Management of Rabbits at Sites where RHD Has Failed

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1. Introduction

Data on rabbit numbers and immune status of populations in Marlborough were interpreted by Landcare Research to provide the Marlborough District Council clear options to advise land owners on what action to take to improve rabbit control. The work was carried out in between April and July 2006 under funding from the Foundation for Research, Science and Technology's Envirolink Small Advice Grant Fund.

2. The Problem

Rabbit haemorrhagic disease virus (RHDV) was introduced to New Zealand as a biocontrol agent for rabbits in 1997. It has generally worked well as a biocontrol because it has persisted with annual epidemics, and has produced a sustained reduction in rabbit numbers in many rabbit-prone areas (Parkes et al. 2002).

However, at a few sites in North Canterbury and Otago, the disease did not reduce rabbit numbers in the initial epidemics, and left a high proportion of rabbits immunised against further epidemics of the disease. These symptoms of failure of the biocontrol (too many rabbits and too many of them with antibodies to RHDV) are now also reportedly becoming evident in sites where the disease had worked well for several years.

The issues are:

- What rules should be developed so that farmers and councils are clear about when conventional control is justified and/or should be imposed in regional pest management strategies.
- What to do about the problem at 'failed' sites?
- Whether there is anything landowners and councils can do at 'successful' sites to stop them moving towards the symptoms of failure?

3. Background Information

There is a lot of misinformation about RHD and its epidemiology that may lead to poor decisions on what to do about rabbits at sites where the disease has failed. Research in New Zealand and elsewhere has shown:

- RHD epidemics appear to break out each autumn at most sites in the South Island (Parkes et al. 2002).
- Rabbits generally breed from the late winter through to the late summer, but generally only the pulse of rabbits born up to the early summer survive to join the adult population.
- The initial epidemics in 1997 typically killed between 70 and 90% of the rabbits in each population, leaving about 30% of the survivors with antibodies to RHD, i.e. **of the**

survivors about 30% had caught the disease and survived and 70% did not catch the disease (Parkes et al. 1999a).

- Young rabbits (up to c. 9 weeks old) are naturally resistant to RHD.
- Young rabbits born to immunised mothers also gain extra protection until they are about 13 weeks old from having maternal antibodies, but this does not necessarily give them life-long immunity. If they are challenged by the virus during this 13-week period they may or may not seroconvert (perhaps depending on the route of infection). If they do seroconvert and survive, they gain their own immunity. If they do not seroconvert they are not immune to later challenge. That is, the maternal immunity is **not** automatically passed on from mother to offspring (Cooke & Fenner 2002; Robinson et al. 2002).
- A rabbit that catches RHD and survives is immune, probably for life, despite the fact that it looses antibodies several months after exposure unless rechallenged by the virus (Parkes et al. 1999b), i.e. our tests for immunity are based on the presence of antibodies but this may give false negative results for immunity when the virus is not active in the area – as in winter.
- There is no evidence that the way the population was first exposed to the virus in 1997/98 (i.e. by natural epidemics, spot-baiting, or biociding) had any effect on the subsequent behaviour of the disease (Parkes et al. 2002). The evidence from Otago that biociding caused higher immunity levels than natural epidemics (O'Keefe et al. 1999) is weak as the evidence was based on changes in rabbit abundance taken from McLean's Scale scores, which were taken by observers who knew which treatment had been applied at each site. The effect was not shown at biocided sites in the Mackenzie Basin.
- At sites where RHD has continued to work, there is evidence (e.g. Fig. 1) that either (a) the rabbits are becoming more resistant to RHD or (b) the virus is getting weaker, or (c) the increasing proportion of older mother rabbits with antibodies are affecting the proportion of their offspring that survive challenge with RHDV (as above). The proportion of young of the year that catch and survive RHD appears to be increasing over the last 8 years (Fig. 1).

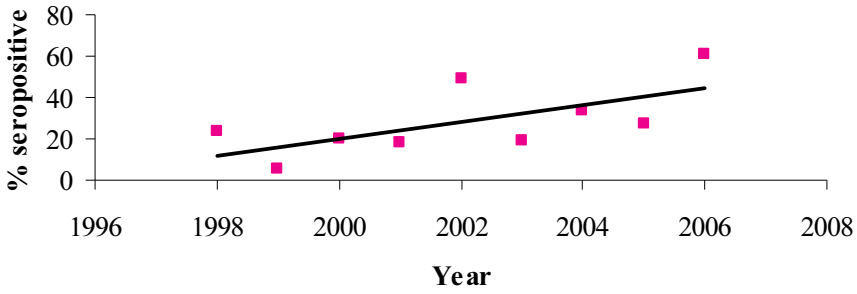

Fig. 1 Trend in the proportion of young rabbits, born in each year since 1997/98 at five sites in the Mackenzie Basin and North Canterbury and sampled in autumn (after RHD epidemics), that have antibodies to RHD. The trend is almost significant ($P = 0.058$).

- Of course the older a rabbit becomes, the more times it has been exposed to RHDV, so that when we sample older age classes of rabbits they show an increase in the proportion immunised because they have passed through two or more epidemics (Fig. 2). This means that rabbit populations with a high proportion of older rabbits will 'accumulate' immune members.

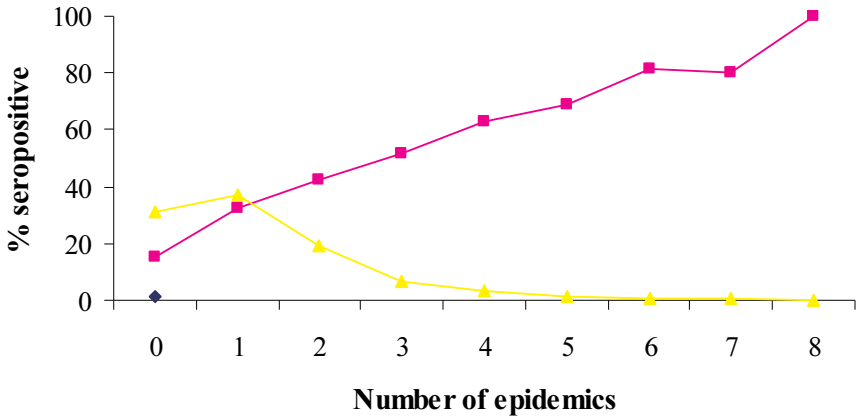

Fig. 2 Percent of rabbits with antibodies to RHDV according to the number of epidemics (assuming annual epidemics) to which they have been exposed (upper line). The lower line is the percentage of such animals in the samples, i.e. very few rabbits are older than 3 years.

- We do not have similar data from sites where RHD has failed so it is possible that either the rabbit or the virus has changed at these sites. However, there are plausible (but untested) alternatives to explain the causes of failure. These include the presence of a pre-existing calicivirus in New Zealand rabbits before RHDV arrived and the assumption that this might impart some imunity to rabbits challenged with RHDV (an hypothesis that the Australians are testing), the route by which young rabbits are infected (by contaminated food or by a biting vector), and the time of year (and so age and presence of maternal antibodies) at which this happens.
- There is no evidence that applying additional conventional control at sites where RHD has continued to work has any benefit either in terms of being cost-effective (it is clearly not where rabbit numbers are very low) or in preventing future failure of the disease. This latter possibility remains untested.

4. Management Options for 'Failed' Sites

A 'failed' site is where rabbit numbers are above acceptable levels AND where too many are immunised. Note: I have no information (other than anecdotal) on the proportion of places where RHD has failed.

Acceptable levels of rabbit abundance are generally determined in councils' pest management strategies and measured by McLean's Scale scores or spotlight counts. For Marlborough District Council, a McLean's Scale of 4 (in the Upper Awatere/Clarence) and 3 (elsewhere) are the triggers at which farmers are obliged to control rabbits (Anon. 2001). A McLean's Score of 4 is equivalent to a spotlight count of c. 15 – 25 rabbits/km and a score of 3 to about 5 – 14 rabbits/km in short-tussock-grassland habitats. It is possible that a good breeding season may result in a high McLean's Scale score taken in say late summer, but this might be reduced to lower levels by a successful RHD epidemic in the autumn.

I note that the obligation to control rabbits in regional pest management strategies based on an index of rabbit abundance is underpinned by the certainty that if rabbits reached a certain trigger level this was sure to lead to even bigger problems in the future and the costs of conventional control were justified, because the predicted damage (let alone the current damage) would outweigh the control costs. RHD has made this logic less certain because the future abundance of rabbits is less predictable depending on whether RHD works or not at the site. Council regulators and farmers now have to judge whether it is worth investing in conventional control when benefits of that control might not outweigh the costs. In other words we need better information on how rabbits at different densities actually affect the resources we are trying to protect.

The measure of 'how many is too many immunised rabbits' requires some thought. Consider three scenarios:

- If we sample a rabbit population and find that most or even all are immunised survivors of an epidemic, but if this represents only a few percent of the original population, this is a sign of success not of failure of the biocontrol. These immune rabbits will not usually pass on the immunity to their offspring (at least once the young are over 3 or 4 months old) so will not compromise future epidemics.

  Management consequence: do nothing.

- Similarly, we might have too many rabbits as measured by a McLean's Score or spotlight count, but a sample shows very few are immunised. This might represent a partial failure of the biocontrol, presumably because the epidemic fizzled out before exposing many rabbits.

  Management consequence: either do nothing and hope the disease will work next time, or if the rabbits are causing unacceptable damage use RHD baits either as an epidemic starter or as a biocide, or apply conventional control.

- We might have too many rabbits AND too many immunised. This is a particular problem if the surviving young rabbits of the year are immunised. This means the disease has exposed a high proportion of the population in previous epidemics but that too many have survived for some reason.

  Management consequence: neither natural epidemics nor RHD-baiting will solve this problem, and conventional control by poisoning or shooting will have to be used. An interesting question is whether successful conventional control re-sets the rabbit population so that RHD will work (at least for several years) in the future or whether the disease will always fail at these sites because of some site factor and more regular conventional control will be needed.

5. Analysis of Data from Marlborough Sites

5.1 Data needs from sites of interest

We need measures of rabbit abundance and trends at the sites. Neither of the two indices used are linear on true rabbit density. McLean's Scales can give rough measures of relative abundance (Warburton & Frampton 1994), but spotlight counts give better measures of trend in abundance over time (Caley & Morley 2002). These are best done after the expected autumn epidemic as they measure the base population that will go into the next breeding season.

We need measures of the percentage of rabbits in each age class that have antibodies to RHD. This is best done soon after any natural epidemics have occurred, usually in the autumn, and the key age class that tells us most about the state of the rabbits is the young of the year – usually born in spring and early summer.

5.2 Current data

We have serological data from rabbits at seven sites in the Awatere/Molesworth region and one site at Burtergill at Seddon (Table 1).

Table 1 Age-specific prevalence of antibodies to RHDV from rabbits shot at six sites in Marlborough. * The 'young of the year' are taken from the previous breeding year as no very young rabbits were shot between 1 July and the date sampled.

Site	Month/year shot	Total rabbits (% with antibodies)	No. young of year (% with antibodies)	No. older animals (% with antibodies)
Tone	May 2005	40 (55%)	34 (47%)	5 (100%)
Tone	May 2006	30 (60%)	25 (56%)	5 (80%)
Molesworth/Alma	May 2005	30 (57%)	19 (63%)	11 (45%)
Molesworth/Alma	May 2006	30 (37%)	20 (30%)	10 (50%)
Honeymoon	May 2005	30 (60%)	20 (55%)	8 (75%)
Honeymoon	May 2006	30 (53%)	18 (44%)	12 (67%)
Yeo Stream	July 1998	30 (27%)	15 (27%)*	15 (27%)
Middlehurst	July 1998	18 (22%)	6 (50%)*	12 (8%)
Camden	May 2006	30 (50%)	14 (14%)	16 (56%)
Burtergill	Aug 1998	20 (20%)	15 (27%)*	5 (0%)
Burtergill	Jan 2000	19 (53%)	6 (17%)	13 (69%)

A crude signal of a growing problem is when the percentage of the young of the year with antibodies is increasing. There is no evidence that this has occurred, e.g. at the three Awatere/Molesworth sites with repeated measures the percentage of young with antibodies in May 2005 was 53.4% but only 44.4% in May 2006.

There are also spotlight count data for the Tone, Honeymoon, and Molesworth sites (Figs 3a, 3b, 3c). These counts show that RHD, which arrived in the area in September 1997, caused a decline in rabbit abundance from between 20–40 rabbits/km to around <5 rabbits/km at the first two sites, but with increasing numbers over the last few years so that current indices are at about the pre-RHD levels. There are no data pre-RHD from the Molesworth site but rabbit indices have remained between 3 and 7 rabbits/km since 1999.

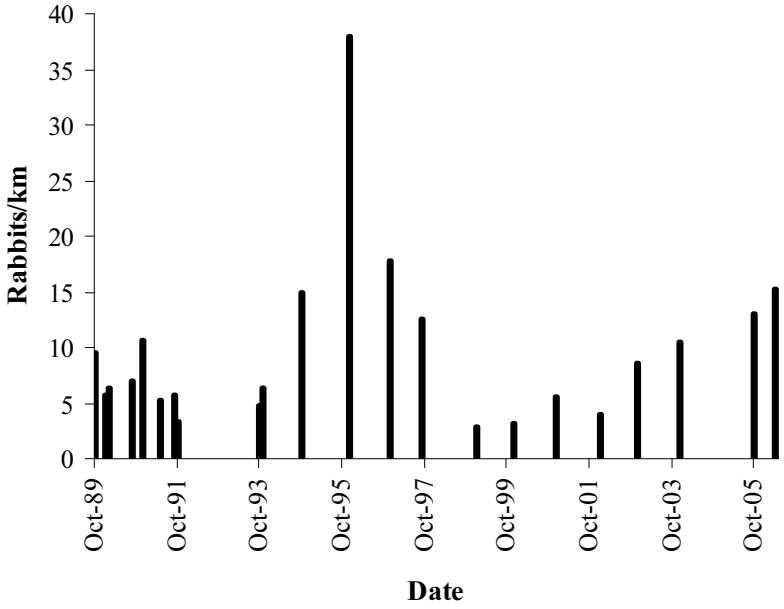

Fig. 3a Changes in rabbit abundance (rabbits/spotlight km) for the Tone site (= Middlehurst No. 1), 1989 to April 2006.

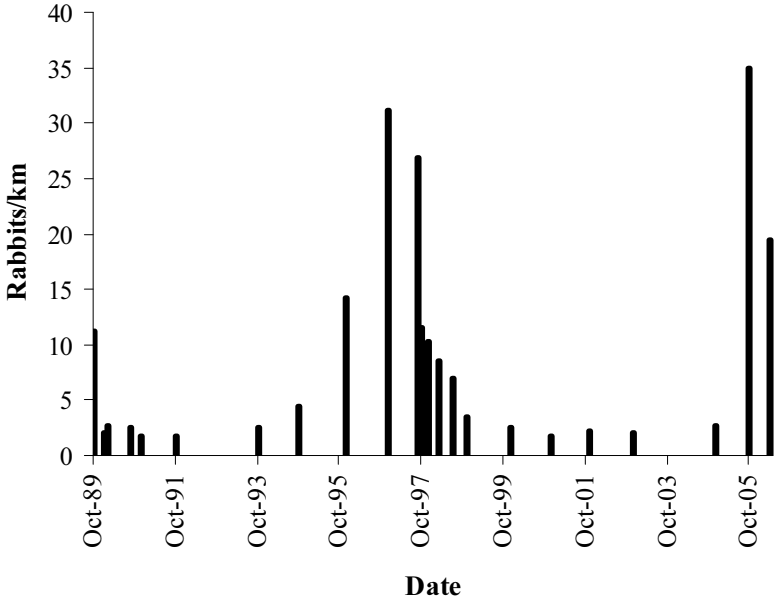

Fig. 3b Changes in rabbit abundance (rabbits/spotlight km) for the Honeymoon site (= Blicks on Muller Station), 1989 to April 2006.

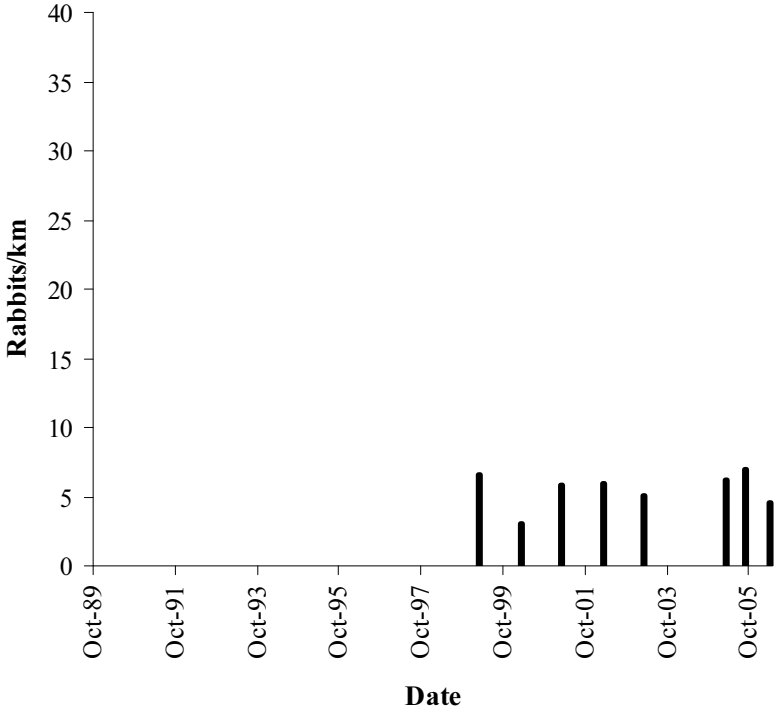

Fig. 3c Changes in rabbit abundance (rabbits/spotlight km) for the Alma site (on Molesworth Station), 1999 to April 2006.

To put these results in context, at 'successful' sites, such as most Mackenzie Basin sites monitored by Environment Canterbury, the current spotlight count indices have remained low since 1997 and the percentage of all rabbits with antibodies has remained about 30% (Figs 4a, 4b). The Marlborough sites show similar levels of immunity with the suggestion that they have remained at about the same levels as seen after the initial epidemics elsewhere (i.e. about 30%), but with the concern that rabbit densities (at least at the Tone and Honeymoon sites) have increased to unacceptable levels.

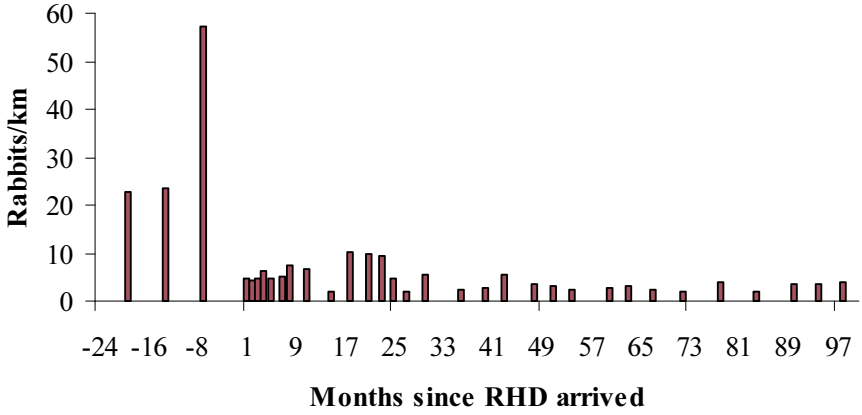

Fig. 4a Changes in rabbit abundance indices at one site in the Mackenzie Basin.

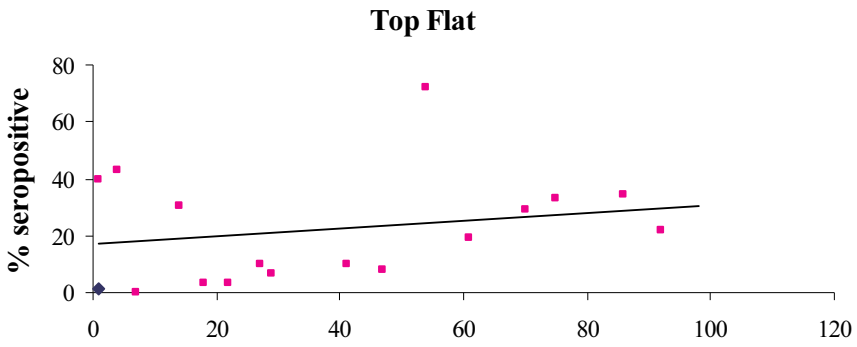

Fig. 4b Changes in the percentage of rabbits with antibodies to RHDV at one site in the Mackenzie Basin since 1997 (time 0 on the *x*-axis). The trend is not significant at this site ($P = 0.43$).

6. Recommendations

6.1 Decision process

Basically, the current rules about thresholds of rabbit abundance in the regional pest management strategy should apply to enforce conventional control, but there some issues that need to be considered in a decision process that meet the issues raised on page 2.

1. Are rabbit indices above the threshold specified in the RPMS? Yes Go to 2; No Go to 5
2. Has there been field evidence of an RHD epidemic? Yes Go to 3 or 4[1]; No Go to 4 or 5[2]
3. Are more than 50%[3] of young rabbits immune? Yes Go to 4; No Go to 4 or 5[4]
4. Apply conventional control
5. No nothing

Caveats and explanations:

[1] If there are too many rabbits after an apparent epidemic, some farmers might go straight to conventional control if the thresholds are greatly exceeded or if the problem area is small and does not justify the costs of sampling and testing rabbits for immunity.

[2] If there has been no evidence of an epidemic, farmers might take a precautionary approach and apply conventional control, or they may delay a decision for a short period. However, note that too much delay constrains their ability to plan and apply some forms of conventional control such as aerial 1080 poisoning (see below).

[3] At sites where RHD has worked, about 20–30% of young rabbits are typically immune when sampled in late autumn. I have arbitrarily picked 50% and above as the prevalence of antibodies that would signal a problem when there are also too many rabbits.
[4] If there are too many rabbits after an epidemic but not many are immune, a farmer might apply conventional control to solve the 'too many' problem, or might apply RHD as a biocide if the geographic scale of the problem allows. If no young rabbits are seropositive in a late-autumn sample that is an indication that RHD epidemics have not occurred at the site.

6.2 The Marlborough case

If we follow the key for the Marlborough data, there are too many rabbits and there is evidence of a natural epidemic of RHD in 2006 (there are many immunised young of the year). However, the proportion of rabbits that is immune is not high so the options are either to wait and see if the next epidemic will kill most of the susceptible rabbits, to use RHD baits as a biocide if the area is not too large, or to apply conventional control if the current damage is too great or if you want to be risk averse.

6.3 Timing of monitoring and conventional control

An issue for some councils is one of timing. The monitoring of rabbit abundance (and immunity levels if that is done too) needs to wait until after the expected autumn epidemic – perhaps in April. If these monitoring results indicate that conventional control is required, the ideal time to use carrot baits with 1080 is in about June. Is there time to arrange the baits and obtain the necessary consents?

6.4 Is there anything landowners and councils can do at 'successful' sites to stop them moving towards the symptoms of failure?

This is unclear. Farmers may continue to apply conventional control at sites where RHD has worked (as a precautionary measure) but they may be wasting their money and killing rabbits that RHD will soon kill anyway, or spending $10 for a $1 benefit.

6.5 Research issues

Marlborough District Council and other councils with sites where RHD has failed and where conventional control is applied should monitor the sites (spotlight counts and measure the prevalence of antibodies in at least 30 rabbits) in c. April/May before the control and thereafter every autumn to determine if killing the 'problem' rabbits re-sets the biocontrol and for how long.

7. References

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Appendix 1 Taking blood samples to test for antibodies to RHD

1. Shoot (or catch) your rabbit.

2. Each rabbit is given a unique identity number (a tag or felt pen on its ear) which is recorded on all samples from it.

3. Note its sex on the sheet provided. Options include measuring body weight, pregnant/lactating, number of embryos).

4. Cut open its chest cavity to expose the heart and take a blood sample as follows. Note: it is best to do this when the rabbit is shot as blood is harder to get once clotting starts.

   The blood is collected in the glass 'vacutainer' tube **using a new needle for each rabbit.**

   Take off the protective cover on the double needle and screw the needle into the yellow holder (the rubber-coated end of the needle goes inside the holder and the sharp end is exposed when you are ready to stick it into the rabbit).

   Stick the needle into the rabbit's heart and then push the other end of the needle into the vacuum seal of the vacutainer.

   The vacutainer has a vacuum and this sucks up the blood. You may have to move the sharp end of the needle in the heart about a bit to get enough blood, but do not allow it to pull out or go right through as this will lose the vacuum. Ideally, you want at least half of the tube filled with blood.

   If you cannot get enough blood from the heart or if the vacuum is lost you can just take blood from the chest cavity or pull off the bung on the tube and add to it.

   Label the vacutainer with the rabbit's identity number.

   Stand the blood-filled vacutainers in a fridge, BUT DO NOT FREEZE, for c. 12 h. The red blood cells separate from the clear serum. Once this has occurred, pour off the serum from the top of the red cells into one of the small microtubes (sample provided) and place in the plastic tray (sample not provided). Ask Tao Zheng (AgResearch) how to order the tubes in the tray as they test these in some standard mass production way.

   Label each microtube with the rabbit's identity number and cap the microtube with the small lids and freeze.

   It is important that the blood samples do not get cross-contaminated so use a new needle for every rabbit and wash the knife etc used to open the rabbits. Sometimes you can get blood through the body wall without opening the chest cavity – a bit of practice in finding the heart is needed.

5. Send the tray of serum samples with the list of Id numbers on the Test Report Form to Dr Tao Zheng, AgResearch, Wallaceville. Ring him at 04 528 6089 to arrange transport (usually in a chilly bin with a freezer pack via courier).

   AgResearch will measure the amount of antibodies in each serum sample, which has been diluted 1:10, 1:40, 1:160 and 1:640 times. They will send you a spreadsheet of results measured as the raw 'optical densities'. These need transforming into a measure called % inhibition. Rabbits that have % inhibitions over 50% at the 1:40 dilution are considered seropositive and immune. This gives a conservative estimate of the percent immune. Alternatively you can request AgResearch to send you the data transformed into % inhibitions.

6. Using a teaspoon and sharp knife, dig out one eyeball from each rabbit and place in the plastic pottle. Add a little 10% formalin to preserve. DO NOT FREEZE.

I can age the rabbits from these. Send to Landcare Research, Gerald Street, Lincoln, and let me know at Parkesj@landcareresearch.co.nz. I can then match the age with the serology results you get from Tao Zheng and give you a quick interpretation.

If you want to age them yourself, leave the eyeball in the formalin for at least a week then cut out the eye lens and dry for 3 days at 80ºC then weigh to the nearest milligram.

The age of the rabbit can be calculated from the formula:
Age (days) = $-57+(181.4/\log_e(314/\text{lens wt in mg}))$

and the birth date calculated from subtracting this from the date shot.

If you want to purchase the various bits of equipment (samples included with this letter):
The vacutainer tubes, needles and holders can be got from Becton Dickinson (0800 572468) and the serum tubes, caps and racks they come in, from Biolab (0800 933 966).