Optimal benefit from RHDV-K5



Introduction

This document presents the hows and whys of getting the best results from a release of RHDV-K5.

As a rule of thumb, RHDV-K5 should be released when:

- RHDV2 is NOT active (RHDV2 is more effective), or
- When rabbits are NOT breeding (if rabbits less than 8 weeks old are infected, they generally survive and develop life-long immunity to RHDV). Rabbit breeding is stimulated by proteinrich, green grass and herbage, so rabbits can be assumed to be breeding if there is actively growing forage available to them.

RHDV-K5 can be a useful part of integrated rabbit control but should be used in conjunction with warren destruction and other targeted control options like fumigation.

Baiting with RHDV-K5 is not a 'silver bullet' itself and, if not done well, can be unrewarding and counterproductive to long-term rabbit control.

Calicivirus strains in Australia

- RHDV1-Czech v351, introduced in 1996
- RHDV2, arrived around 2014
- RHDV1-K5, introduced in 2017

RHDV and rabbits

Strains of RHDV

The original form of the calicivirus (Rabbit Haemorrhagic Disease Virus - RHDV) introduced to Australia in 1996 was the RHDV1-Czech v351 strain. RHDV1-K5 was introduced in 2017. RHDV2 first emerged around 2014 and is now widespread across Australia. Unlike RHDV1, RHDV2 can affect both rabbits and hares.

A benign rabbit calicivirus (RCV-A1) has been present in Australia from before the release of RHDV1.

- RHDV2 is now by far the most common form of RHDV that is naturally circulating and causing death to rabbits in Australia.
- RHDV1-K5 is the strain available for purchase and release in Australia. It does not generally spread far from release sites, due to competition from RHDV2.

Immunity and resistance

Immunity and resistance can arise from:

- **Genetic resistance.** Rabbits and viruses are constantly evolving so rabbits may become more resistant to specific diseases. Levels of genetic resistance are dynamic and vary around Australia.
- Age protection. Young rabbits are resistant to RHDV1 (including K5). Up until three weeks of age rabbits are fully resistant to the effects of RHDV1. This resistance wanes until about 9-12 weeks of age when naïve rabbits are fully susceptible. Young rabbits are susceptible to infection with RHDV2, unless protected by maternal antibodies.
- **Prior exposure.** Animals that survive an infection produce antibodies that can quickly recognise the infectious virus if subsequently re-exposed to it. These antibodies will neutralise the infection and prevent the rabbit from becoming sick. The acquired immunity usually lasts for life.
- Maternal antibodies. A female rabbit that survives a calicivirus infection may pass antibodies on to her young while they are embryos and through milk until they are weaned, giving them temporary protection for up to 10-12 weeks from birth. Young rabbits exposed to RHDV while protected by maternal antibodies can generate their own immunity, lasting for life.

Rabbits visibly affected by, or surviving, myxomatosis will have a weakened immune system and be more susceptible and vulnerable to RHD.

Immunity Terminology

Antigen: Any substance that triggers an immune response, e.g. toxins, viruses and bacteria.

Antibody: Proteins produced by the immune system to bind and eliminate antigens.

Immunity: A body's ability to defend itself from infectious disease through responses that may involve organs, cells and proteins.

Seasonal breeding

Rabbits are 'induced ovulators'; females ovulate in response to the act of mating, which only occurs when they have access to high-protein feed that triggers the release of oestrogen, the female sex hormone.

Typically, feed needs to contain at least 14% crude protein for breeding to occur. Actively growing green herbage is a necessary stimulant for breeding to occur, and the higher the protein level of the feed, the more kittens are born per litter.

Optimising gains from RHDV-K5

When to release K5

Conditions that are most conducive to a successful release of RHDV-K5 are:

- RHDV2 is not already present.
- Rabbits are vulnerable, i.e. high levels of immunity or resistance are not evident.
- Rabbits are unlikely to be breeding, i.e. actively growing green forage is not available.
- The K5 release is part of an integrated rabbit control program.

RHDV2 is the dominant strain of RHDV in Australia. It is very widespread, can infect rabbits at an early age, and is fast acting. RHDV-K5 is a useful adjunct in any areas where RHDV2 is not active. If RHDV2 is already present it will be as effective as RHDV-K5. A 'K5' release would not provide any additional value, and resources could be better applied to other necessary control methods.

Because young rabbits can develop immunity to RHDV, and because breeding is likely to occur whenever green feed is available, RHDV-K5 should not be released when rabbits are breeding or if actively growing green forage is present. Similarly, a release will give disappointing results and be an unproductive use of resources if high levels of resistance or immunity are evident. RHDV-K5 is intended for use as part of an integrated control program involving warren destruction and other targeted control options such as fumigation (see 'Keys to rabbit control' and the 'Glovebox Guide for Managing Rabbits' for more information). It is spread by contact between rabbits and by vectors such as flies, so a release will be most effective when rabbits and vectors are abundant.

To be most effective and reduce the risk of inadvertently increasing immunity levels to RHDV in rabbit populations, RHDV-K5 should only be released in drier months if RHDV is not already present, and as part of an integrated rabbit control program.

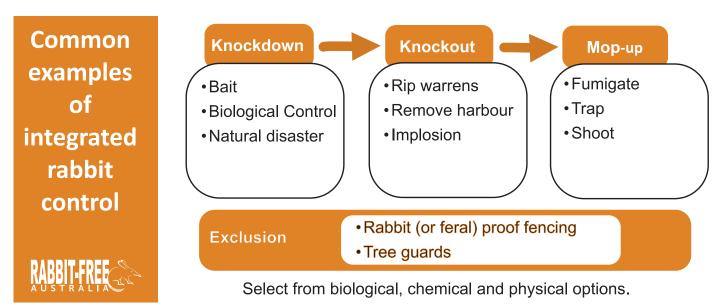
How to release K5

The <u>RHDV-K5 label instructions</u> allow for administration of RHDV-K5 by baiting with medicated feed (intact oats or freshly diced carrots) or intramuscular (IM) injection into rabbits that are returned to the warren from which they came to maximise their acceptance.

Bait delivery of RHDV-K5 is more effective than inoculation in spreading the disease as it directly exposes a greater number of rabbits to the virus. It is also less labour intensive and more humane as rabbits do not need to be caught. IM injection is therefore no longer generally recommended practice. Capture, handling and restraint are significant stressors for wild rabbits that can lead to disruption of social structure, as well as debilitation and sometimes death (NSWRAB SOP, 2022).

In addition, if antibody levels to RHDV (or RHDV2) are high in the population, there is a high likelihood that the captured and injected rabbits are already immune, which means they will not die and are unlikely to transmit the virus. Rabbits cannot be held in traps until their antibody status is confirmed.

Any release of RHDV-K5 must be in accordance with relevant State and Territory Standard Operating Procedures (SOPs), and in accordance with label instructions. Any use of K5 that does not follow label instructions will require an off-label minor use permit from APVMA (the Australian Pesticides & Veterinary Medicines Authority) and is likely to require an animal ethics permit under state animal welfare legislation.



Monitoring rabbit activity and numbers is an important element of rabbit control, helping to direct control activities for maximum effectiveness and efficiency, and as a foundation for follow-up work to ensure sustained success.

Rabbit-virus testing

Testing for rabbit viruses may be undertaken for several reasons using different testing methods.

Purposes

Cause of death. To determine what disease has killed a rabbit it is best to test a small piece of tissue (liver for RHDV or an eyelid if myxomatosis is suspected).

Even if confident of the cause of death from physical signs (see 'Rabbit carcasses' later), submitting tissue samples eliminates any doubt and serves a useful research purpose. Tissue samples help researchers understand the characteristics of viruses and their performance in the field, including which strains of a virus are active and where they are occurring, and how viruses and rabbits are co-evolving.

Tissue testing can be done free of charge by CSIRO, with samples submitted via kits ordered directly from <u>CSIRO</u> or via <u>RabbitScan</u>. Blood and tissue testing can be done for a fee by <u>Elizabeth Macarthur Agricultural Institute (EMAI)</u>, with separate tests required for RHDV1 and RHDV2. With all laboratories there is a necessary delay in getting results due to the time involved in submitting samples, their processing, and the relay of test outcomes. For more information on genetic analysis and guides on how to submit tissue samples, see <u>Rabbit Disease Tissue</u> <u>Testing</u>.

Virus presence of immunity levels. Deciding whether to release RHDV-K5 can be aided by analysing blood samples to determine if the virus is already active or if there are susceptible rabbits about. Blood samples can be tested for the presence of antibodies, indicating that rabbits are, or have been, infected by a biocontrol and hence are likely to be immune.

Methods

RHDV presence or cause of death. Only rabbits that are found dead can be used for these tests. RHDV is relatively quick acting so it is only likely to be found in carcases. While the virus can be detected in the liver of animals that have survived an (initial) infection for some time, this is unreliable as a method to detect current or recent virus activity. Therefore, shot or trapped healthy rabbits are an unreliable source to detect the virus itself.

Myxomatosis presence or cause of death. Only samples from found dead or euthanised rabbits that were clearly infected by myxomatosis, i.e. exhibiting lesions around the eyes, nose or genitals, can be used for these tests. Myxomatosis is slower acting so euthanised rabbits with symptoms may be sampled as well as carcasses. Rabbit carcasses should be placed in a double plastic bag and stored in a freezer while waiting for a test kit. If done as part of a scientific study, euthanasia must be carried out by trained professionals, as required by State and Territory laws, or covered by an animal ethics research licence.

Testing terminology

Serology: Testing blood for the presence of specific disease antibodies, indicating the current or former presence of the specified disease.

ELISA: The Enzyme Linked Immunosorbent Assay is a testing technique used to detect and measure antibodies in bodily fluids.

Seropositive: A blood test result that is positive to the presence of antibodies.

PCR (Polymerase Chain Reaction): A laboratory technique to amplify segments of DNA to detect the presence of specific strains of virus.

RHDV immunity testing. For these tests, blood samples should only come from healthy rabbits collected and killed in accordance with Standard Operating Procedures for the humane destruction of pest animals, such as a headshot by a licensed/ certified shooter. Samples are required from 8–10 different rabbits. Blood samples can be extracted by syringe from the heart.

Immunity or susceptibility tests are not straightforward. Antibody testing complications arise because different tests have different sensitivity and specificity, and getting reasonable estimates on prevalence requires a sufficiently large sample size. For best results liaise with the provider of the diagnostic service regarding sample submission and the interpretation of results.

Rabbit carcasses

Rabbit carcasses can also be used to identify the likely cause of death. RHDV typically leaves dead rabbits with their heads tilted back but few other signs apart from, occasionally, a trickle of foamy blood from the nose. Found dead rabbits can also be dissected to observe tell-tale evidence of calicivirus infection; such as a pale pinkish liver, a darker swollen spleen, or haemorrhagic lungs. <u>'How to' information</u> is available from Rabbit-Free Australia. Myxomatosis symptoms are described above.



Typical RHDV carcass (Image: Peacock D.)



Typical myxomatosis symptoms. (Image: K Patel)

Finding dead rabbits can be difficult as sick animals tend to shelter in burrows and die below ground. They may have to be extracted after detecting the presence of carcasses by blowfly activity at warren entrances or via the 'head down burrow sniff test'. Any found dead rabbits not required for tissue testing can be left in the field, where flies can be attracted and become vectors in the transmission of RHDV. If a rabbit is found that is already decomposed, a (relatively fresh) legbone can also be used for testing (check requirements with CSIRO when requesting a test kit).

Techniques have been developed to test flies for the presence of calicivirus, but further work is required before it can become a mainstream monitoring option.

Welfare and safety

Whether it be the release of RHDV-K5 or collection of rabbit samples for testing, relevant legislation, Codes of Practice, Standard Operating Procedures and label instructions must be adhered to in the interests of animal welfare, human health and safety, and the continuing success of rabbit biocontrols. Accreditation, authorisation and training requirements to handle RHDV-K5 vary by State and must also be complied with.

A general National SOP (NATSOP-RAB009) is available for K5 at <u>pestSMART</u>, along with National SOPs for all <u>rabbit control tools</u>. A general <u>Code of Practice</u> for the humane control of rabbits is also available, including lists of relevant state legislation. Most jurisdictions have equivalent SOPs. The most recently updated SOP for the bait <u>delivery of RHDV</u> comes from the 'NSW Code of Practice and Standard Operating Procedures for the effective and humane management of rabbits' (2022).

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Useful weblinks

- Order tissue test kits from CSIRO (<u>https://research.csiro.au/rhdv/</u> testing/) or RabbitScan (<u>https://www.feralscan.org.au/rabbitscan/</u>).
- Help map sightings of rabbits across Australia with RabbitScan (https://www.feralscan.org.au/rabbitscan/)
- See where recent outbreaks of calicvirus and myxomatosis have been recorded from tissue samples analysed by CSIRO (<u>https://research.</u> <u>csiro.au/rhdv/category/news/</u>)
- Rabbit Management Resources from pestSMART (<u>https://pestsmart.</u> org.au/toolkits/european-rabbits/)
- Rabbit Control from Rabbit-Free Australia (<u>https://rabbitfreeaustralia.</u> org.au/rabbitcontrol/)

More information about Foundation for Rabbit-Free Australia

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