





Rollout of RHDV1 K5 in Australia: information guide

Jason Wishart and Tarnya Cox

An Invasive Animals Cooperative Research Centre Project





















Website: www.pestsmart.org.au

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The 'RHD Boost' research initiative has been delivered through the Invasive Animals CRC, with major financial and in kind resources provided by the Australian government, State governments, and industry and non-government organisations. The partners in this national collaborative project include:

- Australian Government Department of Agriculture and Water Resources
- NSW Department of Primary Industries
- Victoria Department of Economic Development, Jobs, Transport and Resources
- · Primary Industries and Regions South Australia
- Australian Wool Innovation
- Meat and Livestock Australia
- Foundation for Rabbit Free Australia
- · WA Department of Agriculture and Food
- University of Adelaide
- · Queensland Department of Agriculture and Fisheries
- · ACT Transport Canberra and City Services
- · Parks Victoria
- CSIRO

For more information please visit: <u>healthierlandscapes.org.au</u>



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About this Pack

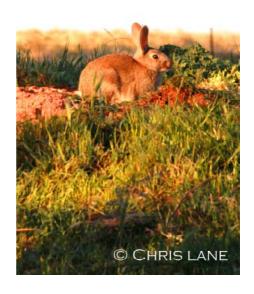
This pack is designed to be a reference guide for your participation in the upcoming release of RHDV1 K5.

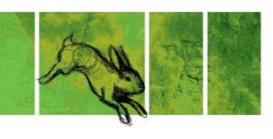
In this pack you will find background information on RHDVs and rabbit biocontrol in Australia, as well as how-to guides for each aspect of the release.

The quick reference timeline is there to help you keep track of where we are up to during the release. Feel free to cut it out of the book and put it on your fridge.

As participants in the program, you have been automatically signed up to our RHD Boost newsletter. We will keep you updated with progress and outcomes of the nationwide release through this newsletter, so keep an eye out for it in your inbox.

The RHD Boost team appreciates your involvement and participation in the release of RHDV1 K5. Thank you.





Rabbits in Australia

Domestic rabbits were brought to Australia on the first fleet. The first wild populations were recorded in Tasmania, where they were numbered in the thousands on some estates by 1827. In 1859, Thomas Austin released 24 wild rabbits on his mainland property, near Geelong Victoria, for hunting purposes. By the 1920s rabbits had spread to all states and territories and their population was thought to be as high 10 billion.

The rate of spread of rabbits in Australia is believed to be the fastest of any colonising mammal anywhere in the world. Rabbits were able to rapidly colonise Australia for numerous reasons, but the use of a warren (protection from elements and predators), their ability to survive in a wide range of habitats and their high reproductive potential were impacting factors. Another reason rabbits were so successful was because there were few parasites and diseases to keep their numbers in check.

Rabbits are estimated to cause over \$200 million dollars in damage to Australian agriculture every year, and they are recognised as a potential threat to at least 304 native threatened species. As a consequence of the damage they cause,



Rabbits around a water trough, South Australia 1938. Image: M W Mules

rabbits are managed wherever they occur. Historically, this was achieved using exclusion fencing and conventional methods such as warren destruction, fumigation and poison baiting. However, broad-scale population level control was not possible until the introduction of two biocontrol agents — myxoma virus (MYXV) and rabbit haemorrhagic disease virus (RHDV).

The Invasive Animals CRC and its predecessor organisations have been successfully leading rabbit biocontrol research in collaboration with government and research agencies and industry partners since the early 1990s.



History of rabbit biocontrol in Australia

MYXV Myxoma virus

RHDV1 Rabbit haemorrhagic

disease virus

RHDV1 v351 Czech variant of RHDV1

used in Australia since

1995

RHDV1 K5 Korean variant of RHDV1

RCV-A1 Benign calicivirus



Myxoma Virus (MYXV)

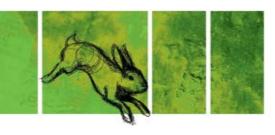
The first field trials of MYXV were undertaken in South Australia's arid zone between 1938 and 1942. The virus killed many rabbits during this time, but it did not spread far. In 1950, the virus was released near Albury NSW, and was more successful. It spread rapidly and by 1954 was present throughout most of Australia's rabbit population, resulting in population declines in excess of 90%. Fleas and mosquitoes are known to be key contributors to its rate of spread the second time.

Within a few years rabbits began to develop resistance to MYXV and their populations began to recover. Today it is estimated that MYXV affects 20 - 40% of the rabbit population and MYXV is still considered to be an important part of integrated rabbit management programs.

Rabbit Haemorrhagic Disease Virus (RHDV1)

RHDV1 was imported to Australia in 1991 to determine its suitability as an additional biocontrol agent for rabbits. RHDV1 causes rabbit haemorrhagic disease (RHD), which is an acute and highly contagious disease that affects only wild and domestic European rabbits (*Oryctolagus cuniculus*). There is no cure for RHD, but there is a vaccine.

The strain of RHDV1 first used to control rabbits in Australia was the Czech 351 strain (RHDV1 v351). In 1995, quarantine trials of RHDV1 v351 began on Wardang Island, South Australia. The virus escaped from the island, likely by flies, and quickly spread across mainland Australia. RHDV1 v351 was officially released the following year and it drastically reduced rabbit populations, although it was less effective in cooler, higher rainfall climates.



Initially, RHDV1 v351 was a suspension formulation that was injected into wild rabbits. In 2006, the use was extended for application on carrots and oats, and in 2015 a new lyophilised formulation (freeze dried) was registered for injection and bait use.

In the 13 years after its release, RHDV1 v351 resulted in nearly \$6 billion of savings associated with reduced rabbit populations and impacts to agriculture. Despite this, rabbit numbers are now increasing due to increasing resistance to RHDV1 v351.



Benign Caliciviruses

Benign or non-disease causing caliciviruses have been present in Australia since the introduction of the first wild rabbits 150 years ago. The benign Rabbit Calicivirus Australia 1 (RCV-A1) was isolated from wild rabbits in Australia in 2009. Varying levels of antibodies to benign caliciviruses have been found in Australian rabbits, although higher levels of antibodies are found in rabbits in higher rainfall areas.

Tests reveal that infection with RCV-A1 reduces the likelihood of death from RHDV1 v351. This explains why RHDV1 v351 was less effective in higher rainfall areas. Scientists are currently investigating the geographic distribution and genetic diversity of this benign strain, and have developed antibody tests to identify its presence in the field.

The cumulative economic benefits for agriculture alone from MYXV and RHDV over 60 years are estimated at \$70 billion.



Invasive Animals CRC rabbit biocontrol program

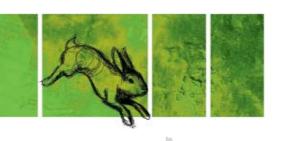
The Invasive Animals CRC (IA CRC) rabbit biocontrol program (2005-2017) is a strategic partnership between the Australian, state and territory governments (particularly NSW and SA), CSIRO, industry through Australian Wool Innovation and Meat and Livestock Australia, University of Adelaide, University of Canberra, University of Queensland, and Rabbit Free Australia. It aims to:

 Discover why RHDV has variable performance in cooler, wetter parts of Australia

- Evaluate, produce and select new RHDV strains to improve rabbit biocontrol effectiveness
- Develop more efficient RHDV delivery systems
- Improve understanding of rabbit genetic resistance and its implications for rabbit biocontrol
- 5. Assess the benefits of additional potential rabbit biocontrol agents.

More information is available at: www.invasiveanimals.com/publications/corporate





HISTORY OF IA CRC RABBIT BIOCONTROL PROGRAM

RHDV1 K5

The proposed national release of RHDV1 K5 to take place to boost rabbit biocontrol in Australia



NEW BIOCONTROL AGENTS

Assessment of new potential rabbit biocontrol agents finished and business case released recommending evaluation of a rabbit parasite (Eimeria) and RHDV2 as two new potential rabbit biocontrol agents



FREEZE-DRIED RHDV

New freeze-dried version of RHDV v351 made available for injection and bait use



RHD ACCELERATOR

RHD Accelerator project demonstrates proof of principle in accelerated evolution of RHDV virus variants, with the aim of producing a continuous supply of virulent RHDV strains



RHD BOOST

Global search for a new strain of RHDV with superior performance in wetter and cooler regions to overcome problems with RCV-A1. 38 strains considered and a selection tested in Australian quarantine facilities. RHDV1 KS was selected





GENETIC RESISTANCE

Genetic resistance by rabbits to RHDV v351 demonstrated



KNOWLEDGE

Co-evolution of rabbit resistance and RHDV evolution knowledge improved



5

RCV-A1

Native benign rabbit calicivirus that impedes effectiveness of RHDV1 v351 in wetter, cooler regions isolated and named RCV-A1. Level of cross protection of RCV-A1 shown to vary nationally, but can be up to 40%



RHDV1 v351

The original RHDV variant released in the mid-1990s, RHDV v351, has new suspension approved for use on carrot and oat baits.





The RHD Boost project

Observations of growing resistance to RHDV1 v351 resulted in the Invasive Animals Cooperative Research Centre (IA CRC) commissioning the RHD Boost project. The objective of the project was for Australian Governments and industry to work together to find a RHDV1 strain to improve rabbit biocontrol effectiveness in Australia. The search and evaluation led to a naturally occurring RHDV1 variant from Korea (RHDV1 K5) being selected for further testing.

RHDV1 K5

RHDV1 K5 is not a new virus; it is a Korean variant of RHDV1 and specific to the European rabbit (*Oryctolagus cuniculus*). The RHD Boost project also found that RHDV1 K5 is likely to work better than RHDV v351 in cool-wet regions of Australia.

Similar to RHDV v351, RHDV1 K5 is a lyophilised formulation that must be reconstituted in distilled water prior to use, and it will be used to either inject live rabbits, or be prepared as carrot or oat bait and fed to live rabbits. Infected rabbits will spread RHDV1 K5 to other rabbits by direct contact or indirectly through faeces and vectors such as insects, specifically bushflies and blowflies.



It is unlikely that RHDV1 K5 will achieve the population reductions that RHDV v351 initially did, as it is not being released into a naïve population. Knockdowns are expected to be on average around 10-15% (ranging from between 0-40%). It is important that RHDV1 K5 is used as part of an integrated multi-technique rabbit management program.

In addition, RHDV1 K5 will be a Restricted Chemical Product (RCP) so its supply will be restricted in accordance with State and Territory legislation (see Persons authorised to access RHDV1 K5 within each state and territory on page 12).





RHDV1 Vaccine

Because RHDV1 K5 and RHDV1 v351 are variants of the same virus (RHDV1), the same vaccine (Cylap®) can be used to protect rabbits from both strains. To further demonstrate this, the NSW Department of Primary Industries implemented a pilot study through the Invasive Animals CRC to determine the suitability of Cylap® to protect domestic and production rabbits from RHDV1 K5.

The experiment compared the mortality of a small number of vaccinated and unvaccinated rabbits that were infected with a high dose of RHDV1 K5. All of the rabbits that were vaccinated with Cylap® survived the infection and none of the unvaccinated rabbits survived. Hence, the experiment indicates that the currently registered vaccine will protect pet rabbits against disease from RHDV1 K5.

The Australian Veterinary Association recommends vaccinating rabbits against RHDV1 K5 and RHDV1 v351 at 10-12 weeks of age, followed by an annual booster and health check. Occasionally, in the face of an outbreak, rabbits may be vaccinated earlier than 10 weeks of age in which case a booster is recommended 4 weeks later.

The vaccine to protect pet and farmed rabbits is available from your local veterinarian.





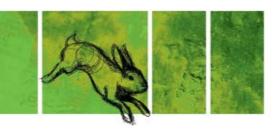
Map: approximate locations where RHDV2 has been detected in both domestic and wild rabbits.

Discovery of RHDV2 in Australia

In 2015, a new RHDV strain was found in wild rabbits in the ACT. It is now present in every state and territory. This virus, referred to as RHDV2, has the same mode of feath as RHDV1 (RHDV1 K5 and RHDV v351), however it is a new strain of RHDV. RHDV2 can cause death in young kittens (11 days old) and the currently available vaccine (Cylap®) is not fully protective against disease from RHDV2.

RHDV2 has been reported to infect certain sub-species of hares in Europe as well as rabbits. In June 2016 it was confirmed in a small number of European Brown Hares in Australia, the only hare species present here. It is currently unclear if these were rare spillover infections from rabbits to hares, or if RHDV2 actually spreads effectively between hares as it does between rabbits.

If the RHDV2 present in Australia is highly virulent, it could benefit rabbit control as it may overcome immunity to field strains of RHDV1 v351. However, if it out-competes the existing strains and replaces them but has lower virulence, leading to increased survival of rabbits during virus outbreaks, it could lessen the impact of current and future biocontrol initiatives using RHDV1 in Australia



The ability to overcome immunity to field strains also means that RHDV2 may be able to overcome vaccination. Preliminary studies from Europe show that the commercially available vaccine at least partially protects against RHDV2, and protection is increased if regular boosters are applied. A revised vaccination protocol to improve protection of rabbits against RHDV2 is available from your veterinarian.

Notwithstanding, it is recommended that the proposed release of RDHV K5 should go ahead and the sampling program around its release should be taken as an opportunity to determine the distribution and potential impact of RHDV2.

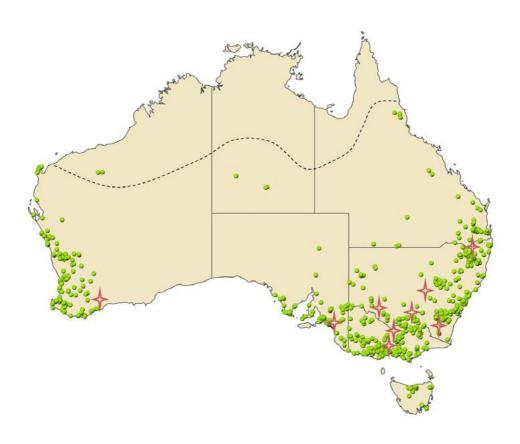
Regulatory approval process for RHDV1 K5 and timeline

The registration and release of RHDV1 K5 requires both national, state and territory approvals. Below is the current state of affairs as of April 2016:

- Commonwealth Quarantine Act 1908: Release from Quarantine was approved in July 2014 by the Commonwealth Department of Agriculture and Water Resources.
- Commonwealth Biological Control Act 1984: In March 2016, Amendments were passed to the Act to clarify the definition of an organism to be consistent with the original intent of the Act.
- Commonwealth Agricultural and Veterinary Chemicals Act 1994 and other associated Acts: In April 2016, RHDV1 K5 was approved as a Restricted Chemical Product.
- Commonwealth The Environment Protection and Biodiversity Conservation Act 1999 (the EPBC Act): In September 2016, the release of RHDV1 K5 was declared "not a controlled action" and did not require further assessment or approval under the EPBC Act.

Now that these approval processes are signed off, the release will be coordinated by operational leads in each state and territory with oversight by the Invasive Plants and Animals Committee.





Release site locations: Green dots indicate successful EOI sites. Stars indicate nationally coordinated intensive monitoring sites. Dashed line indicates approximate northern limit of rabbit distribution.



The Release

During the Expression of Interest process, we received over 700 EOIs encompassing over 900 release sites. It was a fantastic response from the community! Due to the overwhelming response, we increased the number of free vials that were available. After site selection, which was undertaken by each state lead for their state, we have almost 700 sites across the county.

Site selection

Sites were selected strategically in consultation with rabbit experts from each state. Priority was given to sites that were known to be rabbit prone, were not within 50km of the nationally coordinated intensive monitoring sites and were placed to ensure maximum coverage of areas where rabbits are distributed. Monitoring sites were selected from various rabbit affected locations around Australia to enhance spatial replication and ensure RHDV1 K5 effectiveness in various habitats and climates.

Manufacture and distribution

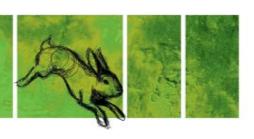
RHDV1 K5 will be manufactured at the Elizabeth Macarthur Agricultural Institute (EMAI), which is the NSW Department of Primary Industries (NSW DPI) Centre of Excellence for Animal and Plant Health. RHDV1 K5 will be delivered to the authorized officers, in each state where monitoring sites are established. A <u>list of authorised officers for each state</u> can be found on page 12.



Persons authorised to access RHDV1 K5 within each state and territory

For the purposes of supply and use of product RHDV1 K5, an 'authorised person' is defined as follows for each State/Territory:

New South Wales	Persons who have undergone appropriate training and been approved as Authorised Control Officers (ACO's) by the Department of Primary Industries.
Queensland	Authorised officers of the Department of Agriculture and fisheries and Local Government.
Victoria	In fee-for-service situations, authorised users include those holding a Commercial Operators Licence with vermin destroyers endorsement or Pest Control Licences authorising the use of pesticides formulated for the control of pest animals. Authorised users for aerial application are those holding a Pilot (Chemical Rating) Licence. In other situations, the use of RHDV is not restricted, meaning it may be used and supplied to any person within Victoria.
South Australia	Authorised officers under the Natural Resource Management Act (2004) who have successfully completed the Vertebrate Pest Management Course, and are suitable qualified officers of the Department of Environment, Water and Natural Resources or Biosecurity SA.
Tasmania	Officers of the Department of Primary Industries, Water and Environment, and persons who hold an Agricultural Spraying Permit issued under the Tasmanian Agricultural and Veterinary Chemicals (Control of Use) Act 1995.
Western Australia	Pest Management Technicians with an appropriately endorsed licence, officers of the Department of Agriculture and Food, officers of Department of Parks and Wildlife and other persons who have undergone appropriate training offered by the Department of Agriculture and Food.
Northern Territory	An authorised person appointed by the NT Chemical Coordinator under the Agricultural and Veterinary Chemicals (Control of Use) Act.
Australian Capital Territory	Authorised ACT Government Officers who have completed appropriate training as determined by the ACT Environment Protection Authority.



State and territory contacts for RHDV1 K5 project

State / Territory	Name	Contact
	Local Land Services	1300 795 299
NSW	Dept. of Primary Industries	02 6391 3834
	Quentin Hart (DPI)	quentin.hart@dpi.nsw.gov.au
Qld.	Dept. of Agriculture and Fisheries	13 25 23
	Peter Elsworth	Peter.Elsworth@daf.qld.gov.au
Vic.	Dept. of Economic Development, Jobs, Transport and Resources	136 186
	John Matthews	john.matthews@ecodev.vic.gov.au
SA	Primary Industry and Regions South Australia	
	Greg Mutze	Greg.Mutze@sa.gov.au
Tas.	Dept. of Primary Industries, Parks, Water and Environment	1300 368 550
	Michael Askey-Doran	michael.askey-doran@dpipwe.tas.gov.au
WA	Dept. of Agriculture and Food	1800 084 881
	Susan Campbell	susan.campbell@agric.wa.gov.au
NT	Dept. of Primary Industry and Fisheries	1800 084 881
	Peter Saville	Peter.Saville@nt.gov.au

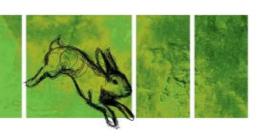


ACT

Transport Canberra and City
Services

13 22 81

Oliver Orgill oliver.orgill@act.gov.au



Key take-home messages

- Rabbits are estimated to cost over \$200 million in lost agricultural production every year. They compete with grazing stock for food, contribute to soil erosion and destabilise the structural integrity of the land potentially leading to injury of livestock.
- Rabbits are linked to the decline of native animals and plant species throughout their range. It is suggested that rabbits impact on 304 threatened species in Australia.
- 3. RHDV1 K5 is a variant of rabbit haemorrhagic disease virus (RHDV1) that causes a fatal haemorrhagic disease in the European rabbit (*Oryctolagus cuniculus*). Importantly, it is specific to the European rabbit.
- 4. RHDV1 K5 is spread by insect vectors, such as bushflies and blowflies. Direct contact between a rabbit and a rabbit carcass with RHDV1 K5 is also an avenue of spread.

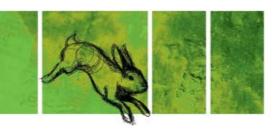
- No variant of RHDV1 (including RHDV1 K5) has ever been found to cause infection in any other animal except the European rabbit However, animals that prey on rabbit carcasses such as foxes, dogs and cats may excrete the virus in their faeces, but will not develop an infection.
- 6. RHDV1 K5 is expected to work in all areas, but success will be variable and based on environmental factors. The greatest benefits are expected to occur in the cool-wet regions where RHDV1 v351 was less effective. An endemic benign calicivirus occurs in these locations and temporarily protects rabbits from RHDV1 v351.
- 7. While it's difficult to predict exact knockdown figures, we do not expect to see population reductions like those seen with the release of RHDV1 v351 in 1996/97. We are not releasing into a naïve population like that in 1996. Knockdowns are expected to be conservative, depending on location and susceptibility of the rabbit population to RHDV1 K5.



- RHDV is one of the more humane methods of controlling wild rabbits. Rabbits basically end up with 'coldlike' symptoms. Post-infection, there may be a rise in body temperature after 24 hours and the animal may exhibit signs of lethargy. In the majority of animals, no symptoms are observed. After the onset of fever, death occurs within 6-12 hours in 70-90% of cases (see *Humaneness* assessment: bait delivery of RHDV). The overall welfare impact prior to death has been assessed as low using the relative humaneness model developed under the Australian Animal Welfare Strategy.
- The existing RHDV1 vaccine has been shown to be effective against RHDV1 K5, however it is advised that pet and meat rabbit owners consult a veterinarian for advice on RHDV prevention and protection.

- Community assistance with the spread of RHDV1 K5 will help ensure that the virus reaches as many rabbit populations as possible and that the best possible knockdown is achieved.
- 11. RHDV1 K5 is not a silver bullet so the community must take advantage of its knockdowns by following up with conventional control tools to achieve sustainable long-term control.
- 12. <u>RabbitScan</u> can be used within your local area to map rabbit problem areas, and to help coordinate control after RHDV1 K5 to maximise the long-term benefits of the virus release.





How-to Guides

This next section includes a series of how-to guides to help you get the most out of your release of RHDV1 K5. More detailed information is also available in video form on the USB that was included in your pack.

All spotlight counts and disease records will need to be submitted via RabbitScan: www.rabbitscan.org.au

RabbitScan is available as a smartphone and tablet app (iOS and Android) or the website can be accessed via your computer. If you have already downloaded the app, please make sure you update the app before you submit your spotlight counts, to ensure you have the latest version.

How to do a spotlight count from a vehicle	
How to do a spotlight count on foot	
How to submit your spotlight count data using RabbitScan	
How to collect samples from a shot rabbit	
How to send your samples	
How to collect a sample from a suspected RHD rabbit	
How to submit your dead rabbit record using RabbitScan	
How to bait with 1080 or Pindone	
How to rip a warren	
How to fumigate a warren	
How to report rabbit sightings, damage and control using RabbitScan	



How to do a spotlight count from a vehicle

When undertaking a spotlight count from a vehicle, be sure to drive the transect during the day before undertaking the spotlight count to ensure you are familiar with the transect.

Transect lengths should be no shorter than 1km (longer is better) and should cover at least 25% of the release area.

Two people are required to undertake a spotlight count—one person to drive and one person to spotlight and record rabbits.

Spotlight counts should be undertaken both before (pre-release counts) and after (post-release counts) the release of RHDV1 K5 so that we can measure the impact of the release. Please check the *Quick Reference Timeline* for details on timing of each of these counts.

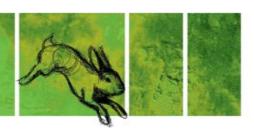
Equipment

- · 4WD vehicle
- 100W (narrow beam) spotlight, either roof-mounted or hand-held
- Pen and paper to record rabbit numbers

Procedure

- Start approximately 30 minutes after sunset from an established starting point
- Drive at a constant slow speed (10-15km/hr)
- Scan in a 180° arc either in front of the vehicle (if counting from the back of a ute) or out of the side of the vehicle (if counting from the cab)
- Record the number of rabbits seen
- Be careful not to count individual rabbits more than once
- Repeat the count completing a total of three consecutive nights of similar weather
- Start all counts as close as practicable to the same time, using the same driver and observer each time
- At the completion of the three nights, upload the count for each night and the length of the transect into rabbitscan as shown in the <u>Howto guide</u> on page 19.





How to do a spotlight count on foot

When undertaking a spotlight count on foot, be sure to walk the transect during the day before undertaking the spotlight count to ensure you are familiar with the transect.

Transects can be any length, however longer is better.

Spotlight counts should be undertaken both before (pre-release counts) and after (post-release counts) the release of RHDV1 K5 so that we can measure the impact of the release.

Equipment

- · Hand held torch
- Pen and paper to record rabbit numbers

Procedure

- Start approximately 30 minutes after sunset from an established starting point
- Scan in a 180° sweeping the torch from side to side as you walk
- Record the number of rabbits seen
- Be careful not to count individual rabbits more than once
- Repeat the count completing a total of three consecutive nights of similar weather
- Start all counts as close as practicable to the same time, using the same observer each time
- At the completion of the three nights, upload the count for each night and the length of the transect into rabbitscan as shown in the <u>How-</u> to guide on page 19.





How to submit your spotlight count data using RabbitScan

Using the App

Step 1: Open up the RabbitScan App and select "Record Rabbits".

Record Report Disease

About Rabbits

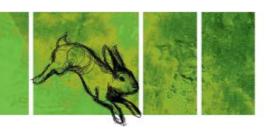
Learn More

PESTSMART

Home Login About Help Pending

Step 2: Select "Enter Sighting"

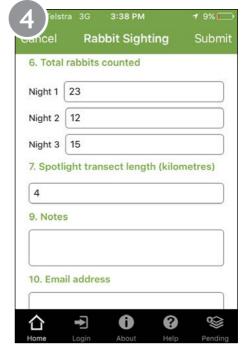




Step 3: Date observed should be the last night of your spotlight count. Tick the box for Question 3 and enter your sight number in Question 4. Your site number can be found on the quick reference magnet that was sent in your kit. Indicate whether this is a pre- or post-release count by answering Question 5.

Step 4: Enter the number of rabbits you counted each night (Question 6) and enter the length of your transect in Question 7. Add any additional notes that you like.

1. Date observed		19/07	/2016
2. Your locatio	n		
Latitude	-33.32121012		No.
Longitude	149.0851	1496	
4. Site Numbe	r (eg. R00	01 or X00	001)
5. Has K5 beer	n released	here yet	?

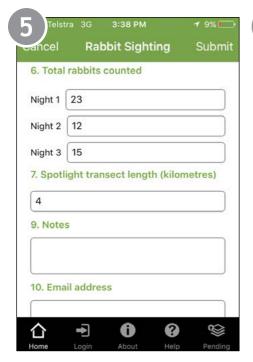




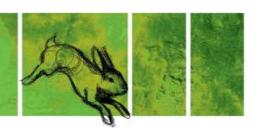
Step 5: If you like, you can upload any relevant photos. Once you've completed the form, select submit to upload your spotlight count data.

Using the Website

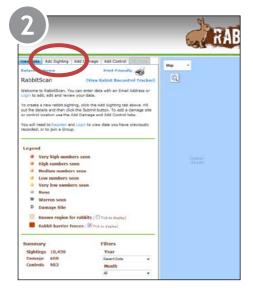
Step 1: Navigate to www.rabbitscan.org.au on your computer and click on "Start Mapping" and log into your account (if you don't have an account you can create one by clicking on "Register" at the top of the screen).







Step 2: Select the "Add Sighting" tab to enter your spotlight data.



Step 3: Place a marker on the map to identify your spotlighting location.



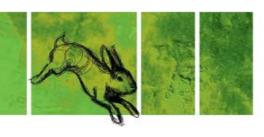


Step 4: Complete all the details on the "Add sighting" tab. Date observed should be the last night of your spotlight count. Tick the box for Question 3 and enter your sight number in Question 4. Your site number can be found on the quick reference magnet that was sent in your kit. Indicate whether this is a pre- or post-release count by answering Question 5.

Enter the number of rabbits you counted each night (Question 6) and enter the length of your transect in Question 7. Add any additional notes that you like.

If you like, you can upload any relevant photos. Once you've completed the form, select submit to upload your spotlight count data.





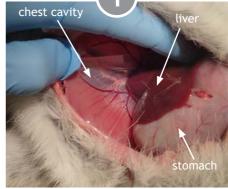
How to collect samples from a shot rabbit

This guide contains information on how to collect liver, blood (for sera) and ear tip samples from shot samples for the broad-scale release sites. Broad-scale release sites should aim to collect samples from 20 shot rabbits prior to the release of RHDV1 K5. All samples except blood should be stored frozen until transport. You have 20 kits in the esky provided. Please use one kit per rabbit and once you have collected the samples (except blood), place them back into the kit bag.

Collecting Blood

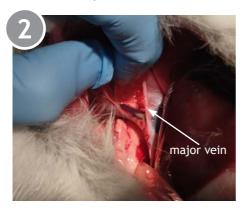
Step 1: Open the rabbit to reveal the stomach, liver and chest cavity







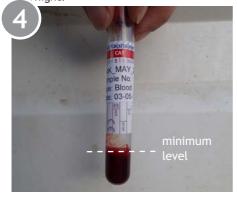
Step 2: Cut into the chest cavity and locate the major vein that runs through the chest cavity. Cut this vein.

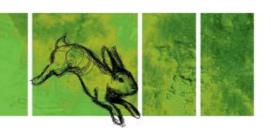


Step 3: Using one of the pipettes provided, collect blood from the chest cavity.



Step 4: Place the blood into the clotting tube. Try to collect at least the minimum level where possible. More is better. Place the tubes in the tube tray provided and let them stand in the fridge overnight.





Collecting Liver

Slice or cut a piece of liver off the side of one of the lobes. Place this into the jar with the yellow lid labeled liver. Place the liver in the freezer until posting.



Collecting Eartip

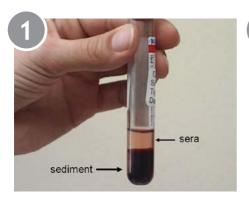
Slice or cut off the top portion of the ear and place it in the microfuge tube labeled eartip. Place the eartip in the freezer until posting.





Collecting Sera

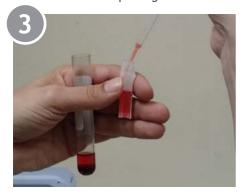
Step 1: After letting the blood sit in the fridge overnight, the sera should separate to the top.

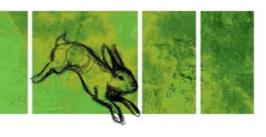


Step 2: Using a clean pipette, carefully extract only the sera from the tube. Try not to collect any sediment. (Sera can be any colour from clear through to red)



Step 3: Gently transfer the sera from the pipette into the small tube labeled sera with the o-ring screw-on cap. Place the sera back into the correct kit bag with the liver and ear tip and put these back in the freezer until posting.





How to send your samples

Please only package up your samples to return on the day you are ready to send them. Confirm with the courier before packing your esky.

Please only send your samples on either a Monday or Tuesday. If you send them too close to the weekend they can sit in the depot and defrost. They will then be ruined.

Step 1: To return your frozen rabbit samples (sera, liver and eartip) and frozen flies (if collected), simply place them back into the styrofoam esky you received your kits in. Line the esky with newspaper or paper, then put in one of the frozen gel packs. Put your frozen samples on top of the gel pack, then cover these with the other frozen gel pack and more paper.



Step 2: Tape the esky closed and affix the address page to the top with tape. Affix the courier note to the side of the esky and contact the courier.





How to collect a sample from a suspected RHD rabbit

This guide contains information on how to collect liver *OR* bone samples from dead rabbits for further analysis. **Please note:** You only need to provide a liver *OR* bone sample per vial, not both.

Please clean your scissors/knife and bench with bleach or warm soapy water between each rabbit.

Please take care when handling scissors or any other sharps during sample collection.

Please ensure you have defrosted the rabbit. This will make sample collection easier.



Safety guidelines for collection of samples

Wild and feral animals may carry diseases that can infect people. Please ensure you are familiar with your state/territory and AVA guidelines, and precautions for personal health and hygiene and to reduce exposure when sampling rabbits.

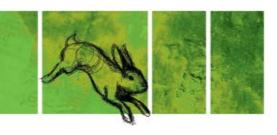
Guidelines to protect yourself against zoonosis are available at:

- Your state/territory health and/or biosecurity department
- AVA—<u>Guidelines for veterinary</u> personal biosecurity (www.ava.com. au/biosecurity-quidelines)

Personal health and hygiene

- Cover cuts and abrasions with a waterproof dressing;
- Wear gloves (provided in your kits);
- Wash and dry hands after handling all samples;
- Do not eat or smoke while handling samples, and ensure that you wash and dry hands before smoking or eating;
- Shower and wash clothing after sampling.

Your kits include a set of latex gloves. Latex can cause skin irritation and allergic reactions such as anaphylaxis, in which case urgent medial aid should be sought.



Seek medical attention immediately if you become ill and a zoonosis is suspected.

Handling samples and disposal

Only people familiar with animal handling and sampling techniques, the risks involved and appropriate safety measures should handle dead rabbits and collect samples for RHDV analysis.

Please take particular care when using scissors, knives or other sharp objects. Ensure the equipment is in good working order and always cut away from yourself.

Place the specimen in a disposable bag, which is then sealed in a disposable airtight container or bag, such as a ziploc freezer bag, and stored in the freezer

Wash all equipment and surfaces with bleach or hospital grade disinfectant after use.

Place all disposable containers, bags, waste and the remainder of the dead rabbit in a bag or container clearly labeled 'biological waste', and place in an external bin.

Handling RNAlater

RNA Later is a non-hazardous substance, however:

- · Always wear gloves
- Do not inhale

- In case of skin contact—wash off immediately with soap and plenty of water
- In case of eye contact—remove contact lenses (if any), rinse thoroughly with plenty of water for at least 15 minutes and consult a physician
- If swallowed rinse mouth with water and obtain immediate medical attention
- Always wash hands immediately after handing RNAlater.

Reporting unusual signs of disease or death in wildlife

To report unusual signs of disease or death in wildlife:

- Contact your state Wildlife
 Health Coordinator (see <u>www.</u>
 <u>wildlifehealthaustralia.com.au/</u>
 AboutUs/ContactDetails.aspx), or
- Ring the emergency animal disease hotline on 1800 675 888.

For advice on human health

If you have any concerns regarding your health or the health of other people incontact with the suspect animal contact your state/territory health department and tell them that you have had contact with a sick animal.

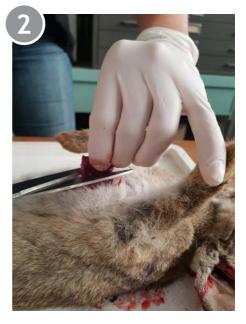


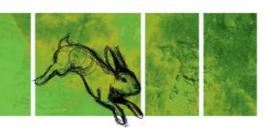
Option 1. Sampling liver

Step 1: Carefully cut open your rabbit carcass and locate the liver. The liver is dark red in colour and located above the stomach.

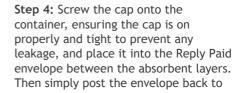


Step 2: Cut off a small piece of the liver (approx. 2cm x 2cm).





Step 3: Insert the liver sample into the sample container. Be careful not to displace the preserving liquid, and ensure the entire sample is submerged in the liquid.









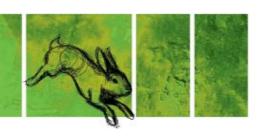
Option 2. Sampling leg bone

Step 1: Select one hind leg of the rabbit, and separate it from the carcass. Remove the fur and flesh, then remove the head of the bone by applying pressure and snapping the bone (the fresher the bone, the easier this is). This is so it fits within the container.

Step 2: Please remove any flesh still on the bone, and insert the bone into the container, broken end first. Be careful not to displace the preserving liquid.

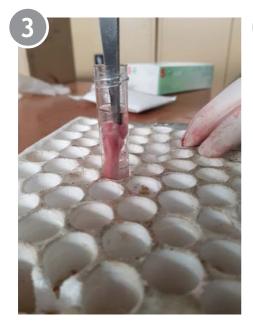






Step 3: Please ensure the entire leg bone is fully submerged in the preserving liquid.

Step 4: Screw the cap onto the container Ensuring the cap is on properly and tight to prevent any leakage and place it into the Reply Paid envelope between the absorbent layers. Then simply post the envelope back to us.







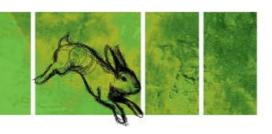
How to submit your dead rabbit record using RabbitScan

Step 1: Open up the RabbitScan App and select "Report Disease".



Step 2: Select which disease you think is present. If you are an official K5 release site, select "Yes" for Question 4. Fill out your site details.





Step 3: If you do not want to submit a sample, go to Step 4. If you do want to submit a sample, select "Yes" and fill in your postal details.

If you are an official release site, please also indicate whether you need a new sampling kit or still have one of the five that were sent to you prior to the release.

Please label the rabbit or sample as per Question 5b and freeze until your kit

Step 4: If you like, you can upload any relevant photos. Once you've completed the form, select submit to upload your disease record.







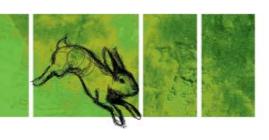
How to bait with 1080 or Pindone

This is a general use guide only. Please check with your local authority on any restrictions and requirements regarding the use of 1080 and Pindone in your area.

Timing of baiting

The best time undertake baiting is when grass is cured and natural feed is limited. Breeding should have ceased and there should be few-to-no young rabbits present in the population.





Poison type

The type of poison you use will be influenced by personal preference, non-target impacts and cost. For broadscale use, 1080 is considered the best option as it requires a single bait night to remove rabbits from the population. Pindone requires a minimum of two feeds of poison bait over 2-3 nights to ensure enough poison to result in death of the rabbit (a single feed of Pindone is unlikely to kill a rabbit).



Bait type

1080 and Pindone for rabbits is registered for use on either carrot or oats. Quite often bait type comes down to personal preference of the user. As long as pre-feeding is undertaken, it should be possible to get rabbits to take either bait.

Bait location

Baiting should be undertaken where rabbits naturally feed. Rabbits do not generally feed on the warren. Look for lawn areas or areas where there are scratching's and diggings to identify feeding areas. These will quite often be at least several meters away from the warren.

Equipment

To undertake broad-scale baiting with either 1080 or Pindone, you will need access to a bait layer. The bait layer has two functions. Firstly it creates a furrow by turning over soil. This turned soil attracts the rabbits to the bait trail. Secondly, the bait layer scatters small amounts of bait across the bait trail allowing more rabbits to access the bait.



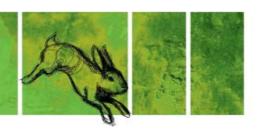
Pre-feeding

For a baiting program to be successful, pre-feeding needs to be undertaken. Pre-feeding (feeding with un-poisoned carrot or oats) allows you to gauge how much bait you need for the poison program, whether you have non-target animals visiting and taking the feed and gives the rabbits the opportunity to discover the bait trail. When undertaking a poison program you want as many rabbits as possible to take the bait.

Poison baiting

Lay poison baits as close to dusk as possible to reduce non-target impacts and to ensure the baits are palatable (particularly when using carrot). Bait for one night when using 1080. Bait for a minimum of four nights when using Pindone. Collect all unused bait and any dead rabbits and dispose of using deep burial.



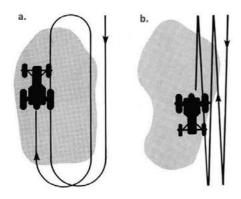


How to rip a warren

Warren ripping should be undertaken when rabbit numbers are at their lowest. For the majority of Australia this is late summer. If rabbit numbers are high, then a baiting program should be undertaken before any ripping. Once the baiting program has been completed, rippers should be in the ground within days to ensure no surface or migrating rabbits take up residence.

Equipment

The type of equipment you use will depend on the landscape, however, at all times you should use the largest equipment the landscape can support. Equipment commonly used includes bulldozers and excavators. Tines should be at least 600mm in length and no more than 500mm apart to ensure complete destruction of the warren.







Procedure

Ripping should be undertaken when grass is cured and soil is friable (to aid in soil flow). Ripping in damp soil will not result in the adequate destruction of the warren.

To ensure your tines are deep enough or your excavator is digging deep enough, it is recommended that you dig a trench to understand the warren system in your area. Warrens can be as shallow as 300mm or as deep as 2600mm, so digging a trench will help you determine the type of equipment required.

There are a number of ways to rip a warren and this may depend on the type of equipment you are using.
Regardless of the approach, the entire warren system needs to be destroyed.
To achieve this, ripping should extend at least 4m beyond the outermost entrance as tunnels within the warren can extend out beyond the entrance.

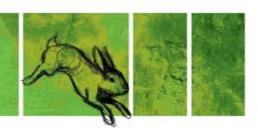
Cross-ripping is also recommended. This is where you rip at right-angles across the ripped warren. This will help ensure complete destruction of the warren complex.



Once you have completed ripping the warren it is important to smooth over or back-blade the ripped warren. This reduces the opportunity for rabbits to dig back in under furrows and raised edges.

Each State/Territory has its own restrictions around the removal of vegetation, earthworks near waterways and activities that may affect cultural heritage values. Please be aware of the requirements in your area before undertaking any ripping or harbour removal program.

Dial before you Dig: 1100



How to fumigate a warren

Fumigation should be undertaken where warrens cannot be ripped, or used as a follow-up tool to treat any re-openings that occur after ripping. Fumigation on its own is not enough to manage a rabbit population.



Equipment

- Suitable respirator for working with toxic gas
- Aluminium phosphide tablets
- Poly pipe
- Water
- Shovel



- Newspaper
- Long rubber gloves
- Overalls
- Warren smoker (where available)



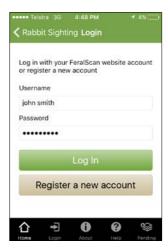
Procedure

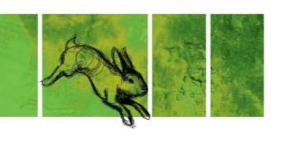
If you have access to a warren smoker, use this to smoke the warren and identify where all of the entrances to the warren are. It is best to let the smoker run for at least 10 minutes (longer for larger warrens) to ensure you can identify all of the entrances.

Step 1: Place the poly pipe down the entrance and seal the entrance with scrunched up newspaper. The newspaper prevents soil from covering the tablets and can help prevent rabbits from digging through the entrance.









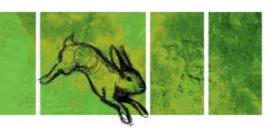






Step 2: Pour the aluminium phosphide tablets down the poly pipe, followed by a generous amount of water. The water will activate the gas production in the tablets. There is not enough moisture in the warren to successfully activate the tablets so this is an important step to ensure successful fumigation of a warren.

Step 3: Pack the entrance with more newspaper to seal the entrance as you remove the poly pipe. Pack the newspaper tightly and then shovel dirt back of the entrance and pack it tight with your foot. Repeat this process for every entrance to the warren.



How to report rabbit sightings, damage and control using RabbitScan

You can use RabbitScan to record information on rabbit numbers, rabbit damage, or any control actions you've undertaken. This can be recorded either via the app on your smart phone or tablet, or via the website.





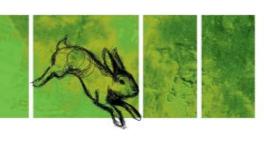
Using the App

When using the app, navigate to the "Record Rabbits" button. From here you can enter information on sightings, including the locations of warrens. damage and control. It is recommended that you set up an account so that you can monitor your entries and therefore your rabbit activity over time. You can set up an account via the app once you select whether you want to record sightings, damage or control. Your phone or tablet will use its GPS to place a marker at your location on the map. Simply complete the details of the form and select "Submit" to upload your information to RabbitScan.

Using the website

Navigate to <u>www.rabbitscan.org.au</u> and either log in to your account or register a new account at the top of the screen. Select the "Start Mapping" button. Select the "Add Sighting", "Add Damage" or "Add Control" tab to enter your information.

Scroll into your location on the map. When you click the map a marker will appear on the map. You can drag this marker to the correct location. Then complete the details of the form and select "Submit" to upload your information to Rabbit Scan.



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PestSmart Factsheet RABFS3: Economic and environmental impacts of rabbits in Australia. Invasive Animals CRC (2012), www.pestsmart.org.au/pestsmart-factsheet-economic-and-environmental-impacts-of-rabbits-in-australia/

PestSmart Factsheet RABFS5: RHD Boost -Enhancing RHDV effectiveness. Invasive Animals CRC (2012), <<u>www.pestsmart.org.au/pestsmart-rhd-boost/</u>>

PestSmart Factsheet RABFS9: The arrival of RHDV-2 in Australia and implications for current rabbit biocontrol initiatives. Invasive Animals CRC (2015), www.pestsmart.org.au/the-arrival-of-rhdv2-in-australia-and-implications-for-current-rabbit-biocontrol-initiatives/

PestSmart Factsheet RABFS10: Rabbit biocontrol in Australia: key facts. Invasive Animals CRC (2015), <www.pestsmart.org.au/rabbit-biocontrol-in-australia-key-facts/>



Weblinks

Visit the PestSmart Toolkit for Rabbits to access this document and listed references online: <<u>www.pestsmart.org.au/pest-animal-species/european-rabbit/</u>>

Boosting rabbit biocontrol—RHDV K5 national release: <<u>www.pestsmart.org.au/boosting-rabbit-biocontrol-rhdv-k5-national-release/</u>>

Register with RabbitScan: < www.feralscan.org.au/rabbitscan>

Subscribe to our mailing list to receive updates: <www.pestsmart.org.au/subscribe/>



