Substantial numerical decline in South Australian rabbit populations following the detection of rabbit haemorrhagic disease virus 2

Greg Mutze,[1] Nicki De Preu,[2] Trish Mooney,[3] Dylan Koerner,[3] Darren McKenzie,[3] Ron Sinclair,[1] John Kovaliskli,[1] David Peacock[1]

Lagovirus europaeus GI.2, also commonly known as rabbit haemorrhagic disease virus 2, was first detected at two long-term monitoring sites for European rabbits, *Oryctolagus cuniculus*, in South Australia, in mid-2016. Numbers of rabbits in the following 12–18 months were reduced to approximately 20 per cent of average numbers in the preceding 10 years. The impact recorded at the two South Australian sites, if widespread in Australia and persistent for several years, is likely to be of enormous economic and environmental benefit.

Introduction

Lagovirus europaeus GI.2,[1] also commonly known as rabbit haemorrhagic disease virus 2 (RHDV2), is a calicivirus belonging to the Lagoviridae that causes fatal disease in wild and domestic European rabbits, *Oryctolagus cuniculus*,[2] and is also known to infect several species of *Lepus*.[3–7] First detected in France in 2010,[2] RHDV2 spread rapidly through southern Europe and by 2014 had largely replaced earlier RHDV strains belonging to the GI.1 genogroups in France[8] and Spain.[9 10] It has become widespread throughout Europe[11–14] and has been detected in domestic rabbits in countries including Canada,[15] Tunisia[16] and Benin.[17]

Initial reports from domestic rabbit farms indicated that RHDV2 caused higher mortality in juveniles than earlier RHDV strains and killed some RHDV-vaccinated domestic adults.[2 18 19] However, initial laboratory challenge studies in susceptible adult rabbits found RHDV2 caused much lower and more variable mortality rates and a longer time to death than were known from RHDV.[8 20] Nevertheless, substantial declines were reported in wild rabbit numbers in their native range as the virus spread through Spain and Portugal[21 22] where some virus isolates from those areas were found to be recombinants with pre-existing RHDVs and non-pathogenic rabbit caliciviruses.[23 24] Mortality rate of RHDV2 cases in Italy also appeared to increase over time and currently circulating strains in Italy have been shown to be highly pathogenic in laboratory challenge studies.[25]

RHDV (Czech strain 351 belonging to the GI.1c genogroup) was introduced to Australia in 1995/1996 as a biological control for pest populations of wild rabbits and caused reductions of up to 95 per cent during its initial spread.[26] Lesser reductions occurred in humid coastal areas and cool, moist areas in south-eastern and south-western Australia[27] and that difference has since been shown to be attributable, at least in part, to the presence in these areas of a non-pathogenic calicivirus, RCV-A1, that provides partial cross-immunity against subsequent RHDV infection.[28] Partial recovery of rabbit numbers began in many areas about eight years after the initial spread of RHDV.[29]

RHDV2 was detected in Australia at Canberra in May 2015[30]; the source of the incursion remains unknown but the virus detected was genetically similar to a Portuguese recombinant RHDV2. The virus spread rapidly in all directions and was detected at several sites up to 500 km from Canberra within six months, reaching South Australia in December 2015.[31] Increased surveillance was then initiated at long-term monitoring sites in South Australia at Turretfield and Ikara-Flinders Ranges National Park (IFRNP), both approximately 1000 km

Veterinary Record (2018) doi: 10.1136/vr.104734

[1]Biosecurity SA, Department of Primary Industries and Regions South Australia, Adelaide, South Australia, Australia
[2]Ardeotis Biological Consultants, Watervale, South Australia, Australia
[3]Department of Environment, Water and Natural Resources, Port Augusta, South Australia, Australia

E-mail for correspondence: greg.mutze@sa.gov.au

Provenance and peer review Not commissioned; externally peer reviewed.

Received October 5, 2017
Revised January 23, 2018
Accepted February 20, 2018

from Canberra, in order to detect the arrival and impact of RHDV2.

Methods

At Turretfield, approximately 50 km north of Adelaide, South Australia, a mark-recapture trapping study of RHDV and myxomatosis epidemiology had operated at 6–10 week intervals since 1996 in a small patch of remnant native woodland surrounded by highly productive agricultural land with a Mediterranean climate (details in refs [29 32]). IFRNP is located approximately 370 km north of Adelaide, South Australia, in the hot arid rangelands where rainfall and plant production are low and highly variable, and high rabbit population fluctuations reflect that variability. Rabbit population monitoring using vehicle-based spotlight transect counts began at IFRNP in February 1992 and the current 23.7 km transects had been counted quarterly in most years since 2002,[29] but with no counts in 2015 and one or two missing counts in three other recent years.

RHDV2 was identified from liver or bone marrow of carcases collected at monitoring sites using PCR and genetic sequencing (details in ref [33]).

Changes in rabbit spotlight counts at IFRNP following the arrival of RHDV2 were analysed with a generalised linear model using Poisson errors and log-link (Statistica V.9, TIBCO Software). Seasonal means (winter, June to August; spring, September to November; summer, December to February; autumn, March to May) were used as data on the few occasions where more than one count was conducted within a season, and season was fitted as a factor with four levels to minimise the influence of missing values.

Direct comparison of spotlight counts and trapping data is complicated by the fact that trapping studies record many juvenile rabbits that suffer high juvenile mortality rates, stay close to warrens and are not detected in spotlight counts until about three months of age. To facilitate comparison with IFRNP, we calculated the number of rabbits above 90 days old (approximately 900 g) known to be alive at Turretfield and analysed the data with a similar model.

Results

RHDV2 was detected at several sites within 15 km of Turretfield in February, March and April 2016, then at Turretfield on May 4, 2016.[33] A myxomatosis epizootic had passed through the population in March and April but none of the four carcases collected at Turretfield during that period contained RHDV or RHDV2. Nevertheless, trapping data from April 2016 were excluded from these analyses because RHDV2 may have been present but undetected. A second epizootic of RHDV2 began at Turretfield in June 2017. Since May 2016, 3/10 carcases recovered in 2016 and 12/14 carcases in 2017 were PCR positive to RHDV2. Seven carcases found in spring 2016 and one in the following summer showed evidence of clinical myxomatosis, and clinical signs of myxomatosis were observed in live rabbits during spring 2017.

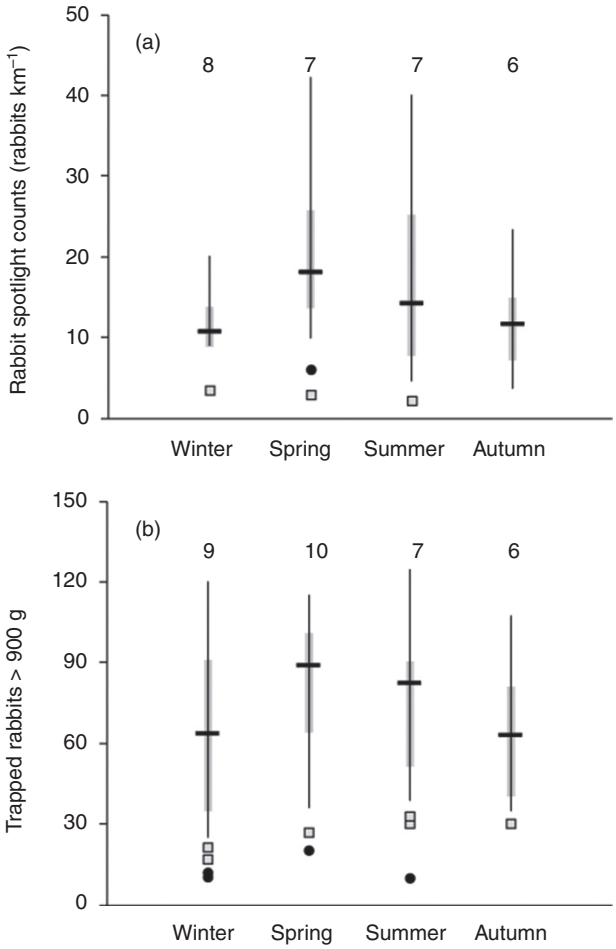

FIG 1: Changes in seasonal abundance of rabbits following the arrival of rabbit haemorrhagic disease virus 2 (RHDV2) at (a) Ikara-Flinders Ranges National Park and (b) Turretfield Research Centre. Data shown as median, 25th–75th percentiles and range for the 10 years before RHDV2 (shaded bar, column and vertical line, respectively, with sample size above), the sampling periods during the first year after RHDV2 was first detected at each site (shaded squares), and during the second year after RHDV2 was first detected (filled circles).

RHDV2 was detected at IFRNP from a single carcase in June 2016. The timing of its arrival is less certain due to the remoteness of the location and irregular monitoring, but RHDV2 had not been detected within 200 km of IFRNP before April 2016.[31]

In the year after the arrival of RHDV2 at IFRNP, all rabbit counts (September 2016 to July 2017) were lower than every individual count during the previous 10 years (July 2006 to November 2014) (Fig 1a). Mean counts were reduced to 2.8 rabbits/km, 18.1 per cent of mean counts during the previous 10 years, 15.5 rabbits/km (Wald x^2_1=27.3, P<0.001) (Fig 1a). During that 10-year period, rabbit numbers had recovered from low post-RHDV levels, peaked in 2010 following a year of extreme rainfall and pasture growth,[29] and then fluctuated in response to variable seasonal conditions between the post-RHDV extreme low and high levels (Fig 2), although mean rabbit counts for 2012 and 2013 (Fig 2a) were probably underestimated because no counts were

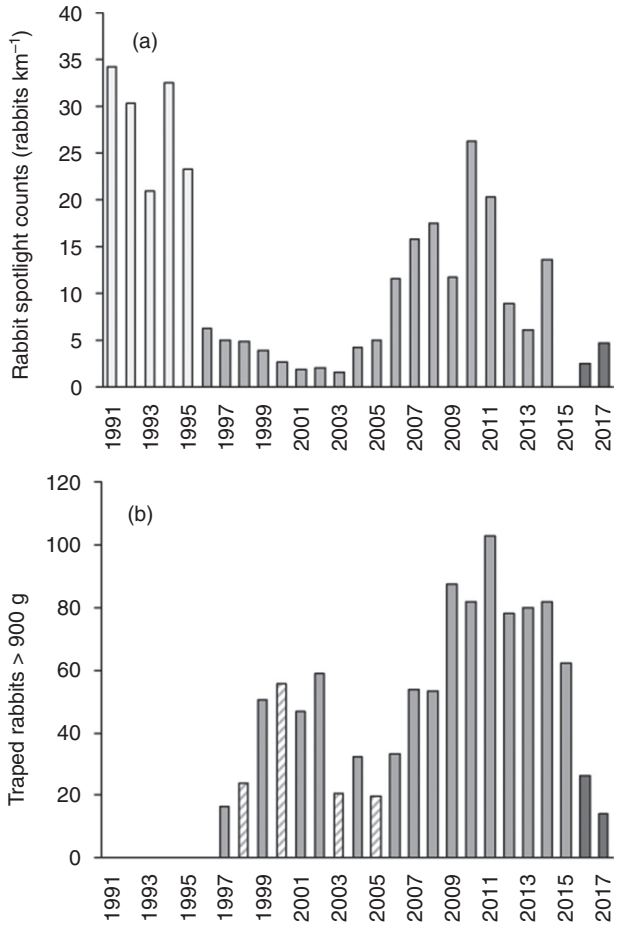

FIG 2: Changes in annual abundance of rabbits following the arrival of rabbit haemorrhagic disease virus 2 (RHDV2) at (a) Ikara-Flinders Ranges National Park and (b) Turretfield Research Centre. Data shown as mean values for the 12-month period beginning in June each year: light grey shading, pre-RHDV; mid-grey, after arrival of GI.1 RHDV but before GI.2 RHDV2; dark grey, post-RHDV2. Diagonal patterning indicates years when no RHDV outbreak occurred at the Turretfield site. No data are available for rabbit numbers at Turretfield before RHDV arrived.

made during spring, when rabbit numbers are typically highest (Fig 1a). Mean counts during the 10-year period were 51 per cent of those recorded at the site in the four years before the arrival of RHDV in 1995, 30.2 rabbits/km (Fig 2a).[29]

In the year after the arrival of RHDV2 at Turretfield (June 2016 to April 2017), the rabbit population in each season was lower than in the corresponding seasons during each of the previous 10 years (June 2006 to February 2016) (Fig 1b). The mean number of rabbits greater than 900 g body mass was reduced to 26, 36 per cent of mean numbers during the previous 10 years, 72 rabbits (Wald x^2_1=155, P<0.001) (Fig 1b). During that 10-year period, rabbit numbers had recovered from low post-RHDV levels, then fluctuated for five or six years around levels close to the post-RHDV peak (Fig 2b). The second RHDV2 epizootic at this site in 2017 killed a number of the older tagged rabbits which survived the 2016 RHDV2 outbreak as adults. The second epizootic continued for at least five-and-a-half weeks and became hard to detect because the population was so low. The number of surviving rabbits has yet to be confirmed so it is not possible to give a precise estimate at this stage, but numbers appear to be approximately half of those in the first year after RHDV2 was detected (Figs 1b and 2b). We have no data for rabbit numbers before RHDV arrived at Turretfield in late 1996 (Fig 2) but they are likely to have been much higher than the post-RHDV levels because ground herbage and adjoining crops were more severely damaged during 1996, before RHDV reached the site, than in any year since.

Discussion

Rabbit numbers were reduced by approximately 80 per cent following the arrival of RHDV2 at two sites where they had already been suppressed by the introduction of RHDV 20 years earlier. These reductions occurred despite above-average rainfall[34] and pasture growth at both sites that usually favour substantial rabbit population increase.

We cannot be certain that the population declines are entirely attributable to RHDV2, even though it has largely displaced earlier GI.1 RHDV variants in Australia[35] and caused high mortality in rabbits that were immune to those earlier variants.[33] Recently emerged, virulent myxoma viruses[36] appear to have contributed to the decline at Turretfield in April 2016. Synergistic effects between the two diseases may also be important if population-level susceptibility to myxomatosis increased due to RHDV2-induced mortality in old rabbits which were immune to both GI.1 RHDV and myxomatosis. Nevertheless, almost all recorded deaths in 2017 were attributable to RHDV2. We have been unable to confirm the spread of RHDV2 in the Flinders Ranges since its arrival due to infrequent visits to the area and rapid scavenging of carcases, and have no evidence relating to the possible impact of myxomatosis at the site, but myxomatosis is generally less prevalent and influential there[37] than in more mesic agricultural areas.

Genetic diversity of RHDV in Australia has also been complicated by the recent unplanned natural incursion in eastern Australia of a Chinese GI.1a variant of RHDV[38] and the Australia-wide planned release of a Korean-sourced GI.1a variant of RHDV, termed RHDV1-K5.[39] However, RHDV1-K5 was not released at Turretfield or IFRNP and has not yet been detected in South Australia outside of release sites, and the Chinese variant has not been detected at all in South Australia.[31]

These detailed long-term monitoring data provide a clear baseline for making comparisons with other Australian sites where extensive shorter term data are available. The impact recorded at the two South Australian sites, if widespread in Australia and persistent for several years, is likely to be of enormous economic and environmental benefit.[40 41]

Funding The authors have not declared a specific grant for this research from any funding agency in the public, commercial or not-for-profit sectors.

Competing interests None declared.

Patient consent Not required.

References

1 LE PENDU J, ABRANTES J, BERTAGNOLI S, *et al*. Proposal for a unified classification system and nomenclature of lagoviruses. *J Gen Virol* 2017;98:1658–66.
2 LE GALL-RECULÉ G, ZWINGELSTEIN F, BOUCHER S, *et al*. Detection of a new variant of rabbit haemorrhagic disease virus in France. *Vet Rec* 2011;168:137–8.
3 PUGGIONI G, CAVADINI P, MAESTRALE C, *et al*. The new French 2010 Rabbit Hemorrhagic Disease Virus causes an RHD-like disease in the Sardinian Cape hare (*Lepus capensis mediterraneus*). *Vet Res* 2013;44:96.
4 CAMARDA A, PUGLIESE N, CAVADINI P, *et al*. Detection of the new emerging rabbit haemorrhagic disease type 2 virus (RHDV2) in Sicily from rabbit (*Oryctolagus cuniculus*) and Italian hare (*Lepus corsicanus*). *Res Vet Sci* 2014;97:642–5.
5 HALL RN, PEACOCK DE, KOVALISKI J, *et al*. Detection of RHDV2 in European brown hares (Lepus europaeus) in Australia. *Veterinary Record* 2016 [Epub ahead of Print 19 Oct 2016].
6 VELARDE R, CAVADINI P, NEIMANIS A, *et al*. Spillover Events of Infection of Brown Hares (*Lepus europaeus*) with Rabbit Haemorrhagic Disease Type 2 Virus (RHDV2) Caused Sporadic Cases of an European Brown Hare Syndrome-Like Disease in Italy and Spain. *Transbound Emerg Dis* 2017;64:1750–61.
7 World Organisation for Animal Health (OIE). Rabbit haemorrhagic disease, Sweden - immediate notification report. Sweden: World Organisation for Animal Health (OIE), 2017.
8 LE GALL-RECULÉ G, LAVAZZA A, MARCHANDEAU S, *et al*. Emergence of a new lagovirus related to rabbit haemorrhagic disease virus. *Vet Res* 2013;44:81–13.
9 CALVETE C, SARTO P, CALVO AJ, *et al*. Letter - Could the new rabbit haemorrhagic disease virus variant (RHDVb) be fully replacing classical RHD strains in the Iberian Peninsula? *World Rabbit Science* 2014;22:91.
10 LOPES AM, CORREIA J, ABRANTES J, *et al*. Is the new variant RHDV replacing genogroup 1 in Portuguese wild rabbit populations? *Viruses* 2014;7:27–36.
11 BAILY JL, DAGLEISH MP, GRAHAM M, *et al*. RHDV variant 2 presence detected in Scotland. *Vet Rec* 2014;174:411.1–411.
12 WESTCOTT DG, FROSSARD JP, EVEREST D, *et al*. Incursion of RHDV2-like variant in Great Britain. *Vet Rec* 2014;174:333.1–333.
13 World Organisation for Animal Health (OIE). Rabbit haemorrhagic disease, Denmark - immediate notification report Ref. Denmark: World Organisation for Animal Health (OIE), 2016.
14 NEIMANIS AS, AHOLA H, ZOHARI S, *et al*. Arrival of rabbit haemorrhagic disease virus 2 to northern Europe: Emergence and outbreaks in wild and domestic rabbits (*Oryctolagus cuniculus*) in Sweden. *Transbound Emerg Dis* 2018;65.
15 World Organisation for Animal Health (OIE). Rabbit haemorrhagic disease - immediate notification report Ref OIE. Canada: World Organisation for Animal Health (OIE), 2016.
16 World Organisation for Animal Health (OIE). Rabbit haemorrhagic disease, Tunisia - immediate notification report Ref OIE. Tunisia: World Organisation for Animal Health (OIE), 2015.
17 World Organisation for Animal Health (OIE). Rabbit haemorrhagic disease, Benin - immediate notification report Ref. Benin: World Organisation for Animal Health (OIE), 2015.
18 DALTON KP, NICIEZA I, ABRANTES J, *et al*. Spread of new variant RHDV in domestic rabbits on the Iberian Peninsula. *Vet Microbiol* 2014;169:67–73.
19 MCGOWAN S, CHOUDHURY B. Update on rabbit haemorrhagic disease virus 2-like variant in Great Britain. *Vet Rec* 2016;178:662.4–3.
20 DALTON KP, NICIEZA I, BALSEIRO A, *et al*. Variant rabbit hemorrhagic disease virus in young rabbits, Spain. *Emerg Infect Dis* 2012;18:2009–12.
21 GUERRERO-CASADO J, CARPIO AJ, TORTOSA FS. Recent negative trends of wild rabbit populations in southern Spain after the arrival of the new variant of the rabbit hemorrhagic disease virus RHDV2. *Mammalian Biology - Zeitschrift für Säugetierkunde* 2016;81:361–4.
22 MONTERROSO P, GARROTE G, SERRONHA A, *et al*. Disease-mediated bottom-up regulation: An emergent virus affects a keystone prey, and alters the dynamics of trophic webs. *Sci Rep* 2016;6:36072.
23 ALMEIDA T, LOPES AM, MAGALHÃES MJ, *et al*. Tracking the evolution of the G1/RHDVb recombinant strains introduced from the Iberian Peninsula to the Azores islands, Portugal. *Infect Genet Evol* 2015;34:307–13.
24 LOPES AM, DALTON KP, MAGALHÃES MJ, *et al*. Full genomic analysis of new variant rabbit hemorrhagic disease virus revealed multiple recombination events. *J Gen Virol* 2015;96:1309–19.
25 CAPUCCI L, CAVADINI P, SCHIAVITTO M, *et al*. Increased pathogenicity in rabbit haemorrhagic disease virus type 2 (RHDV2). *Vet Rec* 2017;180:426.2–426.
26 MUTZE G, COOKE B, ALEXANDER P. The initial impact of rabbit hemorrhagic disease on European rabbit populations in South Australia. *J Wildl Dis* 1998;34:221–7.
27 HENZELL RP, CUNNINGHAM RB, NEAVE HM. Factors affecting the survival of Australian wild rabbits exposed to rabbit haemorrhagic disease. *Wildlife Research* 2002;29:523–42.
28 STRIVE T, WRIGHT J, KOVALISKI J, *et al*. The non-pathogenic Australian lagovirus RCV-A1 causes a prolonged infection and elicits partial cross-protection to rabbit haemorrhagic disease virus. *Virology* 2010;398:125–34.
29 MUTZE G, BIRD P, JENNINGS S, *et al*. Recovery of South Australian rabbit populations from the impact of rabbit haemorrhagic disease. *Wildlife Research* 2014;41:552–9.
30 HALL RN, MAHAR JE, HABOURY S, *et al*. Emerging Rabbit Hemorrhagic Disease Virus 2 (RHDVb), Australia. *Emerg Infect Dis* 2015;21:2276–8.
31 Pestsmart Connect. Rabbit Biocontrol Tracker. 2017. https://www.feralscan.org.au/rabbitscan/map.aspx?mapMode=rhdv (accessed 19 Sep 2017).
32 PEACOCK DE, SINCLAIR RG. Longevity record for a wild European rabbit (*Oryctolagus cuniculus*) from South Australia. *Australian Mammalogy* 2009;31:65–6.
33 PEACOCK D, KOVALISKI J, SINCLAIR R, *et al*. RHDV2 overcoming RHDV immunity in wild rabbits (*Oryctolagus cuniculus*) in Australia. *Vet Rec* 2017;180:280.2–280.
34 Bureau of Meteorology. Twelve-monthly rainfall anomalies for South Australia. 2017. http://www.bom.gov.au/jsp/awap/rain/index.jsp?colour=colour&time=history%2Fsa%2F20150501201704 30&step=4&map=anomaly&period=12month&area=sa (accessed 27 Sep 2017).
35 MAHAR JE, HALL RN, PEACOCK D, *et al*. Rabbit haemorrhagic disease virus 2 (GI.2) is replacing endemic strains of RHDV in the Australian landscape within 18 months of its arrival. *J Virol* 2017:e01374-17.
36 KERR PJ, CATTADORI IM, LIU J, *et al*. Next step in the ongoing arms race between myxoma virus and wild rabbits in Australia is a novel disease phenotype. *Proc Natl Acad Sci U S A* 2017;114:9397–402.
37 MUTZE G, BIRD P, KOVALISKI J, *et al*. Emerging epidemiological patterns in rabbit haemorrhagic disease, its interaction with myxomatosis, and their effects on rabbit populations in South Australia. *Wildlife Research* 2002;29:577.
38 World Organisation for Animal Health (OIE). Rabbit haemorrhagic disease, Australia - immediate notification report Ref OIE. Australia: World Organisation for Animal Health (OIE), 2014.
39 WISHART J, COX T. Rollout of RHDV1 K5 in Australia: information guide. Second edn. Canberra: PestSmart Toolkit publication, Invasive Animals Cooperative Research Centre, 2016.
40 COOKE B, CHUDLEIGH P, SIMPSON S, *et al*. The Economic Benefits of the Biological Control of Rabbits in Australia, 1950-2011. *Aust Econ Hist Rev* 2013;53:91–107.
41 PEDLER RD, BRANDLE R, READ JL, *et al*. Rabbit biocontrol and landscape-scale recovery of threatened desert mammals. *Conserv Biol* 2016;30:774–82.

Veterinary Record

Substantial numerical decline in South Australian rabbit populations following the detection of rabbit haemorrhagic disease virus 2

Greg Mutze, Nicki De Preu, Trish Mooney, Dylan Koerner, Darren McKenzie, Ron Sinclair, John Kovaliskli and David Peacock

Veterinary Record published online March 8, 2018

Notes