BioOne DIGITAL LIBRARY

THE INITIAL IMPACT OF RABBIT HEMORRHAGIC DISEASE ON EUROPEAN RABBIT POPULATIONS IN SOUTH AUSTRALIA

Authors: Mutze, Greg, Cooke, Brian, and Alexander, Peter

THE INITIAL IMPACT OF RABBIT HEMORRHAGIC DISEASE ON EUROPEAN RABBIT POPULATIONS IN SOUTH AUSTRALIA

Greg Mutze,[1] Brian Cooke,[2] and Peter Alexander[3]

[1] Animal and Plant Control Commission, G.P.O. Box 1671, Adelaide, South Australia 5001, Australia
[2] CSIRO Division of Wildlife and Ecology, P.O. Box 84, Lyneham, Australian Capital Territory 2602, Australia
[3] Department of Environment and Natural Resources, P.O. Box 3034, Norwood, South Australia 5067, Australia

ABSTRACT: The calicivirus agent for rabbit hemorrhagic disease (RHD) escaped from an island quarantine station to the Australian mainland in October 1995. Within 2 wk it was detected at an established field study site where wild European rabbits (*Oryctolagus cuniculus*) were being monitored in the Flinders Ranges National Park (South Australia, Australia). During November 1995, RHD reduced the rabbit numbers on the site by 95%. Approximately 3% of the population survived challenge by RHD and developed antibodies. Most of the antibody-positive survivors were 3- to 7-wk-old when challenged. Many rabbits died underground, but counts of rabbit carcasses found on the surface indicated that approximately 1 million rabbits had died above ground in the National Park, and that >30 million rabbits may have died in adjacent areas during the November epidemic.

Key words: Biological control, calicivirus, epidemic, European rabbit, *Oryctolagus cuniculus*, rabbit hemorrhagic disease.

INTRODUCTION

Rabbit Hemorrhagic Disease (RHD), has caused widespread, high mortality of wild and domestic European rabbits (*Oryctolagus cuniculus*) in Europe since it was first described in China in 1984 (Morisse et al., 1991). In 1993, the calicivirus agent for RHD, Rabbit Hemorrhagic Disease Virus (RHDV), was introduced to Australia for consideration as a biological agent for control of wild rabbits, which are severe environmental and agricultural pests. Host-specificity of the virus was demonstrated in quarantine by testing a range of domestic and Australian native animals, and field trials were then begun on Wardang Island in South Australia, to obtain information on efficacy and epidemiology (Lenghaus et al., 1994). During the field trials, the virus escaped to the adjacent mainland. The means of escape is not certain but blowflies, *Calliphora stygia,* collected at the time of escape from traps located immediately outside the quarantine area, were positive for RHDV and wind-borne flying insects are considered to be implicated in the escape and subsequent spread of the disease (Cooke, 1997). Within 2 wk, epidemics of RHD also were confirmed from two sites about 300 km northeast of Wardang Island and more than 100 km from each other. This paper (1) records observations from one of those sites during November 1995, the first month after the disease was detected and (2) makes general observations on the extent of the disease epidemic at that time.

MATERIALS AND METHODS

The RHD epidemic in the Flinders Ranges first was detected only 10 km from an established field site (31°15'S, 138°45'E) on Flinders Ranges National Park (FRNP) and adjoining Gum Creek sheep station (South Australia, Australia). This was an area where the impact of rabbits on rangeland vegetation and soil erosion had been studied since 1991 (Mutze et al., 1995). The disease spread rapidly and most rabbits on the site were subsequently affected.

The 30 km^2 site included four sections (blocks 1 to 4 running approximately north to south) where an index of rabbit abundance had been obtained during the previous 4 yr using spotlight transect counts taken near the beginning and end of each breeding season. All visible rabbits were counted on both sides of fixed 2 km transects within each block by an observer using a hand-held spotlight from a vehicle moving at 10 km/h. The vegetation is generally sparse and most rabbits within 50 m can be counted. Along each transect, seven permanent 7.6 m^2 quadrats were marked out and dung had been collected annually, sorted, air-dried at 40 C and weighed to provide a measure of relative grazing by rabbits, sheep and kangaroos (a

This One

731C-ZJB-G4LQ

combined estimate for red kangaroos *Macropus rufus* and euros *Macropus robustus*) in each block. Rabbits in the area rely on warrens (burrow systems which most commonly have between 10 and 50 entrances) for protection from the harsh environment and the number of warrens and entrances also can be used as an index of rabbit abundance (Parer, 1982). Maps were available showing the distribution and density of rabbit warrens.

The site was visited on 2 November 1995, 4 days after RHD was first confirmed in the general area, and again on 15 November and 28 November 1995. Rabbits were counted by spotlight on the transects and the site was inspected for evidence of RHD and myxomatosis. On the last of these occasions a sample of 32 rabbits was taken by shooting and cage-trapping. Blood samples were collected from the rabbits for RHD antibody analysis (Collins et al., 1994), and eyelenses were collected to determine the age of the rabbits following the method of Myers and Gilbert (1968). Rabbits were assigned to groups according to date of birth. This enabled data from samples collected in the 3 mo after November 1995 to be added in so that eventually, a sample of 70 young rabbits born during 1995 was obtained. The distribution of ages of rabbits which had been challenged with RHD and recovered (seropositive) was compared with the distribution of ages of rabbits which had missed infection (seronegative) to determine whether RHD affected all rabbits within the population to the same degree.

On the basis that mortality is considerably lower among rabbits <8-wk-old than among older rabbits (Morrisse et al., 1991), it would be expected that, after the passage of an epidemic, there would be a higher proportion of seropositive animals among young which had been <8-wk-old compared with those which had been >8-wk-old as the virus spread. On this assumption, and further assuming that all age groups had similar morbidity rates, it was considered that differences in the numbers of seropositive and seronegative survivors in different age classes could provide evidence indicating the approximate date at which RHD had its major impact.

Accordingly, the Kolmogorov-Smirnov two-sample test (Sokal and Rohlf, 1981) was used to compare the age distributions of seropositive and seronegative rabbits. Initially the rabbits were grouped into narrow (3 day interval) age classes to minimise the likelihood of Type 1 errors in the test. Having determined the date, to the nearest 3 days, on which the greatest differences were apparent, the data were regrouped into 2 wk class intervals around that

date for clearer visual presentation. The point of maximum difference was expected to occur approximately 8 wk before the epidemic reached its peak.

Liver samples were collected from dead rabbits found on the study site and across a wide area of northeastern South Australia, and analysed by virus capture ELISA (Collins et al., 1996) to confirm the presence of the virus at specific sites. Confirmed cases were recorded with locality coordinates to provide a distribution map of the area affected by the epidemic.

A further estimate of the impact of RHD in FRNP was obtained by counting rabbit carcasses on a series of 1 ha plots (22 plots at 7 sites). The sites were originally established to record baseline data on vegetation and small vertebrates within representative vegetation associations of FRNP, and had been chosen without reference to rabbit infestation in the immediate vicinity. Staff of the Department of Environment and Natural Resources employed at FRNP conducted a count of the number of rabbit carcasses visible on the ground in 1 to 4 adjacent plots at each site, 2 to 3 wk after RHD was first confirmed in the park. The sites were revisited later to record the density of rabbit warrens in the 1 km² surrounding the plots.

RESULTS

Passage of RHD through the site and impact on rabbit abundance

RHD had already reached part of the experimental site when it was visited on 2 November 1995. Rabbit carcasses could be seen sparsely distributed throughout block 1, at the northern end of the site, and eight carcasses were found in about 0.1 ha near 5 small warrens along the spotlight transect in that block. Moving away from block 1, a few rabbit carcasses were seen in blocks 2 and 3 (1.5 km west and 2 km south of block 1, respectively), but none were seen in block 4 (3.5 km southeast of block 1). Spotlight counts supported the view that RHD appeared to be moving through the site from the northern end. Only 29 rabbits were counted on the spotlight transect in block 1, a decrease of almost 50% since the start of the breeding season 6 mo earlier (Fig. 1). We were accompanied on that night's count by the local kangaroo shooter who was amazed by the lack of rabbits; when spotlighting along the same section of track 10 nights earlier
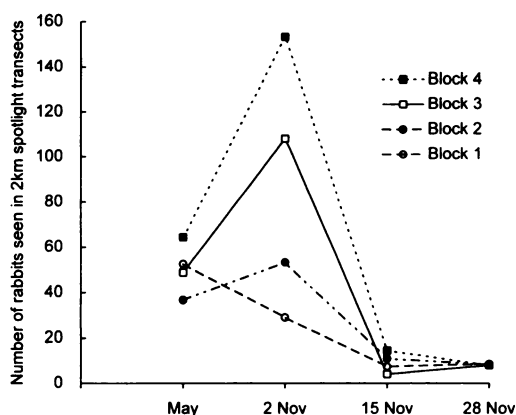
FIGURE 1. Rabbit abundance in the Flinders Ranges of South Australia at the beginning of the breeding season in May 1995 and during the rabbit hemorrhagic disease epidemic of November 1995.

TABLE 1. Numbers of rabbits counted on two 2 km transects by spotlight in the Flinders Ranges of South Australia before, during and after the rabbit hemorrhagic disease epidemic of November 1995.

Date	Rabbits counted		Difference (%)
	Block 1	Block 4	
Spring 1992	83	61	37
Spring 1993	78	98	−21
Spring 1994	86	77	12
May 1995	52	64	−19
2 November 1995	29	153	−81
28 November 1995	9	8	8

he had seen hundreds of rabbits moving across the landscape, "far more than he had ever seen in that area or anywhere else." In contrast to the number of carcasses seen, the number of live rabbits seen increased on moving from block 1 to blocks 2, 3 and 4 respectively (Fig. 1).

Based on the numbers of rabbits recorded at the start of the 1995 breeding season, RHD had not had much affect on the rabbits on blocks 2 to 4 before 2 November. In fact they had increased a little on block 2 and increased substantially on blocks 3 and 4 over the breeding season (Fig. 1). The 153 rabbits counted on block 4 was 30% more than had been counted there on any previous occasion. Rabbit numbers on blocks 1 and 4 had been similar in May (Fig. 1) and during spring in previous years (Table 1). On 2 November, we had expected to count approximately as many rabbits on block 1 as on block 4, and the kangaroo shooter's observations indicate that we would have done so 10 days earlier. However, the number of rabbits counted on block 1 was only 19% of the number counted on block 4. On the basis of these counts, there appeared to have been substantial mortality on block 1; possibly 80% of rabbits had died during the previous 10 days. Abundant feed was available at the time and extremely young rab-

bits were seen which suggested that breeding either had just finished or still was proceeding, so the decline in rabbit numbers cannot be attributed to poor nutrition. A few live rabbits exhibiting clear signs of myxomatosis were seen, but none of the fresh carcasses inspected showed any signs of myxomatosis, indicating that the vast majority of rabbits had died from RHD.

The numbers of rabbits counted in spotlight transects decreased further during the 2 wk between the first and second November visits to the site but did not decrease much further between the second and third visits, except on block 4 (Fig. 1). The average counts for all four sites decreased by 91% during the 4 wk. On block 4, the only area which appeared not to be affected by RHD on the first visit, counts declined by 95% over the 4 wk period.

Serological status of rabbits at FRNP

Analysis of 32 blood samples collected at FRNP and Gum Creek on 28, 29 and 30 November 1995 indicated that 21 rabbits had antibodies to RHD and 11 rabbits had not been challenged. Given that 95% of the population was considered to have died from RHD, the surviving 5% can be divided into two thirds (or about 3% of the original rabbits) which were recoveries and one third (or about 2% of the original rabbits) which had not been challenged. On this basis the morbidity rate, for the population as a whole, is estimated to have
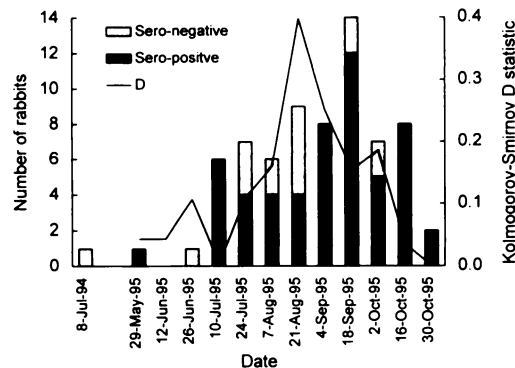
FIGURE 2. Serum antibody status to rabbit hemorrhagic disease virus of rabbits in the Flinders Ranges of South Australia following the epidemic of November 1995. The date of birth given for each sample is the final day of the 2 wk age group. The value of D is the Kolmogorov-Smirnov difference between relative cumulative frequencies up to and including the final day of the 2 wk age group.

been about 98% and the mortality rate is estimated as 95 × 100/98 or about 97%.

The age-distribution of surviving rabbits which had recovered from RHD was clearly different from that of rabbits which had not been challenged (D_{max} = 0.398, n_1 = 16, n_2 = 54, P < 0.05). The differences were at a maximum when rabbits born before and after 21 August 1995 were compared (Fig. 2). Many of the recovered rabbits were young and possibly had been only a few weeks old when RHD arrived on the study site; the majority of them were born between 21 August and 16 October (Fig. 2). Rabbits born in late August would have been about 6- to 7-wk-old in mid October when the RHD epidemic began. Although mortality caused by RHD was generally high, young rabbits <8-wk-old survived challenge a little better than older rabbits.

Morisse et al. (1991) reported that although 80% of adult domestic rabbits challenged with RHD died after inoculation, nestlings were not killed by RHD and about 50% of rabbits of <8 wk of age survived RHD infection. The data from Gum Creek and FRNP indicate that some young wild rabbits <8-wk-old may survive. Nevertheless, very young rabbits (<3-wk-

TABLE 2. Rabbit warren density and numbers of dead rabbits found above ground in Flinders Ranges National Park of South Australia following the rabbit hemorrhagic disease epidemic of November 1995.

Site name	Warrens/ km²	Dead rabbits/ ha ± SD	Number of 1 ha plots counted
Moodlatana	90	33.0 ± 12.0	3
N2/4	55	7.3 ± 10.6	3
N2/5	18	2.0	1
N2/6	55	8.0 ± 2.5	4
N2/7	2	0.5 ± 1.2	4
N2/8	59	7.0 ± 4.2	4
Slippery Dip	10	9.3 ± 2.9	3

old when the disease passed through) also were absent from the wild population (Fig. 2; Cooke et al., 1997). This was probably not because of susceptibility to the virus, but rather because the population of breeding adult rabbits had been greatly reduced. This would lead to fewer young being produced and other young dying from premature weaning.

A few survivors with antibodies to RHD were adults at the time the disease passed through. One rabbit with antibodies to RHD, collected from another site in the November 1995 epidemic, was 3-yr-old on the basis of eyelens weight. It is assumed that these animals survived infection as adults but, in the absence of any serological samples taken from the sites before November 1995, the possibility that the older rabbits with antibodies had survived infection as juveniles during an earlier RHD epidemic cannot be disproved.

Surveys of rabbit carcasses

Rabbit carcasses were counted on 22 plots at 7 sites in FRNP (Table 2). The number of carcasses found varied from 0 to 44 on the 1 ha plots. Overall mean values were calculated from site means to minimise biases due to the number of plots counted per site. The mean of the seven site means was 9.6 ± 10.8 (SD) carcasses/ha.

Linear regression analysis (Crawley, 1993) indicated that the mean number of

carcasses found was positively dependent on warren density in the surrounding 1 km² (R² = 0.58, $F_{1,5}$ = 7.0, $P \leq 0.05$). The areas sampled had a mean warren density of 40 warrens/km², which is lower than the mean value of 50 warrens/km² for the 100 km² of the park which have been surveyed. Rabbit carcasses also were counted on a single transect on block 4 in the experimental site, 4 wk after RHD first appeared in the area. Five carcasses were found in a 600 m long × 10 m wide transect. Although only a small sample, the density of 8.3 carcasses/ha is consistent with the larger data set.

The seven sites on which rabbit carcasses were counted were assumed to provide a representative sample of the whole of FRNP because (1) the sites were chosen to be representative of vegetation associations and independent of rabbit density, (2) the number of carcasses counted was dependent on warren density at each site and (3) the warren densities at the sites sampled were typical of broader areas surveyed in the Park. On that basis it was estimated that almost 1 million rabbits died above ground in the 1000 km² park during November 1995. Furthermore, rabbits dying underground were not visible and therefore were not counted. The putrid odor issuing from the warrens and large number of carcasses which were subsequently found below ground within 1 m of warren entrances suggests that a large proportion of the rabbits probably died inside warrens. That was certainly the case in the Wardang Island trials where the location of all dead rabbits and cause of death were accurately determined, and 75% died underground (Cooke et al., 1996).

Confirmed RHD-positive rabbit carcasses indicated that the epizootic of RHD in the Flinders Ranges and Yunta regions during November 1995 affected more than 35,000 km² of heavily rabbit-infested country. There were no reports of large patches within the recorded extent of spread that had remained unaffected. Within this broad area, the size and den-sity of rabbit warrens varies considerably between habitat types (Mutze, 1991) as it does on FRNP (Table 2). Nevertheless, average warren densities of 60 to 125 warrens/ha have been recorded across a variety of land systems from extensive surveys within the area of the November 1995 epidemic (Cooke and Hunt, 1987; Hunt, 1989; Mutze, 1991) so that an assumed average of 40 warrens/km² is reasonable even if the density is substantially lower in some areas. If it is further assumed that morbidity and mortality rates were similar to those on FRNP, it can be estimated that somewhere in the order of 30 million rabbits died in South Australia during that time.

DISCUSSION

The RHD epidemic occurred at a time of extremely high rabbit numbers (Table 1). The owner of Gum Creek Station counted 75 rabbits sitting on a warren at dusk shortly before the disease arrived and considered that "there were as many rabbits on the property as at any time in the previous 40 yr" (W. McIntosh, pers. commun.). Despite the vast number of scavenging birds which congregated where the disease was active, there were so many carcasses that most were left to decompose largely or completely intact. That made it relatively easy to determine the geographical extent of the epizootic by means of observations and collection of tissue samples. The number of carcasses counted is quite high compared to the warren density but the warrens were full, many rabbits were living on the surface and there is little doubt that >10 rabbits/ha died in FRNP during the November 1995 RHD epidemic. Extrapolating from those data to an estimate of 30 million rabbits killed in the entire affected area is more open to inaccuracies. The figure may have been lower if morbidity rates were not as uniformly high for the whole area but, conversely, if a large proportion of all rabbits died inside the warrens, the epizootic could have killed 50 million rabbits. The

vast majority of rabbits in the area were challenged and killed by the disease during a 1 mo period. It remains to be seen whether future epizootics when rabbits are less abundant will have such high morbidity and mortality rates.

The reduction in the number of rabbits on the study site to only 5% of pre-RHD numbers will be of major ecological significance if it is maintained for an extended period. Transect sampling has indicated that rabbits produce between 70 and 85% by dry weight of mammalian herbivore dung on the site based on mean values calculated from Mutze et al. (1995). Kangaroos produce about 15% of dung on the park, and kangaroos and sheep about 15% each on pastoral land. Rabbits were very abundant on FRNP at the time RHD passed through because of good seasonal conditions (probably 15 to 20 rabbits/ha based on carcasses found). However, even when average rabbit densities are as low as 6 rabbits/ha their grazing pressure is approximately 50 sheep equivalents/km² because rabbits in this country consume about 100g dry matter/day and sheep ingest about 1,200g dry matter/day (Cooke, 1974; Short, 1985). This greatly exceeds the permitted stocking rate of 18 sheep/km² for Gum Creek Station, which is considered to be the maximum sustainable density for the area.

Whether assessed from evidence of dung production or by comparing numbers of grazing animals and the average food intake of each species, rabbits were clearly the major mammalian herbivore at Gum Creek and FRNP prior to the epizootic of RHD. The 95% reduction in rabbit numbers has resulted in a substantial reduction in total grazing pressure and has probably reduced the population to about 1 rabbit/ha. Previous studies have demonstrated that rabbit numbers must be reduced to this critical level to allow regeneration of many of the trees and shrubs which form a vital but ever-diminishing component of arid zone flora (Lange and Graham 1983).

LITERATURE CITED

COLLINS, B. J., J. R. WHITE, C. LENGHAUS, V. BOYD, AND H. A. WESTBURY. 1994. A competition ELISA for the detection of antibodies to rabbit haemorrhagic disease virus. Veterinary Microbiology 43: 85–96.

——, ——, ——, C. J. MORRISSY, AND H. A. WESTBURY. 1996. Presence of rabbit haemorrhagic disease antigen in rabbit tissues as revealed by a monoclonal antibody dependent capture ELISA. Journal of Virological Methods 58: 145–154.

COOKE, B. D. 1974. Food and other resources of the wild rabbit Oryctolagus cuniculus (L.). Ph.D. Thesis, University of Adelaide, Adelaide, Australia, 131 pp.

———. 1997. Analysis of the spread of rabbit calicivirus from Wardang Island through mainland Australia. Project CS.236 report to the Australian and New Zealand Rabbit Calicivirus Disease Program, Meat Research Corporation, Sydney, New South Wales, 25 pp.

———, AND L. P. HUNT. 1987. Practical and economic aspects of rabbit control in hilly semiarid South Australia. Australian Wildlife Research 14: 219–223.

———, K. A. McCOLL, AND N. AMOS. 1996. Epidemiology of rabbit calicivirus disease in experimental populations of wild rabbits, Oryctolagus cuniculus (L), on Wardang Island, South Australia. Final Report to the Proponent Committee of the Australian and New Zealand Rabbit Calicivirus Disease Program, Meat Research Corporation, Sydney, New South Wales, 26 pp.

CRAWLEY, M. J. 1993. Glim for ecologists. Blackwell Scientific Publications, Cambridge, Massachusetts, 379 pp.

HUNT, L. P. 1989. Blade ploughs as an alternative to tined rippers for rabbit warren destruction in semi-arid areas. Australian Rangelands Journal 11: 40–43.

LANGE, R. T., AND C. R. GRAHAM. 1983. Rabbits and the failure of regeneration in arid zone Acacia. Australian Journal of Ecology 8: 377–381.

LENGHAUS, C., H. WESTBURY, B. COLLINS, N. RAT-

NAMOBAN, AND C. MORRISSY. 1994. Overview of the RHD Project in Australia. *In* Rabbit haemorrhagic disease: Issues in assessment for biological control, R. K. Munro and R. T. Williams (eds.). Bureau of Resource Sciences, Canberra, Australian Capital Territory, Australia, pp. 104–125.

MORISSE, J.-P., G. LE GALL, AND E. BOILLETOT. 1991. Hepatitis of viral origin in Leporidae; introduction and aetiological hypotheses. Revue scientifique et technique Office International des Epizooties 10: 297–310.

MUTZE, G. J. 1991. Long-term effects of warren ripping for rabbit control in semi-arid South Australia. Rangeland Journal 13: 96–106.

————, V. LINTON, AND D. POWELL. 1995. Changes in grazing patterns, range condition and soil erosion following rabbit control in South Australia.

10th Australian Vertebrate Pest Control Conference, Proceedings, Department of Primary Industries and Fisheries, Hobart, Tasmania, Australia, pp. 203–206.

MYERS, K., AND N. GILBERT. 1968. Determination of age of wild rabbits in Australia. The Journal of Wildlife Management 32: 841–849.

PARER, I. 1982. European rabbit (Australia). *In* CRC handbook of census methods for terrestrial vertebrates, D. E. Davis (ed.). CRC Press, Boca Raton, Florida, pp. 136–138.

SHORT, J. 1985. The functional response of kangaroos, sheep and rabbits in an arid grazing system. Journal of Applied Ecology 22: 435–447.

SOKAL, R. R., AND F. J. ROHLF. 1981. Biometry. 2nd Edition. W. H. Freeman and Company, New York, New York, 859 pp.