Classification and properties of caliciviruses

Viruses of the family Caliciviridae (Ohlinger and Theil 1991) are small, round viruses with a characteristic appearance in electron micrographs (Fig. 1). Their genome comprises a single positive-sense RNA strand (Ohlinger *et al.* 1990; Parra and Prieto 1990).

Genome comparisons

The genome of RHDV has been completely sequenced and consists of 7437 nucleotides, excluding the poly(A) tail (Meyers *et al.* 1991; Rasschaert *et al.* 1995; Gould *et al.* 1997). The genome of EBHSV has also been sequenced. It is 7442 bases long; alignment of the sequences of RHDV and
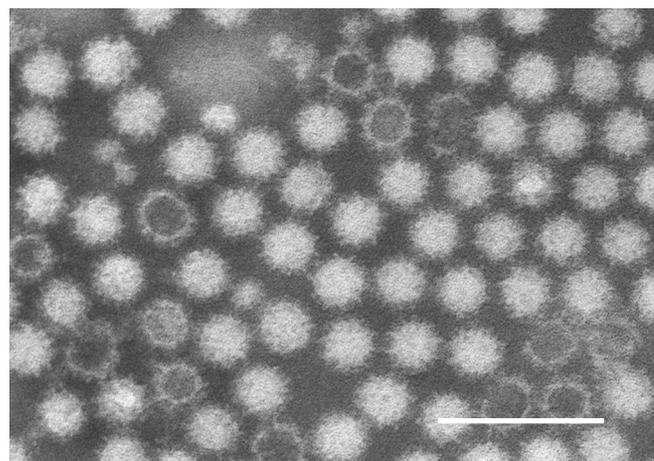

Fig. 1. Electron micrograph of rabbit haemorrhagic disease virus, a calicivirus (from the Latin *calix*, cup or goblet). Bar = 100 nm. Caliciviruses are small round particles characterised by 32 cup-shaped surface depressions arranged in *T* = 3 icosahedral symmetry. Courtesy of H. A. Westbury, Australian Animal Health Laboratory.

EBHSV shows 71% nucleotide identity (LeGall *et al.* 1996). Comparisons of partial nucleotide sequences of the capsid protein gene of representatives of all known groups of caliciviruses show that those of lagomorphs, for which the generic name 'lagovirus' has been proposed, cluster together (Fig. 2). Comparisons have also been made of the relatedness of partial nucleotide sequences from the capsid protein gene of 44 strains of RHDV isolated from cases in several European countries, Mexico, China and Korea between 1989 and 1995 (Nowotny *et al.* 1997; Fig. 3); they show 89–100% identity. Comparisons between 19 strains of EBHSV showed similar homology within that species but there was only 53–60% homology between strains of RHDV and EBHSV.

Host range

Some caliciviruses, notably San Miguel sea lion virus (which produced vesicular exanthema in swine), and the group of serologically related viruses that infect marine mammals, have a wide host range and are readily grown in cell culture. However, like the human caliciviruses, for which the only other susceptible host appears to be the chimpanzee (Wyatt *et al.* 1978) and which cannot be grown in tissue culture (Kapikian *et al.* 1995), RHDV appears highly species-specific and cannot be grown in tissue culture.

Studies in Europe, China and the USA revealed that none of 26 species of animal (other than European rabbits) showed

clinical signs after inoculation with RHDV in various doses and by various routes. Similarly, there have been no reports of illness or disease in humans or domestic animals that were in close contact with sick rabbits in commercial or backyard rabbitries, or RHDV-infected pet rabbits, in China or various countries in Europe. Taken in conjunction with the great difficulty experienced in growing the virus in cell culture, it appears that RHDV is a highly host-specific virus.

Nevertheless, it was essential that its effects should be examined in a range of Australian native species. Extensive studies were carried out on thirteen introduced mammal species and two imported bird species, three native rodents, six species of marsupial, a monotreme, three species of birds and one lizard. One species of bat and one species of bird (kiwi) from New Zealand were also tested. None of these animals showed signs of disease, nor could gross pathology, histopathology, or virus detection by highly sensitive reverse transcription polymerase chain reaction (RT-PCR) detect signs of infection. Serological tests were uniformly negative except for the kiwis, which exhibited a rising titre over the 5-week test period, a result attributed to the use of very high doses (300 000 rabbit lethal doses) of virus in the tests (Buddle *et al.* 1997).

Interestingly, EBHSV, the related lagovirus in European hares, does not appear to be so species-specific. It can infect varying hares (*Lepus timidus*) but it occurs in this latter
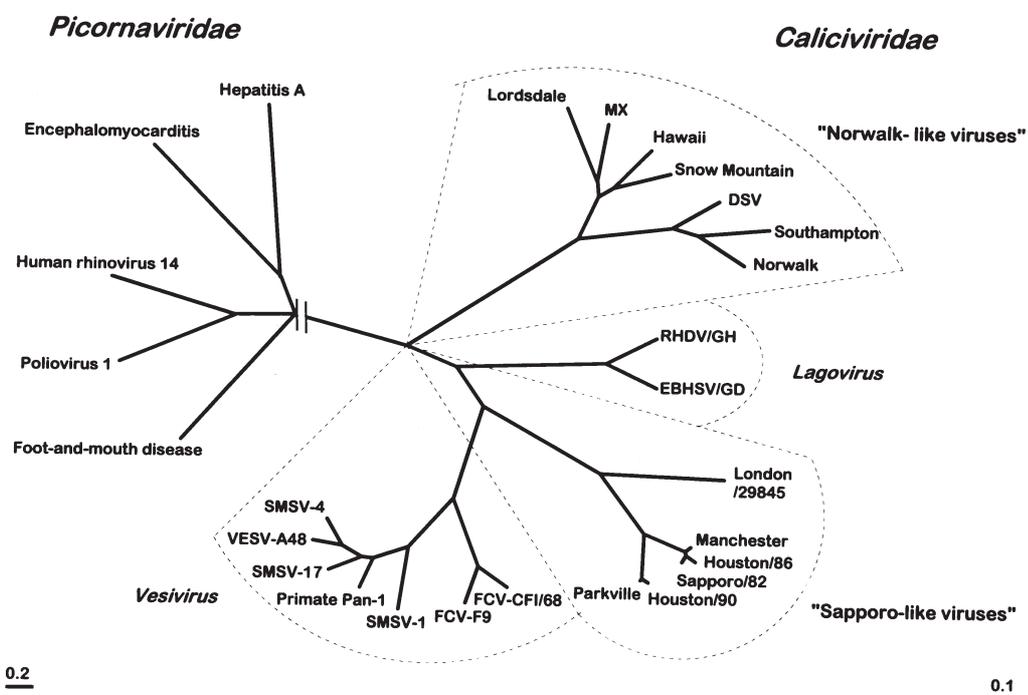

Fig. 2. Genetic relationship of caliciviruses recovered from a variety of animal hosts, based on comparisons of the hypervariable sequences of the capsid protein gene. There are four groups: two groups isolated from cases of human diarrhoea (Norwalk-like and Sapporo-like); the proposed genus *Vesivirus*, comprising caliciviruses of pinnipeds, vesicular exanthema virus and frline calicivirus; and the proposed genus *Lagovirus*, containing rabbit haemorrhagic disease virus and European brown hare syndrome virus. Courtesy of T. Berke.
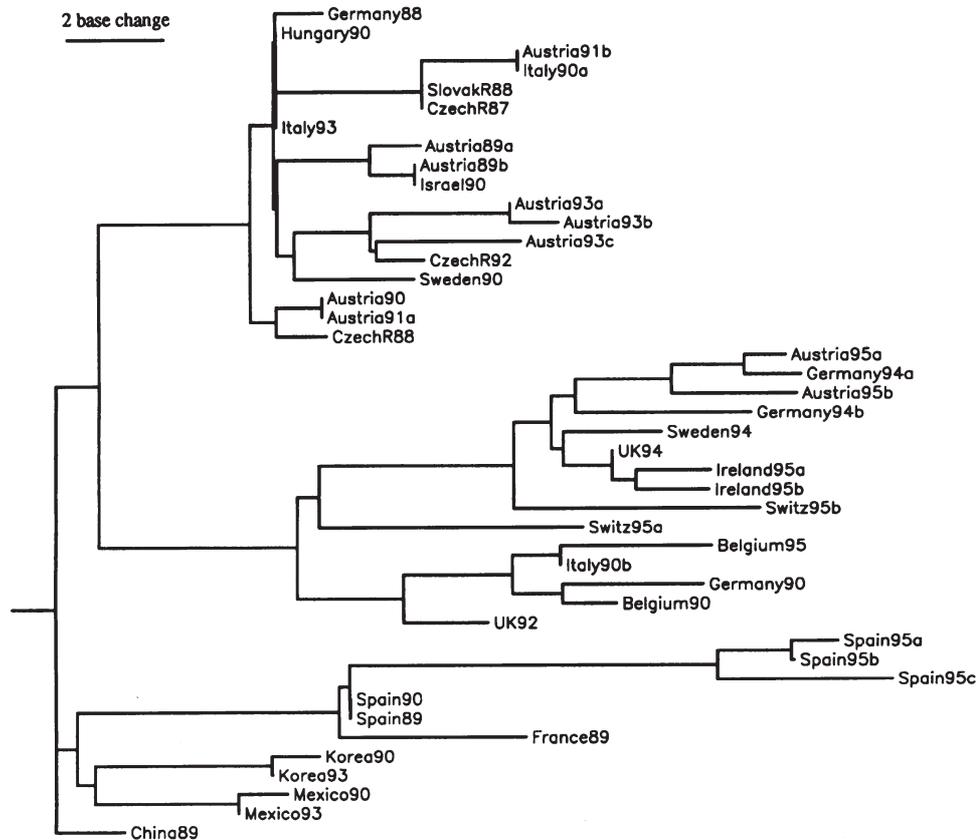
Fig. 3.   Dendrogram showing the genetic relationships of 44 samples of RHDV from various parts of the world, based on a partial nucleotide sequence of the capsid protein gene. Although samples are closely related there are three main branches. From Nowotony *et al.* (1997) with permission.

species only where its range overlaps with that of the European hare (Gavier-Widén and Mörner 1991). The American cottontail (*Sylvilagus floridanus*) can also be infected by EBHSV, sometimes dying as a result (L. Capucci and A. Lavazza, personal communication). A weak antibody response (but no disease and no protection) was evident when hares were inoculated with RHDV or rabbits with EBHSV (Lavazza *et al.* 1996).

Clinical features of RHD

Infection of adult rabbits with RHDV leads to peracute or acute clinical disease in 1–3 days, rabbits infected by the oral route surviving on average for 1 day longer than animals infected by intradermal or intramuscular inoculation (Cooke and Berman 2000). No significant clinical signs are seen in peracute cases, but acutely affected cases appear quiet and show increased body temperature and respiration rate and die within 24 h (Marcato *et al.* 1991). Haematuria and/or vaginal haemorrhage and foamy discharge from the nostrils are occasionally seen, and infected animals occasionally develop signs of central nervous system disease. No animals recover from the peracute disease and the few animals that recover

from the acute disease may exhibit jaundice and die a few days later. Virus is found in all secretions and excretions of diseased rabbits.

Studies at the Australian Animal Health Laboratory (Lenghaus *et al.* 1994) showed that after infection with a standard dose of RHDV, Australian wild rabbits died sooner than laboratory rabbits, within 20–24 h rather than 30–36 h, and apparently with minimal distress. 'Rabbit calicivirus disease' (RCD) was suggested as an alternative name for RHD, and Australian government authorities adopted this name for the disease and 'rabbit calicivirus' as the name for the virus. However, Capucci *et al.* (1996) have since given this name (RCV) to the avirulent precursor virus, and since RHDV is used internationally for the virulent virus, this remains the official scientific name.

Pathology of RHD

The most consistent pathological lesion in adult rabbits infected with RHDV is a necrotising hepatitis that affects the hepatocytes in the peripheral areas of the liver lobules most severely (Marcato *et al.* 1991; Fuchs and Weissenbock 1992). Other characteristic lesions, not seen in all cases, are

lymphocyte depletion and necrosis of the spleen. Disseminated intravascular coagulation produces fibrinous thrombi within small blood vessels in most organs, especially in the lungs, heart and kidneys, in which there are occasionally striking haemorrhages, from which the disease derived its name. Liver, spleen and blood contain high concentrations of virus (Xu 1991). Lenghaus *et al.* (1994) noted that the damage to the liver and spleen in immature rabbits that survived infection did not progress beyond scattered small foci of lytic necrosis, and the extensive blood coagulation seen in adult animals was entirely absent. Measured by PCR (see below), the concentration of virus in the livers of adult rabbits was a million times higher than in those of rabbit kittens.

Clinical diagnosis

Apart from epidemiological features (an acute disease with a high mortality in adult rabbits), the presence of gross lesions of acute hepatitis, a swollen spleen and congested and haemorrhagic lungs suggests RHD. The histopathological lesions in the liver, spleen, kidneys, heart and lungs are highly indicative (Fuchs and Weissenbock 1992).

Laboratory diagnosis

Although it is difficult or impossible to grow the virus in tissue culture, electron-microscopic examination of sections or smears of liver or spleen show numerous small round virus particles, and immunofluorescence or enzyme-linked immunosorbent assay (ELISA) using suitable polyclonal or monoclonal antibodies is diagnostic.

Advances in molecular biological technology have provided sensitive techniques for detecting RHDV. Guittré *et al.* (1995) developed an RT-PCR for detecting a conserved part of the capsid protein and showed that it was 10 000 times more sensitive than ELISA testing for the detection of RHDV. Using similar primers, Gould *et al.* (1997) were able to obtain positive results with material from infected liver at dilutions up to 10^{-10}. RT-PCR is now used routinely to determine whether various species of insect carry RHDV (Asgari *et al.* 1998) and as a step towards sequencing of the RHDV genome. Unfortunately, it does not distinguish between live and inactivated virus. In the absence of a method for culturing the virus, verification that RHDV in tissues is viable still requires the inoculation of rabbits with tissue extracts.

Serological tests

A range of serological tests has been developed to detect virus and demonstrate past infection in rabbits. These include haemagglutination (HA) tests, haemagglutination-inhibition (HI) tests, virus capture ELISA, and several types of competition ELISA (cELISA). The most widely used tests in surveys of wild rabbits are ELISAs based on polyclonal or monoclonal antibodies developed at the RHD World

Reference Laboratory in Italy (Capucci *et al.* 1991, 1995). Using suitable anti-isotype antibodies, it is possible to measure different antibody isotypes: IgG, IgM and IgA (Capucci *et al.* 1997). These can be used to distinguish between past infection with RHDV (IgG, some IgA and possibly IgM in adult rabbits), recent recovery from infection (high titre of IgM, with IgA and IgG at lower titres) and maternal antibody (IgG only, in rabbits younger than 12 weeks) (Cooke *et al.* 2000). Most of these tests are highly specific for RHDV; however, apparent cross-reactivity and anomalous results with IgG isotype tests in some instances have led to the supposition that other RHDV-like viruses might be present in Australia and New Zealand (O'Keefe *et al.* 1998; Cooke *et al.* 2000).

Introduction of RHD into Australia and New Zealand

After observations of outbreaks of RHD among wild rabbits in arid areas of Spain in 1988, the possibility was raised of using RHDV for the biological control of rabbits in Australia. This was widely discussed among appropriate authorities such as the Agriculture and Resource Management Council of Australia and New Zealand (ARMCANZ) and the Australian and New Zealand Environment and Conservation Council (ANZECC). Through the Australian Agricultural Council a combined approach was taken that involved State and Commonwealth governments as well as New Zealand in providing financial support for further investigations of the virus (Fenner and Fantini 1999).

The virus (Czech strain 351) was imported under quarantine into the Australian Animal Health Laboratory in 1991. Tests on a colony of wild rabbits confirmed its lethality. Investigations on a range of domestic and native animals confirmed the high species-specificity of the virus. After conferences involving animal welfare groups as well as scientists and rabbit control authorities, it was agreed by all governments in Australia and New Zealand to proceed to field trials on Wardang Island in Spencer Gulf, South Australia. The objective (Fenner and Fantini 1999) was to find out whether the virus would spread among Australian wild rabbits living in natural warrens and to evaluate (1) the immediate impact of the disease in terms of the humaneness of death and the rates of mortality, (2) rates of transmission, (3) the effect of season on rates of transmission, and (4) persistence of the virus.

A quarantine enclosure of some 50 ha was built, surrounded by an electrified rabbit- and cat-proof fence and containing two pens, one containing six and the other four smaller sites, each 1 ha in size and surrounded by two rabbit-proof fences. Elaborate quarantine protocols were set up to minimise spread by fomites and insect vectors. Staff carrying out experiments washed their boots in footbaths containing glutaraldehyde and changed clothing and footwear on crossing barrier fences between the outer predator-proof perimeter and the inner sites where rabbits

were kept. Rabbits and experimental burrows were treated with Deltamethrin (a residual insecticide), saline swamps were treated with *Bacillus thuringensis israeli* to control mosquito breeding, and baited fly-traps were set up to trap large numbers of flies.

A rabbit-free zone 300 m wide was maintained around the perimeter of the quarantine area, and rabbits elsewhere on the island were kept under surveillance. All experimental rabbits were fitted with radio-collars, and were observed from hides at dawn and dusk. The risk of scavenger birds spreading the virus was reduced by infecting only a few rabbits at a time and by removing any rabbits that died aboveground as quickly as possible at dawn. Rabbits that died underground were located with the radio-collars and removed by digging a hole 15 cm wide vertically into the warrens to minimise disturbance. Carcasses never remained for more than 1 day after confirmation of death.

Tests commenced in March 1995. Each of the 1-ha enclosures, built around natural rabbit warrens, was stocked with four male and six female rabbits, and trials were initiated by inoculating two rabbits on a site and observing the animals for 3 weeks before killing all remaining rabbits.

The experimental rabbits in these enclosures died about 42 h after inoculation (range 21–48 h), usually in their burrows. Behavioural changes were seen in some rabbits 12 h before death; few infected rabbits came above the ground and those that emerged stayed close to their warren (Cooke 2002).

The spread of infection from inoculated to contact rabbits in individual sites varied from nil to rapid killing of seven of the eight contact rabbits. The interval between successive deaths varied from 2 to 7 days, suggesting that virus could persist in a warren for at least 5 days. However, when a site was restocked 7 weeks after an earlier experiment, no rabbit became infected.

Sites being prepared for later experiments were regarded as 'sentinel' sites, and monitored to detect any spread of the virus. On 1 July, as virus was spreading in two experimental sites, it suddenly appeared in a sentinel site, possibly associated with scavenging by a raven (*Corvus* sp.). Rabbits from all three sites were caught and killed and further experiments delayed until it had been confirmed that the virus had not spread further. Tighter precautions were taken to prevent scavenging, and no spreading beyond the experimental site occurred in an experiment in mid-August. However, in an experiment beginning on 13 September, virus again spread to two sentinel sites, and on 29 September a rabbit that had died from RHD was found outside the quarantine area and disease foci developed in the warrens in the area. Accidental spread by staff was ruled out and spread by ravens or other scavengers was considered unlikely; spread by insects could not be excluded. As part of the contingency plans for an escape from quarantine, poisoning with 1080 (sodium fluoroacetate) and ripping of burrows in

that area was carried out; nevertheless, cases of RHD continued to appear on the island until 19 October.

Overall, in the trials on Wardang Island only 13 rabbits became infected from the 14 experimentally inoculated. This suggested that RHD spread rather poorly among wild rabbits living in natural warrens when the only mechanism of spread was by contact between infected rabbits and brief contact with cadavers.

Following the discovery of infected rabbits in sentinel sites on 22 September and outside the quarantine area on 29 September, extensive searches were made on rabbit-infested areas on the adjacent mainland. On 12 October a dead rabbit infected with RHDV was found at Point Pearce, on the mainland about 5 km from the quarantine area, and cases were subsequently found over an area of some 20 ha. In accordance with a pre-planned program agreed with the South Australian government (Operation Garter), rabbit control procedures were begun at Point Pearce, but it was already too late; RHDV was identified from a dead rabbit collected at Yunta, some 360 km away from Point Pearce, on 28 October. On 26 October dead rabbits, subsequently identified as being infected with RHDV, had also been found near Blinman, some 390 km from Point Pearce. Further investigations showed that the Yunta and Blinman outbreaks occurred within 24 h of each other and were probably spatially distinct events that occurred in semi-arid areas of traditionally high rabbit density. They probably began at about the same time as the Point Pearce outbreak. Nucleotide sequencing of RNA from virus samples showed that viruses taken from within the quarantine area, from Wardang Island outside the quarantine area, and from Yunta were identical.

Following a decision of the Consultative Committee on Exotic Animal Diseases that the virus could be considered endemic in Australia, further plans to stop the spread of the virus were abandoned early in November 1995. Following the receipt of submissions from the public early in January 1996, an environmental impact document was produced, followed by a detailed report under the Biological Control Act, in which all submissions from the public were considered. Having also received a document advising that no adverse effects on human health were expected (Carman *et al.* 1998), ARMCANZ agreed unanimously to approve the release of RHDV. In September 1996, the virus was registered as a pest control agent under the Agricultural and Veterinary Chemicals Code Act and quarantine restrictions were lifted.

Deliberate releases, using intramuscular injection of captured wild rabbits, were made at many sites throughout rabbit-infested regions of Australia, starting in Wagga Wagga in New South Wales on 9 October 1996. However, in the months before this official release, the disease had spread over long distances and had been introduced unofficially to other places from Queensland to Western Australia. As a consequence, many inoculations of wild rabbits were made in areas where RHDV was already well established.

Although the New Zealand government was a party to the investigations on RHDV from the outset, on 2 July 1997 it decided not to recommend its use. However, as was the case with myxomatosis in Britain, farmers in areas where rabbits were in plague numbers took the matter into their own hands. On 23 August 1997 the New Zealand Ministry of Agriculture confirmed that RHD had appeared on at least one farm in Central Otago, on the South Island (Thompson and Clark 1997). Tests with PCR confirmed that the virus was similar to that found in Australia (O'Keefe *et al.* 1998). Containment measures were started, but it soon became evident that the virus was present on many farms and that farmers were spreading it deliberately, usually by contaminating carrot bait with suspensions prepared from the entrails of rabbits that had died from the disease. In September 1997 the New Zealand government bowed to the inevitable and made the possession and spreading of RHDV legal. In mid-1998 a local biotechnology company was granted a permit to sell limited supplies of the virus on the open market, to be used on oat or carrot bait.

Epidemiology

Initial spread of RHD in Australia

When RHD escaped from Wardang Island in October 1995, its subsequent spread was recorded from the discovery of dead rabbits and subsequent confirmation of RHDV in liver samples by virus capture ELISA. The presence of antibodies to RHDV in the serum of rabbits that had survived infection was also a useful measure (Cooke *et al.* 2000). Kovaliski (1998) collated Australia-wide data to provide an initial picture of the spread of the virus. The virus spread at over 50 km per week in spring and autumn but more slowly in summer, when fewer new disease foci were recorded. Such a rate of spread is not compatible with normal movements of rabbits, which are territorial animals, nor can movement of scavengers like foxes explain it. Some farmers certainly moved rabbit carcasses about but this did not explain the sudden appearance of the disease in uninhabited inland areas.

Insect vectors

The escape of RHDV from Wardang Island was associated with the first warm spring days of October 1995. Bushflies (*Musca vetustissima*) appeared on the island in large numbers, presumably coming from the warmer mainland areas because these flies do not persist over winter in coastal South Australia. Blowflies (*Calliphora* spp.) also became abundant at the same time. Flies quickly found dead rabbits on the island, and the spread of RHD to the mainland was associated with a cool weather front that could have carried flies back to the mainland. The likely trajectories of wind-borne insects on 12–14 October 1995, when the virus escaped from the quarantine pens, were modelled by

Wardhaugh and Rochester (1996), and these trajectories were consistent with the distribution of RHD when it subsequently appeared on mainland South Australia. As a consequence of this strong circumstantial evidence, Asgari *et al.* (1998) explored the role of blowflies in transmitting the disease. They showed that RHDV could be detected in flies by RT-PCR for up to 9 days in flies that had been fed on livers of rabbits that died from RHD. Furthermore, the fresh faeces (flyspots) from flies that had recently fed on infected liver were infective when fed to susceptible rabbits. It was judged that flyspots contained 2–3 50%-lethal doses (LD_{50}) RHDV. Using PCR, RHDV was detected in several species of flies, in mosquitoes caught in traps and in maggots obtained from rabbit carcasses (Westbury 1996). There was no evidence that flies that developed from maggots laid in RHDV-infected rabbit carcasses contained virus, supporting the idea that caliciviruses of vertebrates do not replicate in arthropods. As with myxomatosis, transmission of RHDV must be mechanical. Within Australia, tests using RT-PCR show that at least eight species of flies readily become contaminated with RHDV (Asgari *et al.* 1998). Heath *et al.* (1998) reported similar results from flies trapped in association with the spread of RHDV in New Zealand. Interestingly, two of the positive fly species reported there, *Lucilia cuprina* and *Calliphora vicina*, are introduced European species.

The potential for mechanical transmission has been further demonstrated in the laboratory for the Australian bushfly (*Musca vetustissima*) and several other non-biting species of flies, which presumably acquire virus by feeding on blood or tissue exudates after the rabbit's death. Mosquitoes (*Culex annulirostris*) and rabbit fleas (*Spilopsyllus cuniculi* and *Xenopsylla cunicularis*) are also capable of transmitting RHDV (Lenghaus *et al.* 1994), reflecting the high titres of virus in the blood of infected rabbits (Xu 1991).

The biology and behaviour of Australian blowflies and bushflies is well known (Norris 1966). Their abundance and behaviour varies according to season. Some winter-active blowflies (*Calliphora* spp.) are partially displaced each year when 'secondary strike' flies such as *Chrysomya rufifacies*, whose larvae prey on other calliphorid larvae, move southwards during the summer months. Several species of blowfly may be present at any given time during the year; consequently, it is not possible to distinguish particular species involved in transmission or particular times of the year when flies might be most likely to transmit the virus. Nevertheless, there is a general peak in fly abundance and activity during the spring and another smaller autumn peak. Blowflies are adept at finding rabbits that die from RHD deep within their warrens.

Mosquitoes have not been sufficiently well studied to confirm whether or not they are regularly involved in RHDV transmission in the field. The only wild-caught mosquitoes

confirmed to carry RHDV are *Ochlerotatus* (*Aedes*) *postspiraculosus*, found positive by PCR and subsequently by inoculating insect tissues into susceptible rabbits (Cooke 2001).

Initial impact of RHD

Rabbit haemorrhagic disease virus was found in wild rabbits in Europe soon after it was first recognised in domestic rabbits. An early outbreak was observed in the province of Almería in arid south-eastern Spain in June 1988 (Rogers *et al.* 1994). Cooke (2002) reviewed the initial outbreaks and spread. In December 1988 the disease was detected to the north of Almería in the province of Murcia. Serological studies carried out at 3-monthly intervals on live-captured rabbits showed that many of the surviving rabbits, both adult and subadult, carried antibodies detectable by HI tests. During the following year the proportion of rabbits carrying antibodies declined as more and more young joined the adult population, but in May 1990 many adults were again seropositive, indicating that a second disease outbreak had occurred.

The spread of RHD across the province of Murcia was patchy, but a broad front could be recognised with the disease spreading at about 15 km per month. Taking advantage of this patchiness, an RHD-affected rabbit population was compared with another RHD-free population nearby. Transect counts were used to follow changes in rabbit numbers and to detect cadavers, and it was shown that between mid-June and mid-July 1990 RHD reduced rabbit abundance by almost 50% in comparison with the unaffected site. HA tests were used to confirm RHD in cadavers found in the affected area, and HI tests were used to detect antibodies in those rabbits that survived. Neither dead rabbits nor rabbits with antibodies were detected on the control site.

At about the same time, the spread of RHD was followed even further north through Alicante, a third adjoining coastal province (Peiró and Seva 1991). Again, RHD spread at about 15 km per month and again, not all rabbit populations were affected as the disease spread; some hunting reserves remained untouched. Furthermore, there was a sharp decline in disease activity at the start of summer. The number of rabbits shot by hunters within the province declined between 1988 and 1989 but recovered to some extent in 1990. By counting rabbits along standardised transects it was shown that on one site the peak counts in June each year fell from 21.1 rabbits km^{-1} in 1988 to 5.2 rabbits km^{-1} in 1989 then recovered to 21.2 rabbits km^{-1} in 1990. Following the initial outbreaks there were less intensive, localised outbreaks of RHD in the late winter (February–March) of 1990 and the spring (April–May) of 1991 (V. Peiró cited in Cooke 2002).

Despite its relatively rapid progress through Almería, Murcia and Alicante, RHD nevertheless took some years to reach all wild rabbit populations across the Iberian Peninsula. Even though RHD had already been reported from Portugal in 1989 (Anon. 1989), the initial spread of RHD through Doñana National Park in south-western Spain only occurred in March–May 1990. Radio-collars were fitted to rabbits to follow its spread in the national park, and it was recorded that 55% of adult rabbits died, with both sexes being equally affected. It was also considered that high temperature in the area in late spring and summer may have curtailed the epizootic. However, the RHD epizootic at Doñana did not appear to be associated with seasonally high mosquito numbers, and it was concluded that vectors did not play an important role in transmission (Villafuerte *et al.* 1994).

Just as RHD took more than 5 years to reach all wild rabbits in Spain (Villafuerte *et al.* 1995; Simón *et al.* 1998) the spread across France was similarly prolonged. After RHD first appeared in 1989, recurrent outbreaks were soon apparent in the Carmargue, Vaucluse and Héralt in the south of France (Rogers *et al.* 1994). However, it was not until 1995 that the first outbreak of RHD was seen at the Chèvreloup arboretum, near Paris, among rabbits monitored since 1989 (Marchandeau *et al.* 1998*a*). The Chèvreloup rabbit population declined to 12% of its initial level in the course of a year and has remained low since (Marchandeau *et al.* 2000).

The initial impact of RHD on wild rabbit populations in Europe was strongly influenced by geography and climate. Certainly, the most obvious declines in rabbit abundance were seen in Spain and Portugal and to some extent France, whereas the virus did not so severely reduce rabbit populations in Britain or other countries of northern Europe.

Spain is the only European country where a major effort has been made to assess the broad impact of RHD on rabbit populations. Hunters were interviewed to determine how rabbit numbers had changed since the arrival of RHD. A nearby site with a rabbit population typical of the general area was then visited and the hunters' assessments of post-RHD rabbit numbers were standardised against quantitative field data. For each site the relative rabbit density was estimated from sightings of rabbits and other signs such as warrens, diggings or dung along a 4-km transect. Data from 311 sites across Spain were compiled, and climatic data, soil types and land use were taken into account when considering the questionnaire results (Blanco and Villafuerte 1994).

Most hunters interviewed considered that the disease recurred annually and mostly broke out in winter or spring. It was concluded that, 5 years after the arrival of RHD, rabbit populations across Spain were being held at a little less than 50% of their former levels. Nevertheless, some rabbit populations made better recoveries than others. In areas that were most favourable for rabbits – generally warmer sites with annual precipitation of about 450–500 mm – there was a small but significant tendency for rabbit numbers to recover. However, in areas that were generally unfavourable

to rabbits there was a strong propensity for populations to remain low. Management of rabbit populations, often involving the planting of supplementary food crops and control of predators, was also thought to be important in facilitating recovery of rabbit numbers.

In Britain, RHD took a long time to establish itself and has had a patchy effect. Well before it became widespread, sera from wild rabbits throughout Britain reacted in HI tests that usually indicated antibodies to RHD (Chasey *et al.* 1997). Some 22 of these seropositive rabbits were challenged with virulent RHDV and all survived. Rabbits throughout France also carried antibodies that reacted in RHD ELISAs, even on islands where RHD had never been detected or suspected (Marchandeau *et al.* 1998*b*). The presence of these antibodies in Britain and France now seems explicable given the isolation of a non-pathogenic RCV from domestic rabbits (Capucci *et al.* 1996).

Following the introduction of RHD into Australia and New Zealand, further reports have appeared on the effectiveness and behaviour of the virus during its initial spread (Mutze *et al.* 1998*a*; Bowen and Read 1998; Saunders *et al.* 1999; Parkes *et al.* 1999; Cooke *et al.* 2000). By far the majority of them indicate significant declines in rabbit numbers, especially in arid and semi-arid regions. In the Flinders Ranges, at Gum Creek, a population decline of over 90% was recorded in the first epidemic in 1995. Naturally occurring outbreaks of RHD have occurred each year between 1995 and 2000 (Mutze *et al.* 1998*a*; Cooke *et al.* 2000), and populations have remained below 15% of pre-RHD levels. A similar picture was a seen at all arid or semi-arid land sites. For example, at Erldunda in the Northern Territory, Muncoonie Lakes in western Queensland, and the Hattah-Kulkyne National Park in north-western Victoria, rabbit numbers have remained very low since the arrival of the virus in early 1996 (Cooke 1999). By contrast, in the Central Tablelands of New South Wales, where average annual rainfall is in excess of 600 mm, the rabbit populations at Euchareena and Lake Burrendong declined by 68 and 87% respectively, but the rabbits near Bathurst increased substantially despite confirmation of the presence of naturally infected rabbits in December 1996 (Saunders *et al.* 1999). In high-rainfall sites in western Sydney, New South Wales, RHD had little impact on rabbit numbers despite several attempts to introduce the disease (Richardson 2001). Introductions of RHDV into Tasmania have also met with little observable effect, although occasional epizootics have been seen (Neave 1999).

Little doubt exists that the initial impact of RHD in Australia decreased as it spread from the arid zone into wetter regions. There also appear to be significant differences in the consequences of RHD once it became established. Along the Coorong lagoon in south-eastern South Australia, rabbit abundance was initially reduced by 73% but is slowly recovering, whereas at Whetstone in south-eastern Queensland the rabbit population was at first reduced by only 53% but has since declined more substantially. At some sites, such as South Stirling in south-west Western Australia, rabbit abundance declined by 70% as RHD first spread. But the disease only breaks out at irregular intervals, allowing rabbits to regain numbers between outbreaks. Such data provide strong evidence that environmental factors have a major influence on the epidemiology and impact of RHD in Australia (Neave 1999).

In New Zealand, the initial epizootics of RHD were mostly initiated by deliberate release. Where they were carefully monitored, these seem to have been generally successful (Sanson *et al.* 2000). Rabbit populations were reduced by about 50% within 6 weeks of release, although results were variable (range 0–77%). In all, 73% of farmers were satisfied with the results as a means of reducing rabbit numbers, and most considered that the virus spread from release sites to areas where it had not been released, although only 27% reported dead rabbits more than 300 m beyond the release point.

In the South Island the effectiveness of deliberately released RHDV was compared with that of a natural epizootic in the dry Central Otago region of the South Island (Parkes *et al.* 1999; Norbury *et al.* 2002). The numbers of rabbits seen on spotlight counts declined by 72% in the area where the virus was spread on baits and 87% on the area of natural spread. On the site where RHDV was spread as a 'biocide', the death rate peaked 3 days after the virus was spread and few new cases of RHD were seen after 40 days. In the natural epizootic the peak occurred 20 days after the disease was first detected and new cases were found for up to 80 days. The disease spread with the prevailing wind at about 200 m per day (6 km per month).

Factors influencing the survival of RHDV

Environmental temperature is important in considering the epidemiology of RHD. Fenner and Fantini (1999) reviewed available data and showed that purified virus survived 4–5 weeks at 22°C but only 3–7 days at 37°C and for only 15 minutes at 56°C. Some virus survived for 105 days in a dried form at 20°C (Rodak *et al.* 1991). Villafuerte *et al.* (1994) and Kovaliski (1998) recorded that the rate of spread of RHD in natural rabbit populations slowed in the hotter summer months. However, this is not related to direct temperature effects on the pathogenesis of RHD in rabbits (Cooke and Berman 2000), as is the case for myxomatosis (Marshall 1959).

Rabbit haemorrhagic disease virus can survive for 3 weeks in rabbit carcasses (Westbury 1996). Nevertheless, the persistence of the virus in rabbit remains or in insect vectors would both be influenced by ambient temperature. Summer air temperatures in the rangeland of northern South Australia commonly reach 40°C, and soil surface temperatures can be 60°C or more for up to 6 h of each day. Even in rabbit

burrows, mid-summer ambient temperatures may exceed 32°C (Cooke 1990).

At the high temperatures encountered in summer, insects are forced to shelter in humid areas during the day, and their behaviour becomes crepuscular (Norris 1966). This effect of temperature on vector behaviour added to the problem of virus survival in hot environments may explain the quiescence of RHD during hot, dry periods. Nevertheless, flies carrying RHDV have been detected in arid regions in Australia during mid-summer (Fenner and Fantini 1999), so it is not possible to dismiss entirely the idea that RHDV may persist by circulating at almost undetectable levels during the hottest months.

In an assessment of 245 sites in New South Wales, Lugton (1999) found that successful releases of RHDV into rabbit populations were associated with relatively low ambient temperatures, high flea infestations and moderate rainfall during the month of release. He also noted that for each week following the cessation of rabbit breeding there was a 7% decline in the odds of an outbreak occurring.

Neave (1999) collated data from sites where changes in the abundance of rabbits were recorded as RHDV spread. Some of these sites were in areas where the virus spread naturally, while in others it had been deliberately released by inoculating captured rabbits. Neave's initial analyses confirmed that although RHD caused high mortality in arid areas of inland Australia, its efficacy in reducing rabbit populations declined as it spread into cooler areas of higher rainfall in eastern Australia. These data have since been analysed in greater detail, again using principal components analyses (Henzell et al. 2001, 2002) and it was concluded that (1) RHD outbreaks are generally less effective at low densities of susceptible rabbits than at high densities; (ii) RHD appears to have relatively low effect in areas of aseasonal or summer rain during summer, although it still caused high mortality in other seasons; (3) RHD causes high mortality in areas of winter rainfall and hot, dry areas irrespective of the season of its arrival, although the number of outbreaks was reduced in summer; and (4) RHD was relatively ineffective in cool, wet areas. These conclusions are mostly consistent with those of other studies reporting climatic correlates with disease effectiveness. Nevertheless, some aspects of epidemiology remain difficult to reconcile. In dry regions, RHD did not break out commonly in summer, yet when it did it caused very high mortality. This may simply mean that the chance of spread was low but the passage of a cool front or heavy rainfall, which stimulated vector activity, altered the rate of spread.

Epidemiology since establishment

Once RHDV became widely established and had spread through most populations of largely susceptible rabbits, changes were forced upon its epidemiology by several factors. Not only were most surviving rabbits immune, but

also further outbreaks were dependent on the subsequent breeding by rabbits and the susceptibility of those young to infection. Breeding in wild rabbits does not occur continuously, but is associated with periods of pasture growth, which occur mainly from autumn to spring across most of southern Australia. Consequently, susceptible recruits reach high numbers in the rabbit population in the spring months (Gilbert et al. 1987). There are still further complications to this picture because very young rabbits have natural resistance to RHD and may be further protected by antibodies of maternal origin from immune mothers. It is the understanding of the timing of births and susceptibility to infection that provide the keys to following the patterns of disease outbreaks.

Response of immature rabbits to infection

European scientists (Morisse et al. 1991; Rodak et al. 1991) reported that even in the absence of maternal antibodies, domestic rabbits younger than 4 weeks did not develop clinical signs or pathological lesions. Nevertheless, there was replication of the virus following infection and the young rabbits developed lifelong immunity. Furthermore, when these rabbit kittens were experimentally infected by intramuscular injection, they excreted enough virus particles to infect sentinel adult rabbits housed in adjacent cages.

As they age, these rabbits become more susceptible (Morrisse et al. 1991). Lenghaus et al. (1994) confirmed this by inoculating laboratory-bred Australian wild rabbits, finding that although all rabbit kittens (less than 10 days old) survived, only six of thirteen 5-week-old and two of eighteen 7- to 9-week-old rabbits inoculated with RHDV survived.

Cooke et al. (2000) considered that the young rabbits' low susceptibility and low mortality following infection with RHDV might be associated with the continuing development and maturation of their immune system. Indeed, it has been argued recently that young rabbits do not become readily infected with RHD because receptors that enable the virus to attach to cells of the intestinal mucosa are not fully developed. Ruvoen-Clouet et al. (2000) found that RHDV binds to antigens of the ABH histo-blood group family. Such antigens occur on human erythrocytes, hence the use of human blood cells in HA and HI tests for detecting the RHDV antigens and antibodies. However, in rabbits such antigens are confined to the mucosa of the upper respiratory tract and intestine and do not become fully functional until rabbits reach about 6 weeks of age.

Despite this explanation of initial resistance to infection, age-specific protection is still seen when the ABH tissue antigen receptors are by-passed, that is, in intramuscularly inoculated young rabbits (Robinson et al. 2002a). Clearly, the ABH antigens form only part of the pathway for infection and there must be at least one further step to enable entry of RHDV into liver cells before it can cause severe disease.

Maternal antibodies

Rabbits that survive RHD initially have high levels of antibody to the virus, although these decline with time. Nevertheless, although such rabbits are unlikely to die if infected again, their antibody titres may be boosted and maintained at high levels by subsequent re-exposure to the virus (Cooke *et al.* 2000). This is important epidemiologically because antibodies can pass across the placenta from the immune rabbit to its progeny, protecting them for a few weeks beyond the waning of their age-specific physiological resistance. Observations by Cooke *et al.* (2000) at Gum Creek, in South Australia, suggested that maternal antibodies persist in young born to immune does for 5–11 weeks. This was subsequently confirmed experimentally by Robinson *et al.* (2002*a*), who demonstrated separate effects of age and the mother's antibody titre on the survival of young rabbits experimentally infected with RHDV. Young rabbits are protected by age-specific resistance until they are 5–6 weeks old but may be further protected against the lethal effects of RHD by maternal antibodies until 13 weeks of age. As with other viral infections, field data support the idea that maternal antibody does not necessarily protect against infection but may assist the young rabbit to recover from RHD and become immune.

Basic epidemiological pattern

Barlow *et al.* (2002) developed a simple mathematical disease–host model, including indirect transmission and juvenile resistance parameters derived from European and Australian data, and found that it generally fitted with New Zealand data collected over the first 3 years following the arrival of RHD. In the model simulations, juvenile recruitment aided population recovery over summer following the first outbreak, and the model predicted a second epizootic in the following winter. However, in the field sites used to validate the model, the second outbreak did not occur until the following spring, suggesting that some model parameters were not properly tuned or that there were additional factors influencing the epidemiology that were not accounted for in the model.

Typically, it might be expected that RHD would begin in spring during the rabbit's breeding season once some young rabbits had lost their maternal antibodies and become fully susceptible. Indeed, this does occur at a low level but it is not until most of the young rabbits have lost their antibodies that the disease becomes widespread. In cooler areas, and areas where rainfall in summer is frequent, outbreaks of RHD in late spring and summer are common. For example, in North Canterbury, New Zealand, RHD occurs between mid-November and mid-December (Reddiex *et al.* 2002) and in Bacchus Marsh, Victoria, epizootics of RHD are often seen in summer (March). However, in hotter inland areas, such as Gum Creek in South Australia, epizootics are apparently delayed by the onset of summer weather and generally begin in late autumn or winter (May–July) when rabbits begin breeding.

Both at Bacchus Marsh and at Gum Creek the mortality caused by RHD among susceptible subadult rabbits is high, and data on the survival of individually tagged rabbits show that few rabbits from each annual cohort survive to contribute to the breeding population in the following year. At Gum Creek, the mortality caused by RHD has been sufficiently high to slowly drive the breeding population of rabbits to levels even lower than those seen immediately after the initial spread of RHDV. However, at Bacchus Marsh, enough young survive to maintain the breeding population only a little below its previous level.

The most comprehensive epidemiological study of RHD in Europe was carried out by Calvete and colleagues (Calvete *et al.* 1995; Calvete and Estrada 2000), who followed epizootics of RHD in a population of wild rabbits near Zaragosa in the semi-arid Ebro Valley in northern Spain. Many of the epidemiological patterns, including a propensity for winter outbreaks, match those seen in semi-arid Australia and those reported from the warm, dry areas of south-eastern Spain (Peiró and Seva 1991).

On the basis of observations made on wild rabbit populations, Calvete and Estrada (2000) argue that resistance to RHD in kittens (age-related or acquired from the mother) determines the pattern of disease outbreaks, and that population density and the consequent level of contact between rabbits determines the impact of the disease. They argued that, as the rate of contact between rabbits increases, mortality from RHD grows because the virus affects a greater number of rabbits. Nevertheless, the increasing rate of spread brings about a reduction in the median age of infection towards the age at which young rabbits are still resistant. Ultimately a point is reached where many kittens are infected while they still have age-specific or maternal antibody protection. This means that many survive and the effect of RHD on the population as a whole is reduced because sufficient numbers of young rabbits survive to maintain the basic breeding population.

At present it remains impossible to say whether climate or rabbit density or both determine the efficacy of RHD as a biological control agent. Certainly in cooler sites like North Canterbury, RHD epizootics can occur in late spring while very young rabbits are still present (Reddiex *et al.* 2002), which may result in a proportion of these being infected while young enough to retain some protection. The chance of late-born young being immunised in this way would obviously decline in areas where epizootics are apparently delayed by warmer weather. Nevertheless, in Australia at least, both rabbit density and production of young are often at maximum levels in areas of high rainfall. As a consequence, the effects of climatic factors are not easily disentangled from those of rabbit density.

While the role of rabbit density in the epidemiology of RHD is still being explored, other options to explain variable effects of RHD are worth consideration. One of the most intriguing is the possibility that there may be other RHDV-like caliciviruses present in the Australian and New Zealand rabbit populations and that antibodies to such viruses may partly protect wild rabbits against the effects of RHDV.

Putative RHDV-like viruses

Evidence of pre-existing RHDV-like viruses in Australia and New Zealand comes from several independent sources. Nagesha *et al.* (1995, 2000) detected cross-reactive antibodies to rabbit haemorrhagic disease virus in rabbits near Bendigo, Victoria, before RHD reached the area. They subsequently demonstrated that rabbits with high levels of these antibodies were less likely to die than seronegative rabbits when challenged with RHDV. Robinson *et al.* (2002*b*) used archival samples to confirm that cross-reactive antibodies were common in New South Wales well before RHDV was brought to Australia. As these reacted with a suite of monoclonal antibodies raised against RHDV, and even some raised against RCV and EBHSV, it was argued that the antibodies must have been raised against a virus related to other lagoviruses. Cooke *et al.* (2000) looked at profiles of antibody isotypes (cELISA, IgG, IgA, IgM) in wild rabbit sera and found that some were exactly as expected from RHDV infection while other aberrant patterns were arguably antibodies to an RHDV-like virus. While this evidence remains circumstantial, longitudinal samples from individual rabbits at Bacchus Marsh, Victoria, showed that some young rabbits lost maternal antibodies, became seronegative, then developed antibodies to the putative RHDV-like virus (i.e. high IgG and a trace of cELISA reactivity but no IgA or IgM). Later, these same rabbits developed typical RHD antibodies (i.e. cELISA reactivity as well as IgG, IgA and in some cases IgM). The simplest interpretation of the data is that these rabbits were infected with the RHDV-like virus before contracting RHD (Cooke *et al.* 2002).

McPhee *et al.* (2002) experimentally challenged wild-caught rabbits with RHDV and found that some, although appearing seronegative by cELISA, did not die. Detailed retrospective testing of their sera showed that many actually had antibodies (IgG) against the putative RHDV-like virus (Cooke *et al.* 2002). However, grouping rabbits according to the titre of these antibodies failed to show conclusively that mortality was reduced among rabbits with the highest titres. Nevertheless, despite the lack of conclusive experimental results it is clear that, unlike rabbits with 'pre-existing' antibodies in Europe that are fully protected against challenge with RHDV (Trout 1999), Australian rabbits with antibodies to the putative RHDV-like virus are not fully protected. This suggests that the putative RHDV-like virus in Australia might be more distant from RHDV than the non-pathogenic viruses in Europe.

Taking these ideas further, Cooke *et al.* (2002) measured antibodies to the putative RHDV-like virus in rabbits shot in different localities across south-eastern Australia at the time that RHDV was first spreading. They found that, where they could be distinguished, antibodies to the RHDV-like virus were generally at higher titres in cool, wet areas of Australia than in inland Australia. This generally fits with the poor initial spread and reduced effectiveness of RHD in cool, wet areas and is consistent with the idea that an RHDV-like virus might be a factor reducing the effectiveness of RHD in the more humid parts of Australia (Neave 1999; Henzell *et al.* 2002).

White *et al.* (2001) develop a similar argument to explain the inability of RHD to spread throughout Britain. Even before RHDV arrived in Britain, many rabbits carried antibodies that reacted in HI tests for RHDV, and subsequent outbreaks of RHD in Britain have been most common and caused the highest mortalities in southern England where the prevalence of the pre-existing antibodies was relatively low. White *et al.* (2001) used modelling to explore how RHDV would interact with an endemic non-pathogenic strain, assuming that each virus had different characteristics enabling spread and persistence. They proposed that the non-pathogenic virus did not replicate strongly but was probably persistent in rabbits, whereas the virulent virus gained benefits from replicating strongly and spreading easily despite killing rabbits quickly.

Despite the serological evidence that a second calicivirus is circulating in Australian rabbit populations, it is clearly necessary to isolate and characterise the putative RHDV-like virus to establish its true identity before further conclusions can be drawn.

RHD and myxomatosis

Some initial concern was expressed as to whether there would be any negative interaction between myxomatosis and RHD. Potentially, this might reduce the full benefits of both agents. These interactions might occur at the level of the individual animal, if rabbits already infected with myxoma virus had an elevated chance of surviving RHD. Or, more likely, the agents might interact at a population level, where reduction in rabbit abundance following outbreaks of RHD influenced the frequency or timing of myxomatosis outbreaks.

No reported evidence exists that myxoma virus or RHDV react in any way within individual rabbits, and so we must assume that the viruses work relatively independently. Nevertheless, there some evidence that interaction between the two diseases occurs at a population level. Mutze *et al.* (2002) suggest that since RHD there have been changes in the patterns of recruitment of young rabbits into the population at two South Australian sites, with fewer

early-born young surviving into the summer and more late-born young being present. This means that rabbit populations are at their lowest ebb in early winter yet relatively high in summer. Associated with this demographic change, the timing of myxomatosis outbreaks has shifted somewhat from late spring to summer.

Ecological consequences of RHD

It is still too early to draw general conclusions about the long-term effects of RHD on rabbit numbers and the consequent effects on native flora and fauna on a national scale. Bomford *et al.* (1998) reported on the overall picture in May 1988, about 2.5 years after the escape of RHDV. At that time there had been significant regeneration of native shrubs in the Flinders Ranges in South Australia where regular outbreaks had occurred. Sandell (2002) also demonstrated that in north-western Victoria, rabbits were no longer inhibiting pasture regeneration. On the basis of such observations it seems that the greatest immediate benefits from RHD are apparent in inland Australia where rabbit populations have been reduced substantially.

Nevertheless, Mutze *et al.* (2002) show that the pattern of mortality caused by autumn–spring activity of RHD has caused significant changes to the annual pattern of rabbit abundance. Instead of the expected rise in rabbit numbers through winter and spring, the numbers seen are often at their lowest ebb in spring but increase moderately during the summer months. Comparing numbers seen on spotlight transects at Gum Creek before and after RHD was introduced, there are now 95% fewer rabbits seen in spring but only 60% fewer in summer. This may mean that rabbit grazing on winter growing annual vegetation will be strongly reduced but rabbit grazing on perennial seedlings over summer may not be so strongly curtailed. Despite RHD, some damage to perennial seedlings in summer should still be expected. In view of this conjecture, Lord (2002) has recently shown that even at low post-RHD densities rabbits are still capable of reducing regeneration of the purple-wood wattle (*Acacia carnei*) in a semi-arid part of New South Wales.

Earlier, there had been concern that, with the decline in rabbits, cats and foxes might begin killing more native animals (Newsome *et al.* 1997), but there is no evidence of a significant relationship between declines in rabbit numbers and changes in small mammal and reptile numbers.

Edwards *et al.* (2002*a*, 2002*b*) report that there were no significant changes in small mammal populations in central Australia following the spread of RHD. Even the combination of RHD and rabbit warren ripping failed to make significant differences to the numbers of small mammals.

Bowen and Read (1998) showed that when RHD spread through inland Australia it reduced rabbit abundance at Roxby Downs to about 3% of former levels. These authors

(Read and Bowen 2001) have since shown that, when rabbit numbers fell, the number of cats recorded on transects fell by approximately 70%, and these predators have since remained low. Foxes, which had previously been abundant, were rarely seen.

Dietary studies at Roxby Downs showed that when the rabbit population fell the percentage of vertebrates other than rabbit in the cats' diet increased from about 50 to 80%; the mean number of non-rabbit vertebrate prey found in cat stomachs increased from an average of 1.3 to 2.7 items. It is apparent from these data that even though individual cats roughly doubled the number of native prey (lizards, small marsupials and birds) they ate, this was offset by the fact that less than half the original cats were present. In short, major benefits to vegetation from reduced rabbit grazing were achieved while the total level of predation by cats on native fauna remained roughly the same or more likely declined overall. Foxes were not able to maintain themselves when rabbits were few, and fox predation on native fauna was markedly reduced as a consequence.

It seems clear that at Roxby Downs, cats can maintain themselves even when the density of rabbits is too low to support foxes. For foxes, the greater difficulty in obtaining rabbits as prey is quickly reflected in changes in diet. Holden and Mutze (2002) found that in the Flinders Ranges, where RHD did not reduce rabbit abundance as markedly as at Roxby Downs, foxes nevertheless ate more invertebrates and carrion to replace rabbit. By contrast, cats did not change their diet so readily. A small decline in rabbit in the diet was to some extent offset by increased predation on house mice (*Mus musculus*) rather than increased consumption of invertebrates. Interestingly, although the numbers of both cats and foxes declined, Holden and Mutze (2002) considered that foxes maintained good body condition whereas cats were generally found to be in poorer body condition than before RHD. Such observations support the idea that foxes are opportunists and probably leave areas where food is scarce, whereas cats are more conservative, specialist hunters of vertebrates.

Observations on predator populations and their response to RHD have given some new and interesting insights into the role of rabbits in supporting predators. Nevertheless, cats and foxes have not declined in all situations. Edwards *et al.* (2002*a*, 2002*b*) note that no changes could be detected in the numbers of either species as RHD became established in central Australia. However, in areas where rabbit warrens were ripped, there were significantly fewer signs of both cats and foxes.

Economic benefits from RHD

Increases in livestock production

In the Flinders Ranges, South Australia, the effects of rabbit warren ripping on sheep grazing capacity was assessed some years before RHD spread through Australia (Mutze *et al.*

1998*b*). It was shown that sheep spent 42% more time grazing in areas where rabbits had been removed than in unripped areas where rabbits remained. These observations generally paralleled the doubling in sheep numbers seen on nearby Arkaba station, where rabbits had been eliminated by warren ripping some years earlier (Hunt and Rasheed 1991). In that case the sheep carrying capacity of the station was quickly restored to a level close to the district average, and vegetation cover improved even in previously eroded areas.

When RHD spread, rabbit numbers on unripped areas fell by at least 85% and have since remained at a low level. Once again, sheep responded to the lowering of rabbit abundance. On Gum Creek station, sheep moved from the stony hills and spent more time grazing in the more fertile valley floors where rabbits had been most abundant. They doubled their use of these areas as judged by the extra sheep dung deposited (Saunders *et al.* 2002). The data indicate that over the last 5 years, with rabbits held low, RHD has had an effect approaching that previously achieved by warren ripping.

Limits to the number of stock that can be grazed on leasehold properties mean that it is not always possible to increase sheep numbers to regain rabbit control costs or to gain immediate benefit from RHD. Nevertheless, there are some options in terms of changing flock structure to increase gains (e.g. more wool per sheep, extra lamb production). Furthermore, the numbers of perennial plants on vegetation transects increased significantly after rabbit warren ripping, and similar increases have been seen on unripped sites since RHD spread (Mutze *et al.* 1998*b*; Saunders and Kay 1999). One of the primary benefits from RHD for properties that have been severely overgrazed in the past should be the re-establishment of perennial vegetation to provide more-stable productivity during drought. RHD clearly provides a step in that direction.

It is also important to note that benefits to the wool industry are not confined to arid lands. Saunders and Kay (1999) estimated the effect of RHD on the value of pastoral production at Euchareena in the Central Tablelands of New South Wales. Even assuming a high rate of compensatory pasture growth offsetting the effects of rabbit grazing (0.7), they calculated that RHD should have enabled extra wool production worth \$7.42 ha^{-1} on a site that had previously been heavily infested with rabbits.

In high-rainfall areas, the benefits of RHD can further be seen in terms of reducing farm production costs. Saunders and Kay (1999) compared the quantity of 1080 rabbit baits used in New South Wales after RHD with the amount used in 4 climatically similar years before its introduction. Total 1080 carrot baits used in 1987–90 amounted to 2388 tonnes but only 412 tonnes in 1996–99 following the spread of RHD. They conservatively calculated that the direct savings in the cost of rabbit poisoning for landholders in New South Wales amounted to \$1.2 million each year.

A survey of Rural Lands Protection Boards in New South Wales (Saunders and Kay 1999) showed that 58% had reductions in the use of 1080 poisoning as a direct result of RHD. Reduced warren ripping was reported in 29% of Boards and 27% reported that they had reduced other rabbit control methods such as fumigation. However, 16% of boards reported increased ripping, mainly to capitalise on the opportunity presented by RHD (see above).

In South Australia, the use of 1080 oat baits fell from an annual average of 82 tonnes (1987–96) to about 26 tonnes (1997–99). It was estimated that primary producers across the state saved about \$560 000 annually in the costs of rabbit poisoning. Figures were also broken down according to agricultural regions. These showed that use of 1080 poison fell by more than 70% in the Murray Mallee and on Eyre Peninsula but only 47% in the south-east of the state. This is consistent with the fact that RHD had less impact in cool, high-rainfall areas. Not only did the number of landholders using poisoned oats decline by 49%, but also those still applying baits used less.

The future of RHDV as a biological control agent

It is too early to predict how long RHDV will remain a useful adjunct for rabbit control in Australia and New Zealand. It has achieved excellent results over large areas, especially in hot, arid parts of Australia, but poor results in other areas. As among wild rabbits in Spain, it appears to have become enzootic in most parts of Australia, recurring naturally in successive years.

Over the long term, its future depends on whether it retains its high virulence, and whether rabbits become genetically resistant. Although detailed investigations have not been conducted in Europe, even a decade after its appearance there are still no reports of either virus attenuation or increasing genetic resistance among wild rabbits. The physiological resistance of immature rabbits complicates forecasts of the future evolution of RHD. If an epidemic occurs when there are many rabbit kittens dependent on their mothers, this factor is of little importance, because unless they were survivors of an earlier epidemic, the mothers would die of RHD and the kittens would die from neglect. Physiological resistance would diminish the impact of RHD if there were many young rabbits that were independent of their mothers, since a proportion of such animals would survive and be resistant in future outbreaks. However, there would not be any elements of genetic resistance in such animals or their offspring; any resistance would be physiological rather than genetic, associated with their age, or due to maternal antibody.

As with myxoma virus, whether the virus retains its high virulence depends primarily on what governs its transmissibility. If transmission, and especially the spread of virus between warrens and between districts, depends primarily on the airborne movement of insect vectors, and

such insects become contaminated by feeding on exposed internal organs of fatal cases, there could be a selective pressure for sustained virulence. However, if animals that recover owing to physiological (age) resistance excrete virus for substantial periods, and if such virus is important for transmission, there would be no selection for high virulence, nor would there be any selection for viruses of reduced virulence.

Acknowledgments

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