Celebrating 26 years of the SA Turretfield Rabbit Biocontrol Study

Ron Sinclair and David Peacock, 2022.

Sometime in mid-to late 1995, Rabbit Calicivirus Disease (RCD), later to be known as Rabbit Haemorrhagic Disease (RHD) escaped off Wardang Island and rapidly spread across arid and semi-arid South Australia. In October 1996, Governments proclaimed their respective Biological Control Acts and began to systematically release RHD virus (RHDV) by injecting up to 20 wild caught rabbits per site across the country. On 22nd October 1996, the Chair of a local Animal and Plant Control Board, Ms Penny Hopper, officially released the first rabbits injected with RHDV in SA. The rabbits had been trapped from a population living in a small, hilly area of remnant peppermint gum woodland on Turretfield Agricultural Research Centre, 50 km north of Adelaide.

At the time, there was no intention to initiate a research project based on this release, but out of curiosity Ron Sinclair returned to the site 3 days later to find many dead rabbits, some of whom were marked (had been injected) but also a few that were not marked. This suggested that the virus had already spread and killed new rabbits. Over the next few days, many more freshly dead rabbits were found and the need to understand this spectacular event initiated the world's longest study of viral biocontrols in free-ranging wild rabbits by PIRSA (Primary Industries and Regions, SA), working independently and in partnership with Australian and overseas researchers and organisations. 22nd October 2022 marked 26 years of continuous monitoring of this somewhat isolated population of rabbits to study the epidemiology (behaviour and impacts) of RHDV and myxomatosis.

Much of the field work involved a capture, mark, release program. Trapping trips took place at approximately 8-10 week intervals and would usually last for 4 or 5 days depending on capture rates and the weather. Cage traps were permanently located on about 15 rabbit warrens spread across approximately 12 ha of the site. The traps were baited with chopped carrot when we wanted to catch rabbits. Captured rabbits were weighed, sexed, reproductive status assessed, checked for clinical signs of myxomatosis, and given a uniquely numbered ear-tag if not previously tagged. A small blood sample was taken for laboratory testing to see if antibodies to myxomatosis or RHDV were present, indicating survival from a challenge by one or both of these diseases. Rabbits were released back into the warren at which they had been trapped.

Between trapping trips, we often visited the site to look for sign of virus activity indicated by seeing rabbits showing signs of clinical myxo, or by finding or smelling dead rabbits and/or seeing meat ants or blowflies moving in and out of burrows as they feasted on underground carcases. Confirmation of the presence of RHDV via PCR (Polymerase Chain Reaction) testing required obtaining a tissue sample (liver, heart, kidney or hind leg bones for marrow extraction) from a carcase.

During outbreaks of RHDV, we could easily sample carcases located on the surface, but because many rabbits died underground, we had to spend a great deal of time sniffing burrows to locate dead rabbits. With a crowbar and shovel, we dug (or hooked) out over 150 often very smelly, maggoty rabbits just to get a small sample for PCR testing. Tissue samples also provided an opportunity to obtain material for genetic studies on the rabbit population, in particular looking at the rate at which the virus was evolving and the familial relationships between individuals and how this might influence disease transmission.

We have no estimate of the population size prior to the 1996 release of RHDV nor quantification of the impact of rabbits on the local environment, but make the following observations.

- In 1996 there was significant rabbit grazing damage to the cereal crop bordering the site, no young trees or shrubs present, and the ground surface was almost completely denuded of vegetation. Much of the site looked like it had been cultivated with a scarifier, but it was rabbits digging up and eating bulbs of onion grass (*Romulea rosea*).
- Post 1996, in parts of the site that were fenced off to exclude sheep, we saw regeneration of Eucalypts, Golden Wattle (*Acacia pycnantha*) and Sheoak (*Allocasuarina verticillata*) and damage to the cereal crop has been minimal.
- Today, even at the end of summer there is good ground cover of leaf litter, dry grass and cryptogams, and some native and introduced grasses and herbs persist.
- In September 1999, we had more than 300 rabbits alive and ear-tagged immediately prior an outbreak of RHDV. During the outbreak we recorded 86 carcases of which only half were ear-tagged suggesting that the population was likely to have been around 600 rabbits (possibly about 50 rabbits/hectare).
- Rabbit damage across the site in 1999 seemed to be less than that seen at the time of the initial virus release in 1996, suggesting that the population in 1996 might have been even higher than in 1999.

PIRSA staff and volunteers sniffing for evidence of RHDV.

During the first 10 years of the study, RHDV outbreaks occurred in late winter or spring every second year except in 2002 and 2003 when there was an outbreak in both years. In the intervening years we only found 1 or 2 dead rabbits that tested PCR positive to RHDV in late winter/early spring despite there being many susceptible rabbits (i.e. no antibodies to RHDV) present. Furthermore, in subsequent trapping trips, a small number of rabbits had an increase in antibody levels indicating recent exposure to RHDV. It seemed that virus transmission had failed.

From 2007 to 2015, things changed markedly with annual outbreaks in late winter/early spring providing many samples from dead rabbits testing PCR positive. In 2016, things changed again. In May of that year and in June the following year (i.e. late autumn/early winter), a small number of dead rabbits were found and confirmed by PCR to have the 'new' RHDV2 variant.

After 2017, we are less certain if and when outbreaks occurred due to problems obtaining reagents for PCR testing and less time spent on the site, but it seems the original variant (now called RHDV1) has been replaced by RHDV2.

Work at the site has continued since Dave and I left PIRSA, with Matt Korcz, then Dr Kandarp Patel taking over.

In June this year, Dave Peacock presented a poster at the 9th World Lagomorph Conference in France showing some of the results from our Turretfield work and this attracted the attention of several very prominent ecological geneticists from a range of European countries and institutions. Dave has opened dialogue with these scientists and initiated collaboration with them which is likely to result in several new projects looking at host/parasite interaction and evolution utilising the Turretfield database that Dave originally created. This work will greatly increase our understanding of rabbit pedigree genetics and individual survival, work critical to future work on potential additional agents for the biocontrol of wild rabbits.

In brief, some highlights of the research at Turretfield demonstrating the value of such long-term research in providing data to help understand how existing biocontrols impact recruitment of the next generation, and its importance to modelling the likely effects of future biocontrol strategies:

Virus transmission:

- The discovery that flies in Australia (especially blowflies) were capable of transmitting RHDV via oral and/or anal excretions (flyspots). A single flyspot administered orally was able to cause RHD (Asgari *et al*. 1998).
- RHDV does not persist in the population between outbreaks, with a new strain imported each time, most likely via flies. The rate of RHDV evolution recorded was around 4 times higher than elsewhere in the world (Kovaliski *et al*. 2014, Schwensow *et al*. 2014).

Rabbit biology

- A female rabbit lived 7.6 years old at last capture, at that time the longest lifespan recorded for a European rabbit in the wild (Peacock & Sinclair 2009). We now have another female at 9.5 years old, and our six oldest rabbits are female. Only 50% of young reach 3 months of age and only 8% make it to 12 months.
- A strengthened understanding of climate change impacts on rabbit range and abundance achieved by accounting explicitly for potential synergisms between disease dynamics and climate (Fordham *et al.* 2012).
- Provided insights into how environmental variation can influence disease-affected population dynamics of rabbits by revealing the importance of seasonal matching between recruitment and timing of disease introduction (Wells *et al*. 2015).

RHDV

- Evidence of the development of resistance in wild rabbits to RHDV (Elsworth *et al*. 2012).
- Evidence that the virulence of RHDV had not attenuated in Australia in the 18 years since its release and that virus-laden rabbit carcasses are a likely source of virus transmission via flies (Elsworth *et al*. 2014).
- RHD epizootics started to occur earlier, became more frequent and prolonged, and the age of rabbits dying of RHD declined. Most rabbits are challenged by RHD in their year of birth, but because age-specific mortality rates are low in young rabbits we hypothesised that their survivors may be the source of recovery in rabbit populations across Australia (Mutze *et al*. 2014).

RHDV2:

- Reported the first documented cases of wild rabbits immune to the original strain of RHDV being infected with and/or succumbing to RHDV2 infection (Peacock *et al*. 2017).
- Rabbit numbers were reduced by approximately 80% following the arrival of RHDV2 despite above-average rainfall and pasture growth that favours rabbit population increase (Mutze *et al*. 2018).
- Evidence that RHDV2 can kill rabbits immune to the original RHDV, kills young rabbits, produces outbreaks earlier in the year and changed the timing of myxomatosis outbreaks (Mutze *et al*. unpublished).

Myxomatosis:

- Rabbits previously exposed to myxoma virus had 10% lower survival during RHDV outbreaks than rabbits never exposed to either virus. The reverse was not true (Barnett *et al*. 2018)
- Contributed to the finding that the Myxoma virus had changed dramatically over the last 20 years, with increased virulence and differences in its clinical impact on rabbits (Kerr *et al*. 2019).

Genetics:

- Identified the evolution of genetic sites likely to be responsible for selection for resistance to RHDV (Schwensow *et al*. 2016, 2017).
- Development of a large family tree from tissue collected in 2013 & 2014 showed:
    - that more kittens survived if they were from a big warren to a mother with myxo antibodies but age at an RHD outbreak was more important for survival than was the mother's antibodies to RHDV.
    - that young males often disperse into close neighbouring warrens.
    - evidence of more than one male siring kittens from a single litter! (Iannella 2018)
- Contributed DNA data for a genetics study of rabbits around Australia which found six genetic clusters possibly from multiple introduction events (Iannella *et al.* 2019). Additional work found a possible genetic factor that may partly explain the east/west divide in effectiveness of RHDV1 and RHDV2 (Iannella manuscript under review).
- Identified candidate genes for RHDV resistance that have evolved under natural conditions, and over a time span that would not have been feasible in an experimental setting (Schwensow *et al*. 2020).

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We would not have managed to run the field work without the help of dozens of volunteers including our own families, friends and colleagues, school children, university students and staff, NRM Board officers and anyone else we could drag in to ear-bash about rabbit damage and management, and environmental conservation.

We are grateful to the staff of the SARDI Turretfield Research Centre for free access to the study site and for the help they provided whenever we needed it. The State Government of SA provided the majority of the staff and funding to run the site. Several student projects were also supported by funds from Rabbit-Free Australia.

We also acknowledge the great work our recently deceased colleague and friend Greg Mutze put into writing up much of the data from the study site.

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