Monitoring and Release Strategy for the national release of the K5 strain of RHDV

Endorsed by the Invasive Animals and Plants Committee
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Introduction

The RHD-boost project aimed to identify new strains of Rabbit Haemorrhagic Disease Virus (RHDV) with high lethality to rabbits immune to Australian Rabbit Calicivirus (RCV-A1) and rabbits resistant to infection with Czech 351 derived RHDV strains. The project was a strategic response to the apparent rising genetic resistance to the RHDV CZ 351 strain released in 1996, and its limited effectiveness in temperate regions due to the endemic RCV-A1, which protects many rabbits from the RHDV strain.

This Monitoring and Release Strategy (hereafter referred to as 'the Strategy'), is designed to maximise the benefit gained from the characteristics of the selected RHD-boost strain known as K5. This draft outlines the objectives and desired outcomes of the Strategy, suggests sampling methods that may be utilised, and provides a number of monitoring options that may be adopted. It should be noted that this is a release of a strain of RHDV into a population where three pathogenic strains are already present, with two exotic strains recently confirmed in wild rabbit populations (Chinese variant strain confirmed in NSW in 2014, RHDV2 confirmed in the ACT in July 2015). As such, it is likely that results from the release of the K5 strain will be more subtle than that seen after the escape in 1995. The design of the Strategy takes this into consideration by: (1) using information on rabbit abundance, virus efficacy and predicted spread of benign and released strains of RHDV to estimate the minimum number of sites required to detect changes (outlined in Ramsey, 2013), and (2) using a combination of intensive and broad-scale community-based monitoring. This approach will maximise the ability to measure the efficacy of virus release as well as promoting community-led integrated rabbit management.

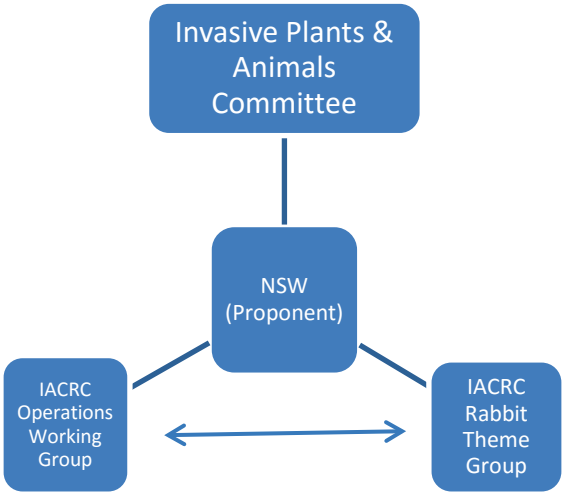
The Strategy was developed with funding from the Caring for Our Country initiative of the Australian Federal Government, and administered through NSW Primary Industries, and the Invasive Animals Cooperative Research Centre.

Scope of the Strategy

This Strategy is designed to inform on the scientific process of releasing the K5 variant of RHDV into Australia's rabbit population. This Strategy provides information on the timing of release, the appropriate monitoring methodologies for sites and how data should be collected. This Strategy also defines the roles and responsibilities of both state and federal bodies and governance groups involved in the release of K5. Key messages, risks, consequences and opportunities around the release of K5 have been developed in a separate Communications and Engagement Strategy.

Roles and responsibilities

The Invasive Plants and Animals Committee (IPAC) is responsible for policy coordination around the release of K5. It is the responsibility of each jurisdiction (through their representatives on IPAC) to ensure necessary legislative and regulatory requirements are met within each state and territory, and to develop their own policy on the release of K5, using both this strategy, and the Communications Strategy to inform their decisions. Both the Invasive Animals Cooperative Research Centre (IA CRC) and the Operations Working Group (OWG) provide support and recommendations to the New South Wales Department of Primary Industries (DPI), who are responsible for the registration of the K5 variant, and for the development of this release strategy (Figure 1). Project leaders at NSW DPI will resource and manage monitoring sites through consultation and collaboration with state leaders and contacts.



REGULATIONS

Governance

Invasive Plants and Animals Committee, IACRC Research Director and NSW (Proponent)

Responsibility

Coordinate the policy and approvals processes for a release of K5.

IMPACT OF OWG

Develop a communications and engagement plan.

Incorporate citizen science approach, integrating learnings from the Melbourne Workshop.

Use to manage partner/stakeholder expectations.

Use to mobilise resources for the science and IPM, where there is potential for high return on investment.

OPERATIONS

Governance

Operations Working Group

Responsibility

Clarify CRC partner/stakeholder drivers, needs and requirements for the national release of RHD.

Undertake communication and engagement planning, and oversee citizen science of the national release, drawing on multidisciplinary CRC partner/stakeholder perspectives and expertise.

Make recommendations to NSW (Proponent) and IACRC Research Director

Coordinate operational processes to guide national release.

SCIENCE

Governance

CRC Rabbit Theme Group

Responsibility

Report and make recommendations to the Invasive Plants & Animals Committee via NSW (Proponent) and IACRC Research Director, CRC partners and stakeholders on the science: objectives, aims, methods, results and conclusions.

Seek input through the OWG on stakeholder drivers, needs and requirements for the science, and logistical matters to assess science-related feasibility, risks and benefits; and the options available to achieve the science objectives.

Figure 1: Governance arrangements for the K5 release.

Objectives and outcomes of the Strategy

Surveys have confirmed that since 2003 rabbit numbers have been increasing, and investment in conventional control has been escalating. The current apparent genetic resistance to the Czech 351 strain, and the reduced efficacy in temperate regions due to the endemic RCV-A1, led to the evaluation of new candidate RHDV strains under the RHD-boost project. This Strategy was developed to monitor the impacts of the release of the RHD-boost virus (K5) on rabbit populations throughout Australia. Vegetation and non-targeted animal monitoring is outside of the funded scope of this project. The Strategy has a number of objectives and outcomes that will be met through the establishment of a monitoring program.

The objectives of the strategy are:

1. To evaluate the impact of K5 on rabbit populations at release sites across Australia
2. To evaluate the success of multiple biological control programs on the continued suppression of rabbit numbers throughout Australia.
3. Promote community ownership of the RHD program (coinciding with conventional control) through the joint adoption of the communication strategy

The outcomes will include:

A. More effective rabbit control throughout a range of Australian landscapes currently affected by rabbits.
B. An understanding of the impact of a second phase release of RHDV for the biological control of rabbit populations in Australia
C. On-ground knowledge to feed into decision support systems to allow land managers to be better informed, particularly in regards to financially based decisions on how best to control the impacts of rabbits.

Background

Rabbits in Australia and their impact

Since the successful introduction of the European rabbit (*Oryctolagus cuniculus*) onto the mainland in 1859, rabbits have had a devastating impact on Australian ecosystems. With an estimated economic impact of $200 million p.a. (McLeod 2004), rabbits cause significant damage to agricultural and pastoral industries, and the environment.

Rabbits spread quickly through the Australian environment, and within 62 years a population of 24 had grown to an estimated 10 billion (Invasive Animals CRC, 2010), spreading at an average rate of 70 km per year. Rabbits are now found in all states and territories (Figure 2) (Department of Primary Industries and Fisheries 2008). Currently their distribution across Australia is estimated at 5.3 million km^2 or 69% of the continent. Rabbits can colonise a wide range of habitats and have successfully colonised Australia's arid interior. Rabbits compete with native and stock animals for food resources, and overgraze native plants. Their grazing and burrowing activities continue to destabilise soil systems and undermine geomorphic processes; contributing to erosion and undermining soil integrity, as well as altering the structure and composition of vegetation communities (Department of the Environment Water Heritage and the Arts 2008). Rabbits are also highly fecund and their high population numbers help to support populations of other introduced species such as foxes and cats (Bowen and Read 1998).

The activities of rabbits' impacts negatively on many native species: 35 species of animal (19 birds, 13 mammals, 2 reptiles and 1 insect) and 121 species of plant are directly threatened by the activities of the rabbit. Of these plants and animals 69 are vulnerable to extinction, 78 species are in danger of extinction, and 9 species are in critical danger of extinction (Department of the Environment Water Heritage and the Arts 2008). Many more animals, plants and vegetation communities are indirectly affected by rabbits and their activities. Competition with, and land degradation caused by, rabbits are listed as key threatening processes under the Commonwealth *Environment Protection and Biodiversity Conservation Act 1999*.

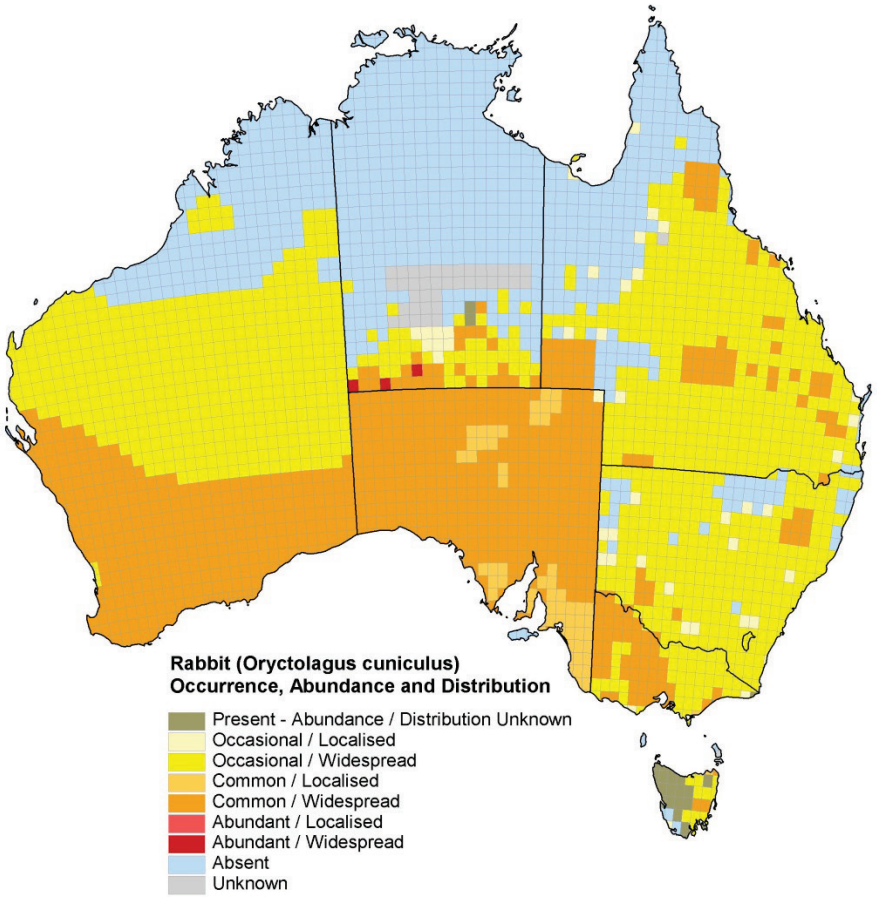


Figure 2: Rabbit occurrence, abundance and distribution across Australia (National Land & Water Resources Audit and Invasive Animals Cooperative Research Centre 2008)

RHDV in Australia

After the escape of the virus in 1995, and its subsequent release in 1996, Czech 351 RHD caused a decline in rabbits of between 0–80%, with variability occurring both within sites and between sites (Mutze *et al.* 2010). Variability may have been due to differences in contact rates between individual rabbits locally (within-groups) and globally (between-groups) (Marsh 2009). RHDV had its greatest impact in arid and semi-arid inland Australia (Henzell et al. 2002). However, its performance in the southern agricultural areas and more temperate agricultural areas of Australia has been highly variable (Neave 1999, Mutze et al. 2010), with RHDV least effective in coastal areas, in cool moist areas, and during summer in areas of summer rainfall (Henzell et al. 2002). The patchy success of RHDV has been, for the most part, attributed to the presence of the benign calicivirus RCV-A1 (Rabbit Calicivirus – Australia 1) which has long been suspected of reducing the effectiveness of RHDV in Australia (Cooke *et al.* 2000, Nagesha *et al.* 2000, Cooke *et al.* 2002, Robinson *et al.* 2002). Early serological work suggests that some populations of rabbits had prior exposure to RHDV or RHDV–like viruses, with rabbit sera collected before the presence of RHDV in Australia cross-reacting to RHDV (Nagesha *et al.* 2000, Robinson *et al.* 2002, Bruce and Twigg 2004). However it was not until 2008 that RCV-A1 was identified and characterised (Strive *et al.* 2009). RCV-A1 causes a prolonged infection in rabbits as young as 6 weeks (unlike RHDV

which appears to only infect animals of 12 weeks or more) and offers partial cross-protection to RHDV (Strive *et al.* 2010).

1996 Monitoring Program

The 1996 monitoring program was quickly established after RHDV escaped quarantine testing facilities on Wardang Island in South Australia in 1995. Due to the viruses' escape, control sites could not be established, therefore the main criteria for monitoring site establishment was the existence of pre-RHD data; as well as their broad bioclimatic zone and land use type (Neave 1999). Ten intensive, two supplementary and 54 broad-scale monitoring sites were set up to monitor the effects of the virus on rabbit populations. In addition to these 66 monitoring sites, more than 780 Czech 351 release sites were monitored for changes in rabbit abundance.

Intensive monitoring sites

The 10 intensive monitoring sites included one Queensland site (Muncoonie Lakes), one Tasmanian site (North Tasmania), one Victorian site (Hattah), one Northern Territory site (Erldunda Station) two Western Australian sites (Nullarbor and South Stirling Range), two South Australian sites (Flinders Ranges and Coorong), and two New South Wales sites (Lake Burrendong and Central Tablelands) (Figure 3). Within these intensive sites a number contained sub-sites, with two sub-sites at Nullarbor, two sub-sites at South Sterling Ranges, two at Flinders Ranges, three at Central Tablelands, four at Erldunda Station and six sub-sites at Hattah, totalling 23 intensive monitoring sites. For all of these sites and sub-sites, pre RHD CZ 351 release rabbit indices were available and the sites were monitored for changes in rabbit abundance, disease prevalence and impacts on flora, fauna, predators and agricultural production (at some sites).

Broad-scale monitoring sites

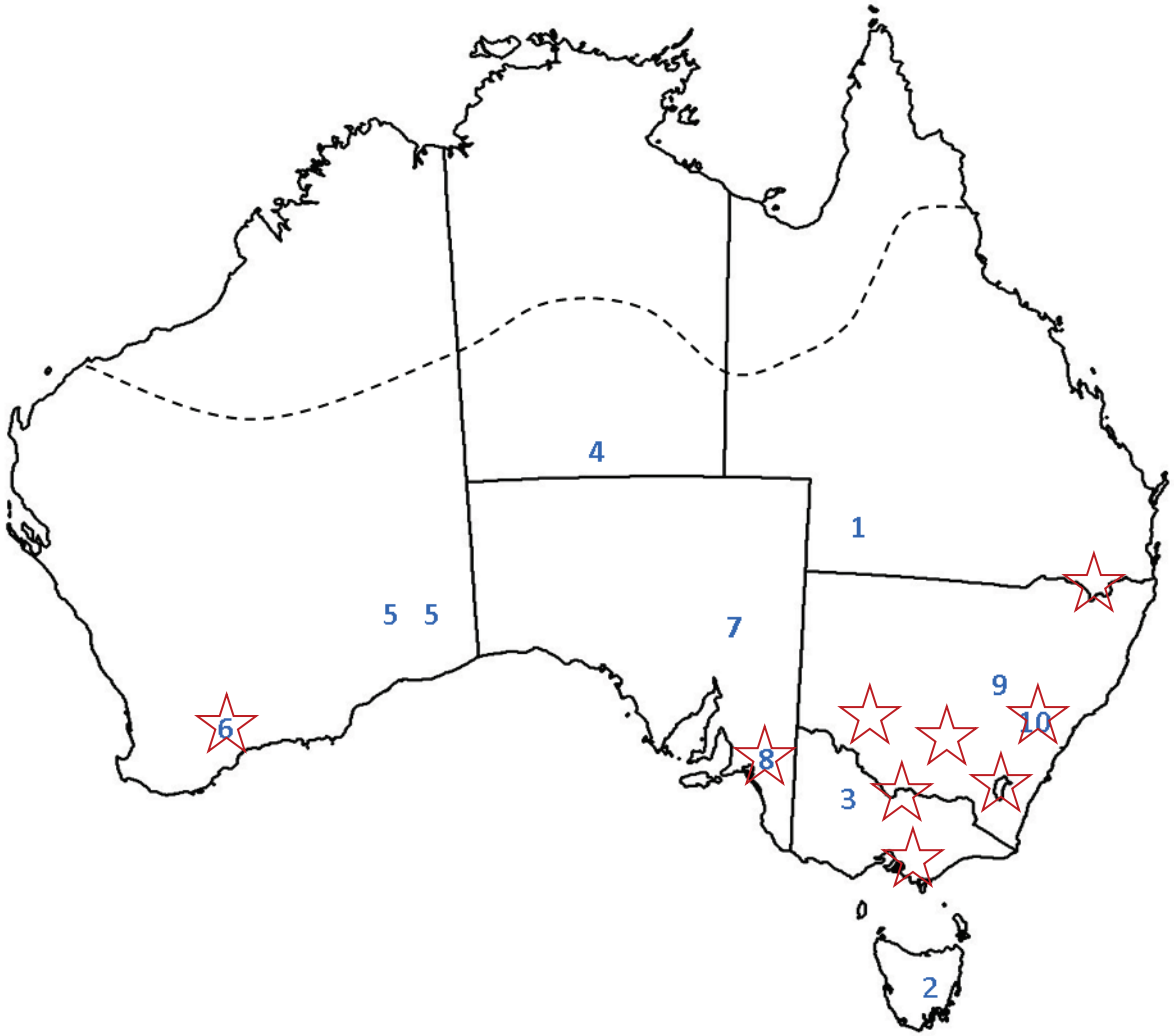
A number of broad-scale sites were selected throughout the rabbits' distribution to monitor changes in rabbit abundance. Of the 54 sites, four sites were in Tasmania, seven sites were in the Northern Territory, nine sites were in Western Australia, six sites each were in the Australian Capital Territory and Victoria, 12 sites were in New South Wales, and five sites each were in Queensland and South Australia (Figure 4). Within these sites, a number contained sub-sites, with 16 sub-sites in the Northern Territory and six sub-sites in South Australia, totalling 67 broad-scale monitoring sites.

Supplementary monitoring sites

In addition to the intensive and broad-scale sites, two supplementary sites were monitored for changes in rabbit abundance, and breeding success of birds of prey. These two sites were Cattai near Sydney in New South Wales, monitored for changes in rabbit abundance and genetics, and, Strzelecki in the Strzelecki Drainage System in South Australia, monitored for changes in rabbit abundance and breeding success of birds of prey.

Release sites

Czech 351 was released at over 780 sites (not including intensive, broad-scale or supplementary sites) throughout Australia. At these release sites, rabbit populations were monitored for any change in abundance.



Intensive monitoring sites

1. Muncoonie Lakes, Queensland
2. North Tasmania
3. Hattah, Victoria
4. Erldunda Station, Northern Territory
5. Nullarbor (two sub-sites), Western Australia
6. South Sterling Ranges, Western Australia
7. Flinders Ranges, South Australia
8. Coorong, South Australia
9. Lake Burrendong, New South Wales
10. Central Tablelands, New South Wales

Figure 3: indicative locations of intensive monitoring sites used in the 1996 release of Czech 351. The dotted line indicates the northern limit of rabbit distribution. ☆Stars indicate approximate locations of the current intensive monitoring sites (paired) established to monitor the release of K5.

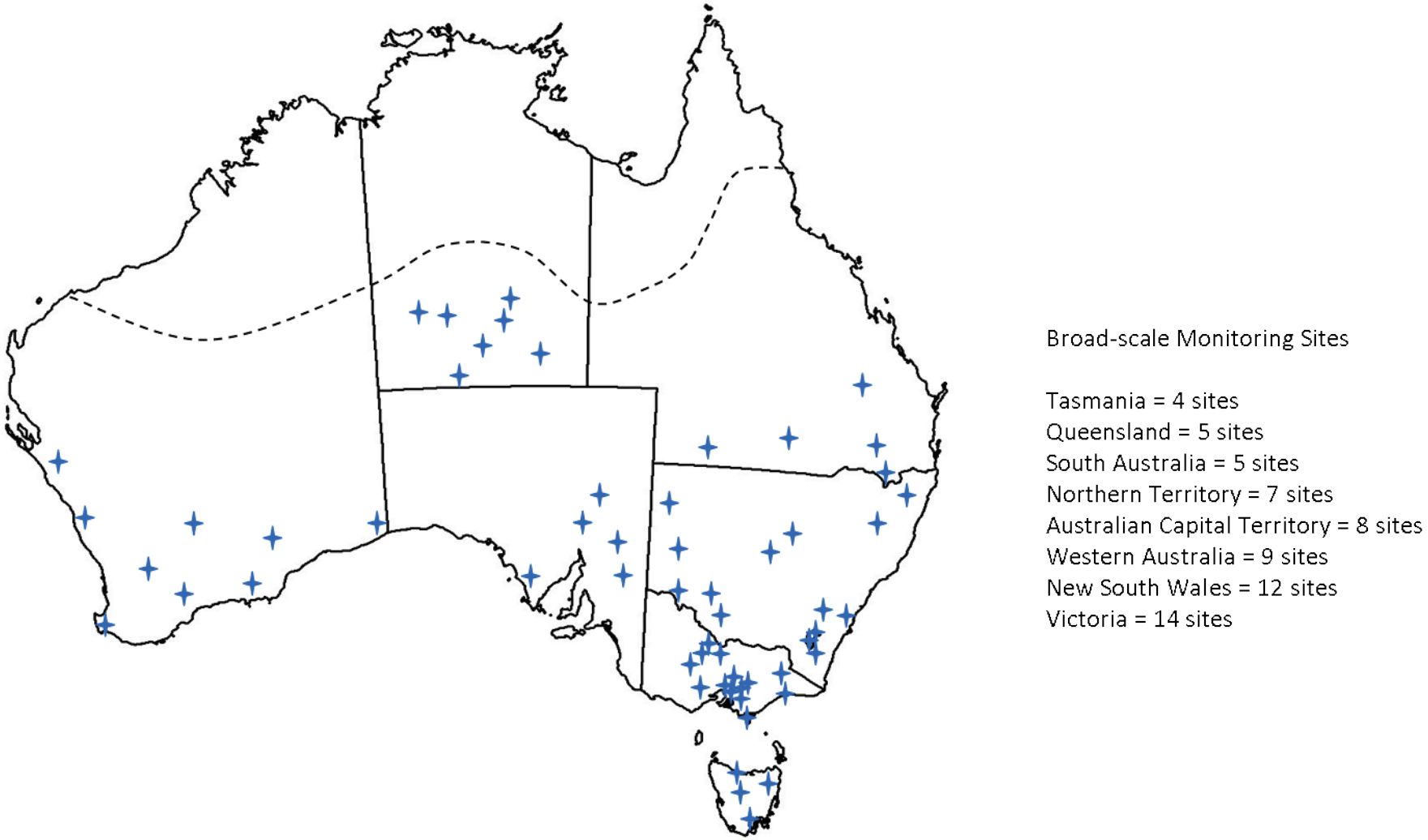


Figure 4: Indicative locations of broad-scale monitoring sites used in the 1996 release of Czech 351. The dotted line indicates the northern limit of rabbit distribution.

K5 monitoring sites

The establishment of sites is dependent on funding and the level of government, NRM groups and community support. Currently 18 (9 paired) intensive monitoring sites (9 release sites, and 9 nil treatments) are in place. Broad-scale and release sites are being established, ready for release once registration of the K5 strain has been achieved.

Site selection

The selection of monitoring sites for the K5 release was dependent upon two aspects; the nature of the K5 strain and, meeting the objectives and outcomes of the Strategy. Where appropriate, the 1996 sites will be used as intensive sites for the monitoring program. This allows for comparison with historical datasets. Where additional intensive sites need to be established, sites that have some measured and recorded history of rabbit populations should be selected where possible.

Intensive sites

There are some minimum requirements that need to be met for sites to be suitable as intensive sites. At each location, two sites are required (one release site, one non-treatment site). Sites should be close enough to experience similar environmental variation but far enough apart to limit the (immediate) risk of spread of K5 from the release to the non-treatment site (e.g. sites can be 50-100km apart, or separated by a significant barrier such as a ridgeline). Each site should contain an equilibrium population abundance of at least 100 rabbits. Spotlight transects need to be at least 1 km in length and the transect needs to cover between 25-50% of the site.

Broad-scale sites

The requirements for broad-scale sites are less restrictive. Broad-scale sites will also be subject to release of K5, but do not have an associated non-treatment site. Spotlight transects need to be at least 1 km in length and the transect needs to cover between 25-50% of the site.

Release sites

Release sites are additional sites subject to release of K5. A release site is any site where rabbits are present and their relative abundance can be measured by spotlight counts (walked or driven) as a minimum, however other recognised method (dung estimates and active warrens) may also be incorporated.

Experimental design

The Strategy will be implemented with pre- and post-release monitoring periods. The pre-release monitoring will allow for the collection of site-specific baseline information prior to release, with post-release monitoring focusing on the impact of K5 on these representative rabbit populations. The design has an 80% probability of detecting a 20% level of population suppression and a 60% probability of detecting a 10% level of population suppression (Ramsey 2013).

While rate-of-spread and field-establishment needs to be determined, the establishment of satellite sites for monitoring K5 spread may be somewhat hit and miss, as RHDV is spread by flying insect vectors. In order to pick up this information, early consultation with, and involvement of, NRMs, LLS, CMAs, Landcare and their officers on the ground, as well as the general public, in the collection of rabbits from suspected RHD outbreaks, would facilitate the collection of rate-of-spread and field-establishment information.

Pre K5-release data collection

The collection of information prior to the release of K5 will allow for quantitative assessment of the impact of K5 on rabbit populations, to meet the objectives and outcomes of the Strategy.

Intensive sites

All K5 pre-release data should be collected no less than within the one year period prior to the estimated virus release date for intensive sites. To standardise the data across the regions, data collections should occur once every season in the middle of the season (January, April, July and October). A full list of data collection methods used during the 1996 surveys are listed in Appendix 1, however, at a minimum, spotlight counts and shot samples (with full sample collection) are required at each site.

Broad-scale sites

All K5 pre-release data should be collected within one month of the estimated virus release date for the broad-scale site. Spotlight counts should be undertaken and pre-release shot samples should be taken with blood (for sera) collection the only sample requirement.

Release sites

All K5 pre-release data should be collected within one month of the estimated virus release date for the release site. Pre-release rabbit abundance should be measured by spotlight counts (walked or driven) as a minimum, however other recognised method (dung estimates and active warrens) may also be incorporated.

Spotlight counts

(Objectives: 1, 2, 3; Outcomes: A, B)
Spotlight counts should be undertaken at all sites to ascertain rabbit densities prior to K5 release. Where spotlighting is already conducted, the same protocol should be continued to ensure comparability with historical data. Adaptation of existing protocols to include any of the following information (where that is not already collected), is encouraged. Where an existing protocol is not in place, spotlight counts should be done using 100 watt spotlights, travelling at uniform speed (10–15 km/hr) over a set transect with a width of 100 m. The same observer should be used for each count at each site. Transects should be established that are representative of the monitoring area and should cover the different land and vegetation types. Counts should be conducted over three consecutive nights of similar weather, commencing at least 30 minutes after sunset. Data collected should include information such as; the numbers of large vertebrate fauna (kangaroos, wallabies) as well as the number of predators (foxes, cats, dogs/dingoes) seen within the range (up to 100 m), and all environmental variables including rainfall, wind, cloud cover, temperature, time and moon phase. The average of the counts divided by the transect length gives a simple index of abundance (animals/km), with the maximum number counted in any of the three nights

giving the minimum known to be alive (MNA) (procedures for spotlight counts are modified from Mitchell and Balogh (2007)).

Shot samples

(Objectives: 1, 2, 3; Outcomes: B)
The collection of shot samples allows for a rapid, time and cost effective method of sampling a random cross-section of the local rabbit population. Recent sampling sessions around Australia utilising this method have obtained serological and demographic information from rabbits as young as 55 days (average age sampled = 161 days). Blood (for sera), duodenum (first 5 cm from the pyloric junction, Appendix 2), liver, ear tip and whole eyeball should be collected from shot samples. For broad-scale sites, the collection of blood (for sera) is the minimum sample required. Any carcasses should be investigated for signs of myxomatosis and RHD. Bloods should be centrifuged upon collection and sera collected and frozen. If bloods cannot be centrifuged upon collection, then they should be stored chilled or frozen for no more than 24hrs before sera is collected. Sera samples will then be tested using ELISA to determine the antibody status of rabbits at RHD-boost virus release sites, and for the presence of RCV-A1. Duodenum, liver and ear tip samples should be frozen. Eyeballs should be stored in 10% neutral buffered formalin. Any collected samples and incidences of diseased carcasses should have the site name, date of collection/observation and disease recorded on the field data sheet (Appendix 3).

Insect vectors

(Objectives: 1, 2, 3; Outcomes: B, C)
The monitoring and analysis of insect vectors such as blowfly (Calliphoridae) and bushfly (Muscidae), provide adequate information on the spread of RHDV and prevalence of other field strains (Appendix 1). Monitoring of these species will be used during the pre K5-release to establish the presence and spread of RHDV K5 and other field strains.

Rabbit disease surveillance App

(Objectives: 1, 2, 3; Outcomes: A, B, C)
A cross-platform rabbit disease surveillance app with off-line capability will be established to aid in land manager and general public reporting. This app will be linked into the RabbitScan website (http://www.feralscan.org.au/rabbitscan/) which will provide an extra disease layer of information – identifying where disease outbreaks are occurring, and which strain or disease is currently moving through the area. The app will allow users to record the deaths of animals, assign those deaths to a disease (either myxomatosis or RHDV), provide images of the dead rabbit, and elect to collect samples for further evaluation. The mobile devices inbuilt GPS will provide accurate location information, and information collected will provide valuable broad-scale data on rate of spread of the K5 strain of RHDV after its release.

Release of K5 virus

The timing of the release of K5 will be dependent upon a number of factors. Ideal climatic conditions for the persistence and longevity of RHDV in the field consist of a temperature range of 11–24°C with a minimum relative humidity of 40%. Proportional rabbit survival is also reduced in areas of winter rainfall, if outbreaks occur in summer, and in hot dry climates (provided the basic temperature range requirements are met). Persistence of RHDV in the field is considerably shortened (< 3 hrs) in temperatures above 36°C. Efficacy is also reduced in cool wet climates, warm coastal areas and in areas of summer rainfall, most likely due to the presence of RCV-A1 (Liu et al. 2014).

Almost 90% of kittens (< 50 days) are resistant to Czech 351 infection (Cooke 2002). This resistance is most likely due to maternal antibodies, and is of great concern as a sub-lethal infection boosts the immune response and creates an immune adult (Cooke 2002). Because of this, previous RHDV release efforts have been timed to coincide with the pre-breeding season (generally autumn) when kitten numbers are at their lowest.

The added consideration of kitten survival, in conjunction with optimal climatic conditions (based on historical climate data (1961–1990) (Bureau of Meteorology 2010)) for longevity and lethality of the RHD-boost virus, results in a four-staged regional-release program (Figure 4). The first stage of virus release should be in the southern parts of Victoria, south-west and south-east Western Australia, Tasmania, and in the highlands of New South Wales, when temperatures average between 9–24°C and rainfall is between 50–600 mm. The second stage of virus release should occur in the south-central part of Western Australia and parts of south-western and mid-eastern South Australia in autumn (late March to early May). During this time these regions experience average temperatures of between 15–21°C with rainfall of between 25–50 mm. The third stage of virus release would occur during late autumn (May) in central Australia, north-west New South Wales and south-eastern Queensland. In late autumn these areas experience average temperatures of between 12–18°C and between 10–200 mm of rainfall. The final stage of release would occur in the northern reaches of rabbit distribution during early winter (June) 2013. Average temperatures are 15–21°C with 10–200 mm of rainfall. Currently, all intensive field sites are in Zone 1 (Figure 5) except for the Hay field sites which are in Zone 3.

An additional factor to consider in the release is the presence of insect vectors. Flies (Calliphoridae) are known vectors of RHDV (Asgari et al. 1998) and their presence in the environment would certainly facilitate the spread of K5 beyond the release sites, and likely enable the establishment of K5 into Australia's rabbit population. The abundance of fly's declines over the winter period, particularly in the southern parts of Australia, suggesting there may be a lack of insect vectors available during an autumn release. For this reason, it may be necessary to undertake a second release in October/November, to establish K5 in the field. An October/November release would allow the kittens from the first litter of the season to reach a susceptible age, thus providing less chance of vaccinating the next generation, and may allow the virus to spread more rapidly across the country due to the presence of insect vectors. A limitation with an October/November release is the high likelihood that a field strain of the Czech strain may move through the population before K5 is released, potentially removing any susceptible population. It is because of this risk that

autumn is the preferred time for release (when natural outbreaks are uncommon), and that an October/November release is undertaken only in the instance when K5 has not established. Where this recommendation may not hold true is in those locations where rabbits are surface dwelling, such as in Western Australia.

The warren system provides an ideal situation for contact transmission of RHDV. When animals die from RHD in the warren, the presence of that carcass, which is full of virus, in the warren system, allows other animals that use that warren system to come into direct contact with the virus. Terminally ill rabbits also shed significant amounts of virus in the faeces. Under these circumstances, the lack of an insect vector is not likely to limit the spread of RHDV to other individuals. This form of contact transmission is less likely to occur where rabbits do not use warren systems. In these circumstances, the chances of healthy rabbits coming into direct contact with RHD carcasses are greatly reduced. Therefore insect vectors likely play a critical role in the spread of RHDV from one rabbit to another where there are surface dwelling rabbits. For this reason, it may be more prudent to release RHDV only when insect vectors are present.

Release recommendations

While releases should be undertaken under the ideal climatic conditions (see Figure 5), and ideally, when kittens are not or are less likely to be present, there is a good scientific basis for a release in either spring or autumn. While there is likely to be a higher proportion of young rabbits in spring, the increasing vector activity would facilitate enhanced spread. An early spring release would allow K5 to spread prior to existing field strains and RHDV2, maximising its effectiveness and its establishment in the rabbit population. The presence of RHDV2, which is known to remove very young rabbits from the population, may well reduce the likelihood of young rabbits being vaccinated by a spring release of K5. An autumn release, while ideal for ensuring kitten numbers are at their lowest (under normal climatic conditions) may also require a second release of K5 in early spring to allow the virus to establish, particularly if a late autumn release is undertaken while insect vector numbers are low and establishment does not occur.

Virus delivery

The K5 strain will be available as a freeze-dried product from the Elizabeth Macarthur Agricultural Institute, and should be reconstituted as per label instructions. The K5 strain will be delivered to rabbit populations as per the current method of delivery for the Czech strain – on carrot or oat baits. Pre-feeding of rabbit populations (a minimum of three feeds over one week) should be undertaken to ensure rabbits are taking the bait and that the correct amount of bait is being supplied. Baited carrot or oats should then be used.

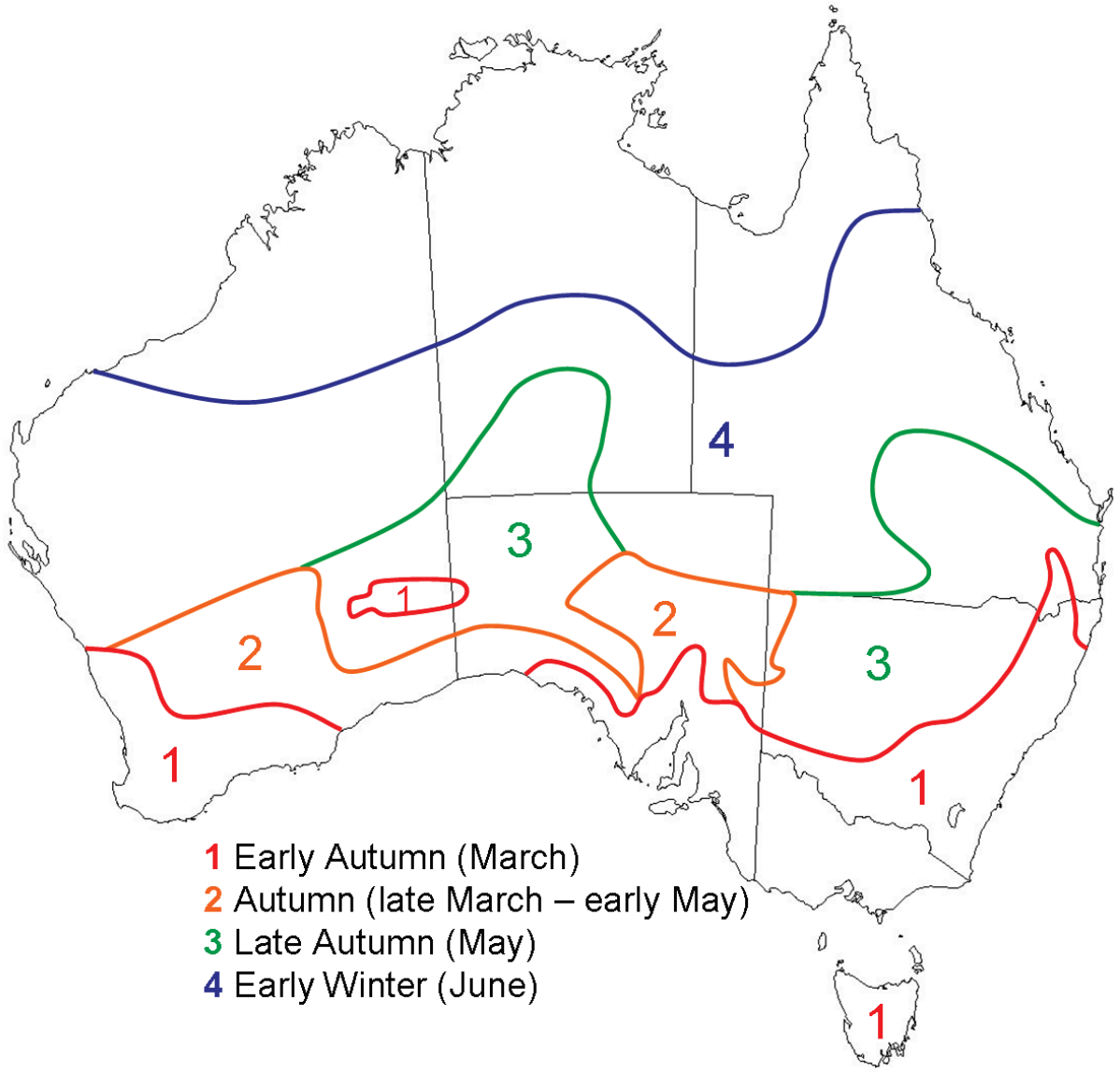


Figure 5: The staged-regional-release of the K5 virus between March and June, to ensure maximum longevity and lethality of the virus, based on optimal climatic conditions and minimal kitten numbers.

K5 post-release monitoring program

Post-release monitoring should repeat the pre-release monitoring. This will aid in the evaluation of the impact of RHD-boost on rabbits and the success of RHD-boost in achieving the objectives and outcomes of the Strategy.

Intensive sites

To standardise the data across the regions, all K5 post-release data collections should occur once every season in the middle of the season (January, April, July and October). The same methods utilised during the pre-release monitoring should be continued in the post-release monitoring (at a minimum, spotlight counts and shot samples (where rabbits remain) with full sample collection). It is proposed to continue post-release monitoring until 2018. An intensive search for rabbit carcasses should be undertaken for up to four weeks post-release and samples taken (see **Rabbit Mortality**).

Broad-scale sites

Spotlight counts should be undertaken one month after the release of K5 at the broad-scale site. Shot samples should be taken where rabbits remain. An intensive search for rabbit carcasses should be undertaken for up to four weeks post-release and samples taken (see **Rabbit Mortality**).

Release sites

All K5 post-release data should be collected one month after the virus release date for the release site. Post-release rabbit abundance should be measured by the same method as was used to determine pre-release abundance. An intensive search for rabbit carcasses should be undertaken for up to four weeks post-release and samples taken (see **Rabbit Mortality**).

Rabbit mortality

Following the release of the virus via baited carrot, the populations at all sites (intensive, broad-scale and release) should be monitored for carcasses for the next four weeks. Any carcasses should have liver collected (where possible), or leg bone (if liver not available), and samples should be placed in individual bags labelled with the date and location and frozen.

Additional mortality studies at intensive sites

At each of the intensive monitoring sites (treatment and control) radio telemetry collars should be fitted to a sample of captured rabbits. A minimum of 10 rabbits should be collared at each site. Transmitters should weigh no more than 5% total body weight. Ideally, transmitters should transmit two signal types; one to indicate movement (i.e. life), and one to indicate a lack of movement after a timeout period (7 hrs) suggesting the animal may be dead and collars should be able to reset if movement occurs, however standard VHF collars to locate animals are sufficient. Following the release, signals from collars should be checked regularly and any transmitters giving a 'dead' signal, or animals that have not

moved location after 24 hours (using VHF) should be retrieved and a cause of death determined. If possible, the retrieved collar should then be disinfected and fitted to another rabbit. Non-collared animals should also be collected when found. Sites should be monitored for four weeks following virus release. Any carcasses should have liver collected (where possible), or leg bone (if liver not available), and samples should be placed in individual bags labelled with the date and location and frozen. Subsequent monitoring sessions should also monitor for carcasses where possible.

Contingency actions

Any increase in non-target predation is likely to occur in the short term immediate post-release as per the 1995 release, however, predator populations are expected to adjust (decrease) in accordance with the reduction in prey numbers over time (as per the situation seen since the 1995 release), particularly for foxes, but less so for cats (Read and Bowen 2001, Doherty *et al*. 2015). Additionally rabbit population reductions are only expected to be in the order of 25% on average. At all times land managers are reminded that an integrated pest control strategy to remove both introduced predator and prey species is the recommended best practice.

Where threatened or vulnerable native species are present, it is strongly recommended that concurrent predator control programs are undertaken at the time of the release of K5 to limit prey-switching impacts on these species. In the long term it is preferable to maintain rabbit numbers at consistently low levels to avoid large swings in numbers and to help keep predator numbers low.

Experimental design

During the post-release monitoring period it is possible that non-treatment sites will acquire the K5 strain due to natural spread from the release site. Both treatment and non-treatment sites should continue to be monitored for the effects of K5. Some differences in the success of the original Czech 351 strain were seen between sites where the virus was released and where the virus arrived naturally (Neave 1999). Continued monitoring of non-treatments sites after the arrival of K5 may again highlight whether there are differences between sites where virus is released, or arrives naturally.

A Communications Strategy has also been developed to inform stakeholders about the national release and potential impact of K5 as part of a best practice integrated rabbit management program.

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Appendix 1

The following is a description of a number of data collection methods to monitor rabbits and their impacts, many of which were used during the 1996 monitoring period. These methods may be applied to achieve the objectives and outcomes of this Strategy (using ACEC approval requirements and research standard operating procedures as a guide). Procedures for spotlight counts, live trapping and warren counts are modified from Mitchell and Balogh (2007).

Live trapping

Given the variability of trap success and the published findings that trap responses are highly variable depending on age, sex and breeding season (Daly 1980), live trapping with cage traps should only be used when attempting to capture kittens, or to trap adults which are to be fitted with a radio collar. While trap success may be improved by leaving traps locked open in situ for the entire period of trapping, this approach may not be feasible at many sites, and could result in a high turnover of traps due to theft and loss. Where live trapping does occur, traps should be placed at the entrance holes for each warren (where warrens are used). Traps should be locked open in position for 2–3 days to allow for habituation. During this time pre-feeding of rabbits with diced carrot should occur. Traps should then be set each night for four nights and checked and closed each morning. Captured animals should be individually ear tagged and basic biological and epidemiological information collected (weight, length, sex, body and breeding condition, presence of fleas, clinical symptoms of myxomatosis and blood collection) and recorded on the Live Trapping standardised data sheet. Animals should be classified by age class as kittens (25–700 g), sub-adults (700–1200 g) and adults (> 1200 g).

GPS tracking

GPS collars (fitted with VHF transmitters) fitted to individuals may help ascertain the likely spread of RHD boost virus throughout populations of rabbit. Collars should weigh no more than 10% total body weight and may include a number of features including a GPS logger, activity logger, proximity logger, temperature logger and a break-away feature. These collars should transmit two signal types: one to indicate movement (i.e. life), and, one to indicate a lack of movement after a timeout period (7 hrs) suggesting the animal may be dead. Collars should be able to reset if movement occurs. Signals from collars are to be checked regularly and any transmitters giving a 'dead' signal retrieved using VHF. A cause of death should be determined. The retrieved collar can then be disinfected and fitted to another rabbit. Similarly, as collars are retrieved for battery changes, they should be disinfected and fitted to another rabbit. A minimum total of 50 rabbits should be collared at each site over the pre- and post-release monitoring periods. The collected information is then mapped to show animal dispersal distances, warren visitations etc. for both males and females at different times of the year (e.g. breeding, dispersal, etc.).

Warren activity

Recordings of warren activity consist of simple warren counts, active entrance counts and warren activity around vegetation sampling plots.

Warren counts

Warren counts consist of a several parallel straight light transects where the number of warrens (active and inactive) 10 m either side of the transect are recorded. Transects encompass all land types but avoid roads and running along the edge of land types. Warren density (*D*) and the standard error (*S.E.*) per land type can then be determined, along with the number (*Y*) and *S.E.* of warrens and the total number (*T*) and *S.E.* of warrens across land systems (Mitchell and Balogh 2007).

Active entrance counts

For active entrance counts, sites are to be 1–5 ha and have more than five warrens, each with more than three entrances. Mark and identify each warren with a star picket in the centre and determine the width of the warren from NE–SW. Count the number of active and inactive entrances for each warren. An active entrance may have a smooth floor, recent soil disturbances, feet and/or claw impressions in the soil, fresh urine or faecal pellets, or hair. Inactive entrances usually have leaves, grass-heads or weeds on the floor, have wind-blown or rain-washed soil, have old faecal pellets, or spider webs. The same warrens and the same observer are to be used for each count at each site. Around vegetation sampling plots, active entrance counts should be recorded for the three closest warrens.

The number of active entrance numbers can then be converted into the number of rabbits for each warren by *dividing* the number of active entrances by the appropriate conversion factor (Table 1).

Table 1: Conversion factors based on season for estimating rabbit densities based on warren counts (Mitchell and Balogh 2007).

Season	Conversion Factor
Breeding	3.0
Non-breeding	1.6

Vegetation surveys

Vegetation sampling methods are based on national standards of the National Vegetation Information System. All floristic information (species occurring at a site) is to be recorded if possible. Vegetation is described in terms of structural information (e.g. growth form: tree, grass etc.), height (growth form measured in metres), and cover (percent cover of each growth form). This information, along with floristic information, should be provided for each stratum (layer of vegetation). While traditionally three strata have been used (i.e. upper, mid and lower/ground), more complex structures (particularly in the southern regions of Australia) have resulted in national guidelines that recognise up to eight strata/sub strata (Table 2).

Insect vector surveys

Species of blowfly (Calliphoridae) and bushfly (Muscidae) are known vectors of RHDV in Australia (Asgari *et al.* 1998, McColl *et al.* 2002). Rabbit fleas (*Sphilopsyllus cuniculi* & *Xenopsylla cunicularis*) and mosquitos (*Culex annulirostris*) may also be important vectors in the field (Lenghaus, 1994). Virus has been found in the gut of flies up to nine days post-feeding on RHDV-infected rabbits, and fresh fly spots can contain 2–3 times the median lethal dose (LD_{50}) of virus; enough to infect a rabbit (Asgari *et al.* 1998). Flies should be trapped monthly over a one week period. Traps are to be set and checked each afternoon one hour before sunset.

Stocking density

Patterns of domestic grazing stock (where present) are to be recorded. The collection of this information is necessary to explain any dramatic changes in pasture production due to changes in stocking rates.

Pasture growth and off-take

A 300–400 m grid is to be established at each monitoring site. Within each grid, nine 0.25 m^2 quadrats are randomly placed. Three of these quadrats are to be accessible to all grazing species, three are to exclude all vertebrate grazing species and three quadrats are to allow rabbits to graze, but exclude large herbivores (differential exclosures). Each quadrat is to be scored for pasture biomass and height, bare ground, litter cover, dominant and other species present, and presence/absence of rabbit scat.

Non-target surveys

The surveying of non-target species is important to determine the impact of the reduction in rabbit numbers on the populations of native and other introduced animals.

Small mammal trapping

Trapping will be undertaken quarterly over a period of four consecutive nights. A minimum of 200 traps per site is suggested. Traps are to be laid in transects, and where possible, encompass all land use types. Traps within transects are to be 10 m apart and each transect a minimum of 20 m apart. Traps will be baited with a peanut butter/flour/vanilla essence mixture and captured animals will be individually marked (ear tagged, ear clipped etc.) and basic biological information (species, sex, weight, breeding status) collected.

Predator and raptor surveys

Predator surveys can be undertaken during rabbit spotlight counts. Raptor surveys can be undertaken both opportunistically, and on the same day that rabbit spotlight counts are undertaken. Raptor surveys should be undertaken every 30 minutes, where the sky is scanned for the presence/absence of raptors. Surveys should take place between 10:00 am and 4:00 pm and, where possible, raptors identified to species level.

Appendix 2

This appendix provides information on the anatomy of the rabbit and in particular the location of the duodenum and liver of the rabbit, to ensure that sample collection is standardised across the collection areas.

For liver collection: locate the liver. The liver in the rabbit can be found quite high in the chest cavity above the stomach (Figure 6).

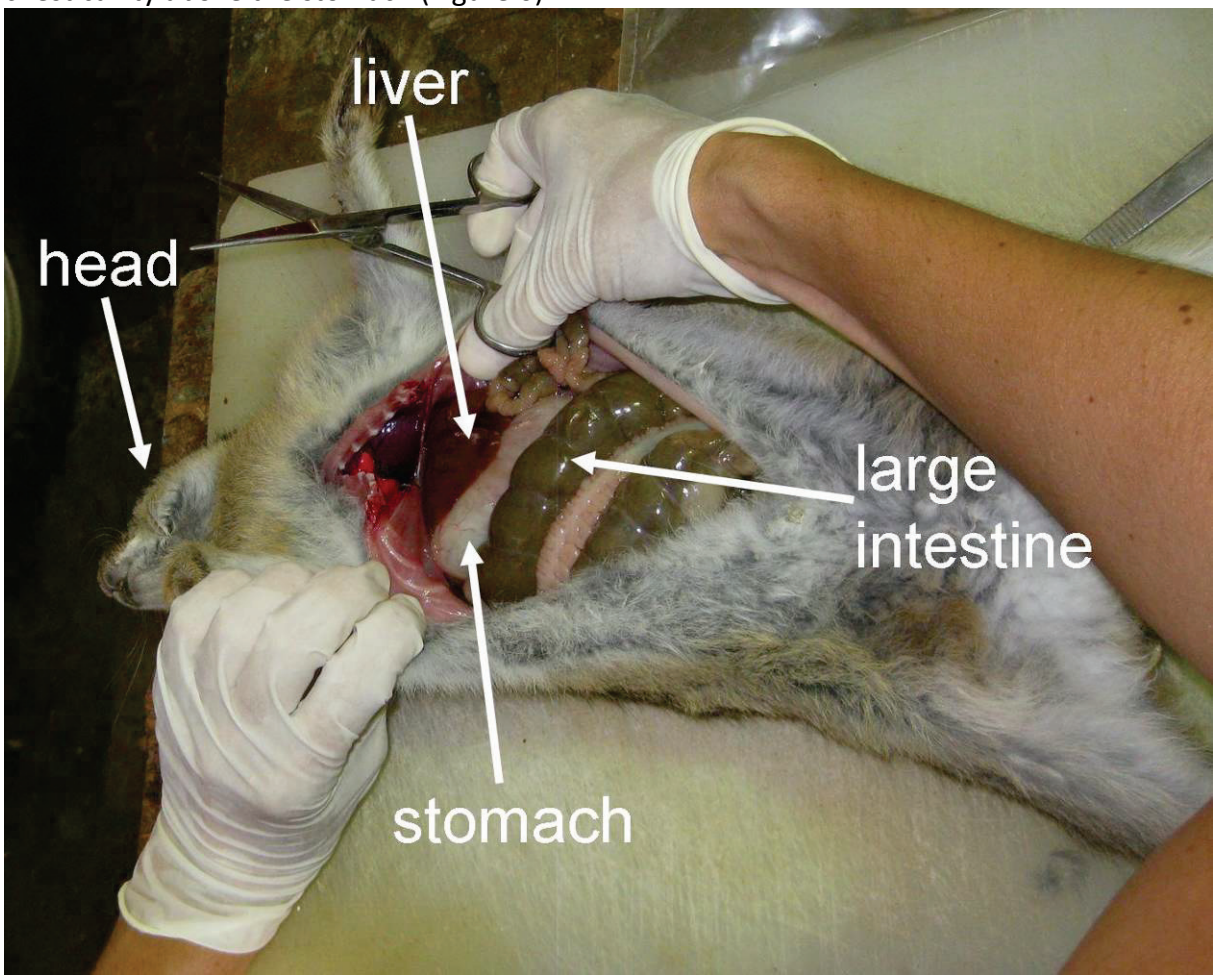

Figure 6: ventral view of a dissected rabbit showing the location of the liver in relation to the stomach and the large intestine (Photo: Brian Lukins).

The liver of a rabbit that has died due to RHD infection will have a cream-coloured reticulated pattern (Figure 7).

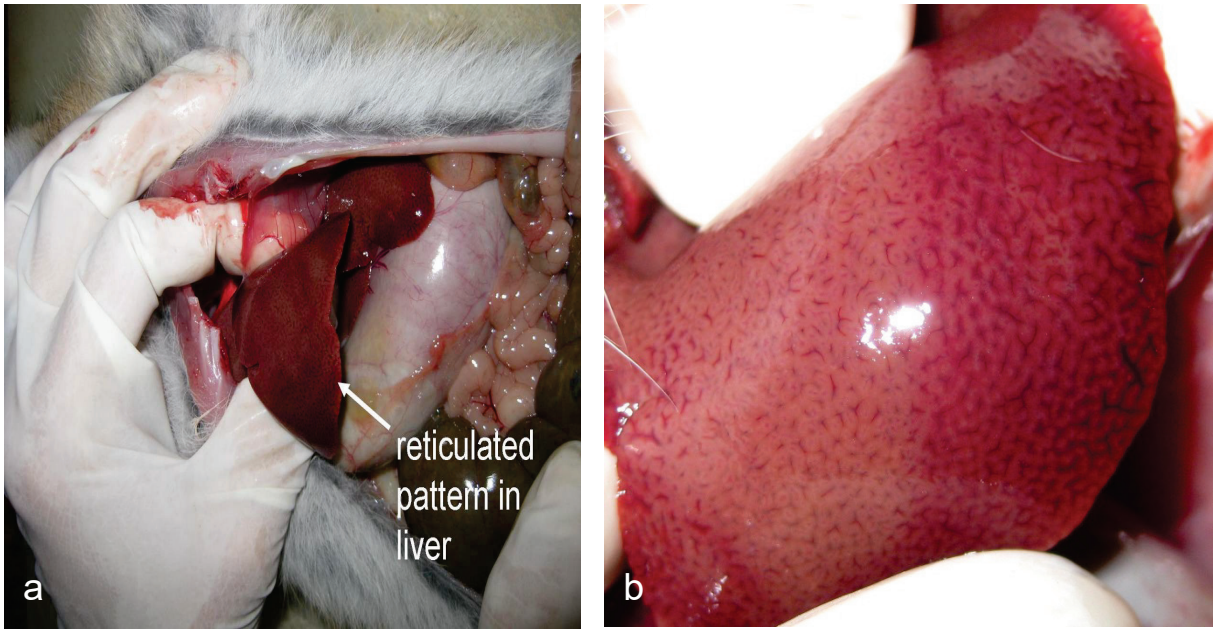

Figure 7: (a) & (b) The cream coloured reticulated pattern of a RHD infected rabbit liver (Photos: Brian Lukins).

Collect one lobe of the liver (Figure 8) and seal in a plastic bag or container. Label the bag or container, including the location and date of collection and store the liver sample in the freezer.

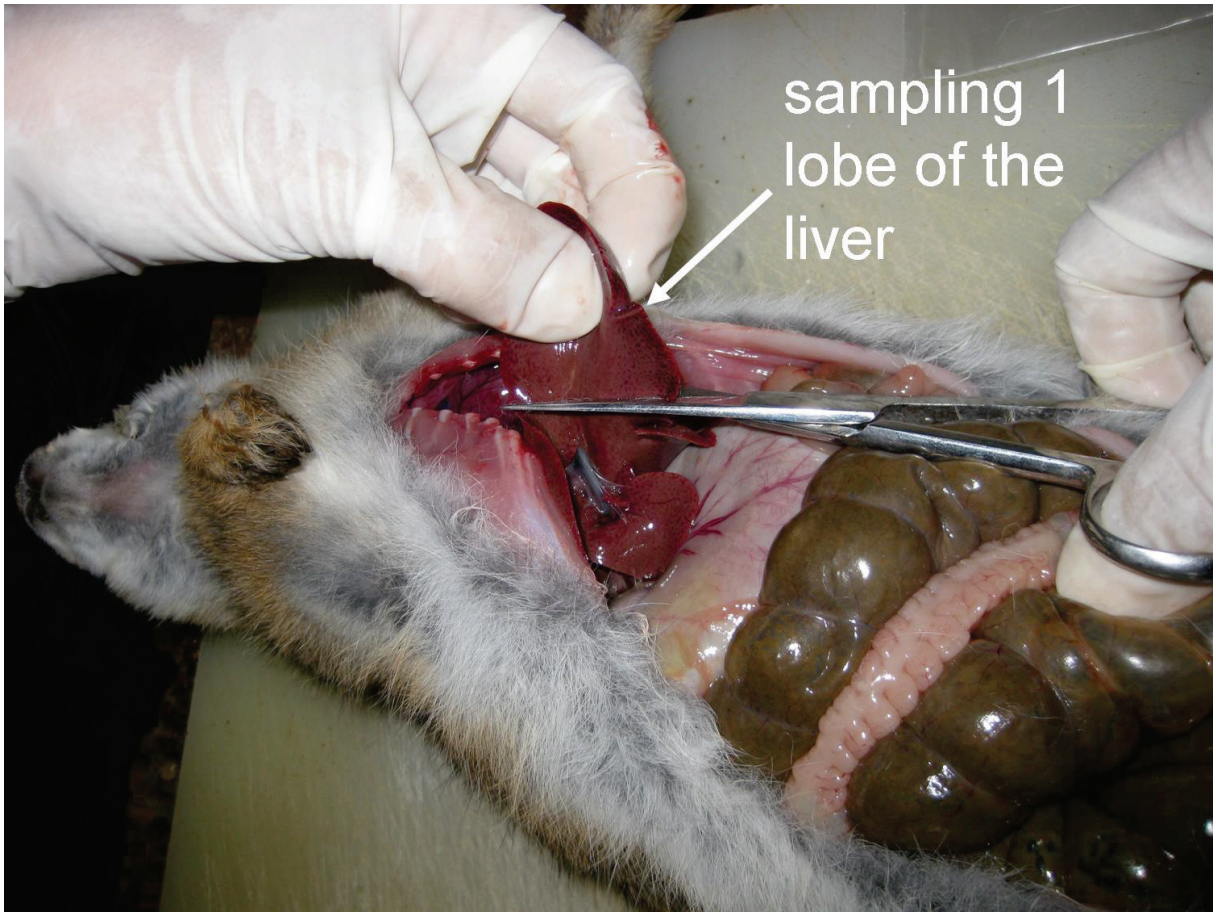

Figure 8: Sampling one lobe of the liver in the rabbit (Photo: Brian Lukins)

For duodenum collection: locate the pyloric junction, which is the junction between the stomach and the duodenum (first part of the small intestine) (Figure 9). Collect a 5cm piece of the duodenum and seal in a plastic bag or container. Label the bag or container, including the sample number, location and date of collection and store the sample in the freezer.

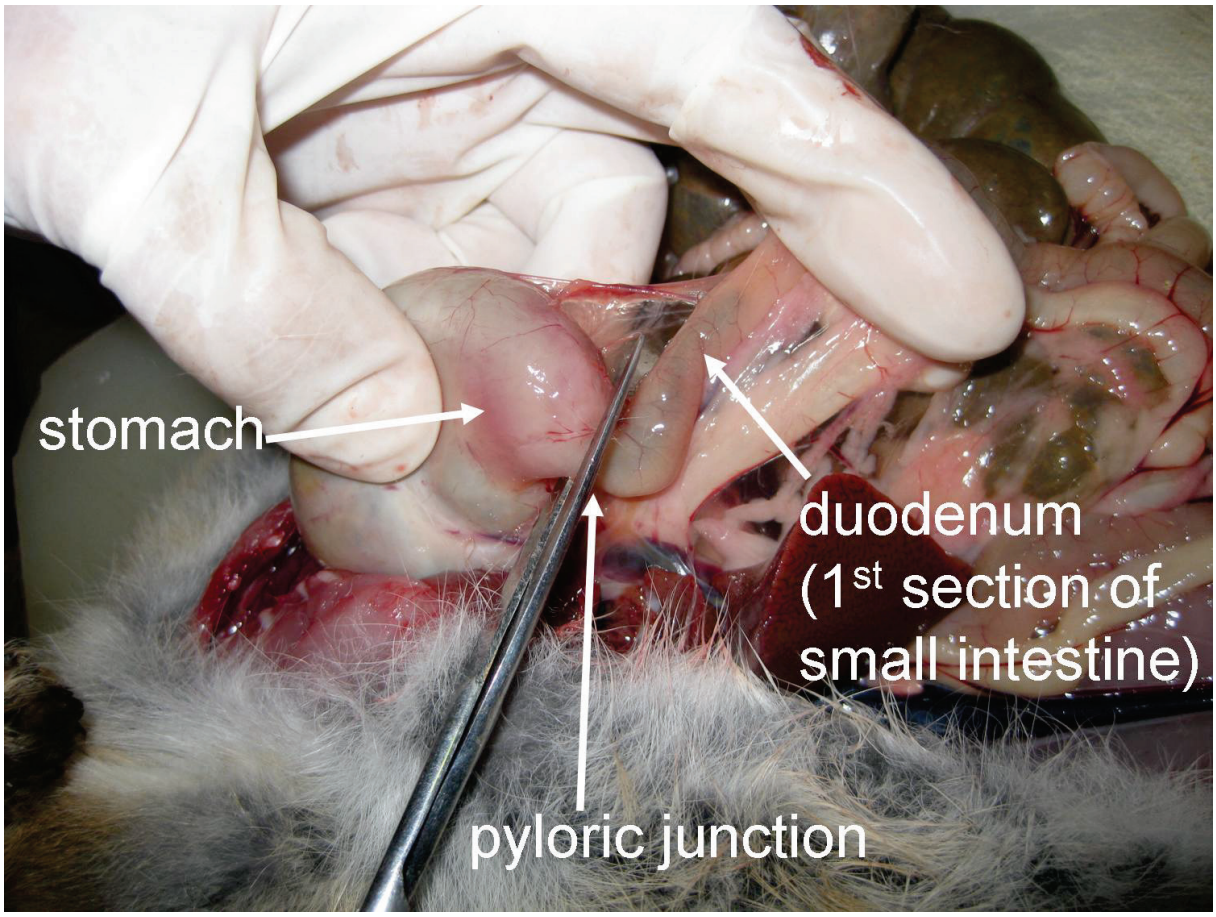

Figure 9: The pyloric junction is where the stomach ends and the duodenum begins. To collect duodenum for RCV-A1 research, a 5cm section of the duodenum from the pyloric junction should be collected (Photos: Brian Lukins).

Appendix 3

Field data sheet

Data collection sheet

Site:

Observers:

Season:

Wind: nil light medium strong **/ Dirn:**

Cloud: nil 20% 40% 60% 80% 100%

Rain: > week / this week / yesterday / today / now

Rain Intensity: light / medium / heavy

Moon: new 1/4 half 3/4 full

Night 1	Night 2	Night 3

Spotlight counts				**Shot sample collection**										
Start Date:			**End Date:**	**Samples collected**										**Comments (disease, pregnancy)**
Transect Length (km):			**Start time:**	**Date**	**Sample**	**Sex**	**Blo**	**Liv**	**Duo**	**Eye**	**Bile**	**Ear**	**Grams**	
Night 1	**Night 2**	**Night 3**	**Comments**		1	M F								
					2	M F								
					3	M F								
					4	M F								
					5	M F								
					6	M F								
					7	M F								
					8	M F								
					9	M F								
					10	M F								
					11	M F								
					12	M F								
					13	M F								
					14	M F								
			← Total per night		15	M F								
Abundance index					16	M F								
Rabbits per kilometre =			Avg (Night 1 + 2 + 3) / length of transect (km)		17	M F								
					18	M F								
					19	M F								
Total rabbits per kilometre					20	M F								