

Standard Operating Procedure RAB001: Innoculation of rabbits with rabbit haemorrhagic disease virus (RHDV)

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Background

Rabbit haemorrhagic disease virus (RHDV) is used to minimise the impact of the introduced European rabbit (*Oryctolagus cuniculus*) on agricultural production and the environment. Other rabbit control methods include poisoning, warren destruction, surface harbour removal, shooting, trapping, exclusion fencing and biological control with myxomatosis.

RHDV causes rabbit haemorrhagic disease (RHD), an acute, highly contagious disease that infects wild and domestic European rabbits (*Oryctolagus cuniculus*). In most adult rabbits the disease progresses rapidly from fever and lethargy to sudden death within 48-72 hours of infection. The virus causes acute liver damage with resultant blood clotting abnormalities. Death occurs due to obstruction of blood supply in vital organs and/or internal haemorrhages. The virus has a high mortality rate, killing upwards of 70% of susceptible rabbits.

The deliberate release of RHDV into wild rabbit populations can be used to initiate outbreaks in an attempt to maximise the impact of the disease. A sample of wild rabbits is collected from a target population, and inoculated with RHDV to induce infection and disease. The rabbits are then released back into the population to spread the virus among uninfected rabbits.

As RHD is now prevalent in the majority of Australian rabbit populations, high levels of

immunity to the virus is expected to occur periodically. Attempting controlled release of the virus in a population of rabbits with high immunity may have minimal impact.

This standard operating procedure (SOP) is a guide only; it does not replace or override the legislation that applies in the relevant state or territory jurisdiction. The SOP should only be used subject to the applicable legal requirements (including OH&S) operating in the relevant jurisdiction.

Application

- Introduction of RHDV is used as part of an integrated approach to rabbit management and is not meant to be used as a stand-alone method.
- Where RHDV is used as a control agent, follow up control of remaining rabbits should be undertaken to ensure long-term effects.
- The inoculation procedure should only be performed by personnel who have received training by a veterinarian. Rabbit trapping and handling should be performed by personnel with training and experience in these procedures.
- Release of RHDV should take into account level of existing immunity, rabbit breeding patterns including presence of young rabbits, natural spread of the virus and insect activity.
- Only healthy, non-pregnant or non-lactating rabbits should be inoculated.





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Rabbits visibly affected by myxomatosis should not be inoculated because they are less likely to spread RHDV.

- Young rabbits less than 8 weeks age are not susceptible to RHDV and should not be inoculated.

Animal Welfare Considerations

Impact on target animals

- In most rabbits, death from RHD is sudden. Some animals show no signs of illness prior to death whilst others will have elevated temperature, anorexia, apathy, dullness, prostration and reddened eyes. Respiratory signs (eg rapid respiration, bloody nasal discharge) and occasionally nervous signs (eg convulsions, paralysis, squealing) may be seen in the later stages. 5 to 10% of rabbits may show a chronic or subclinical course of disease. These animals may have jaundice, weight loss and lethargy for up to 1 to 2 weeks before dying.
- Effective handling and restraint techniques must be used to minimise the risk of injury and to reduce the intensity of distress to the rabbit. Improper restraint, especially of distressed animals, may lead to major and potentially fatal physiological disturbances. Minimising stress on the rabbit is not only essential for the welfare of the animal but is important for effective transmission of the virus. Debilitated, disorientated and distressed rabbits may not re-enter an established warren system and may significantly reduce their normal movements, therefore limiting contact with other rabbits.
- Captured rabbits that have been injured in a way which will threaten their survival should not be released. Such animals should be euthanased either by neck dislocation, or, stunning, with a sharp blow to the back of the head, followed by neck dislocation. Death of the animal should always be confirmed by observing the following:
 - absence of rhythmic, respiratory movements
 - absence of eye protection reflex (corneal reflex) or 'blink'
 - a fixed, glazed expression in the eyes
 - loss of colour in mucous membranes (become mottled and pale without refill after pressure is applied).
- Euthanasia should be performed by trained operators who have practiced on an anaesthetised or dead animal. For more information on euthanasia techniques refer to [GEN001 Methods of Euthanasia](#).

Impact on non-target animals

- All rabbits in Australia are derived from the European rabbit (*Oryctolagus cuniculus*) and are therefore

potentially susceptible to infection. Farmed and pet rabbits should be vaccinated against RHDV. Vaccinations are available from veterinary practitioners. There is no evidence that RHDV causes infection in other species of native and domestic mammals and birds.

Health and Safety Considerations

- Long sleeved, heavy duty overalls and long trousers should be worn to protect the operator from being scratched by rabbits during handling.
- Protective gloves may be used if required, although these may hinder dexterity.
- Operators must be protected by tetanus immunisation in case of infection of scratches and bites.
- Accidental administration of the inoculum to an operator may result in an adverse reaction. Seek medical advice if this occurs.
- Good personal hygiene is encouraged when handling wild animals. Routinely wash hands and other skin surfaces contaminated with virus, blood and other body fluids.
- All needles and syringes should be disposed of in a hard plastic 'sharps' container. Non-sharp waste contaminated with blood and/or virus must be contained in sealed plastic bags. Full sharps containers and other contaminated waste should be disposed of at a biomedical waste disposal facility. DO NOT take contaminated materials to hospitals or veterinary practices for disposal. Contaminated waste must NOT be disposed of at domestic waste facilities.

Equipment Required

- The following items are used for the inoculation procedure:
 - cotton bag for restraining rabbit
 - vials of RHDV inoculum (one vial contains 10ml, sufficient for inoculation of 20 rabbits). Vials are supplied by NSW DPI, Elizabeth Macarthur Agricultural Institute, Woodbridge Road, Menangle, NSW: Phone 02 4640 6333.
 - sterile 1 ml insulin syringes with 27 gauge (0.41 mm x 12.7 mm) needles
 - esky and freezer brick
 - plastic 'sharps' disposal container for disposal of needles and syringes
 - thick lock-seal plastic 'biohazard' bag for disposal of contaminated material (non-sharps)
 - surgical gloves

Procedures

Handling of rabbits

There is a serious risk of spinal injury to the rabbit and scratches to the operator if rabbits are not handled correctly.

- Operators should have experience and be confident in the handling of rabbits.
- Rabbits may kick out and scratch the handler with the claws of their hind feet.
- Rabbits are prone to spinal injury as a result of struggling during inappropriate or prolonged handling. Sudden kicking with the hind legs while restrained can result in fractures of the vertebrae and even paralysis.
- Rabbits should not be lifted by the ears, neck or back legs. Doing so may result in injury to the operator and/or rabbit.
- There are 2 approaches to handling which should be used as appropriate:

Bagging

- This technique should be used for restraint prior to inoculation.
- A strong cotton bag is placed over the rabbit whilst in a trap or whilst it is 'stunned'. The bag securely holds the rabbit without risk of suffocation. It will often rest quietly because its eyes are covered. Use only one rabbit per bag. Expose the back legs, whilst leaving the head covered, during the inoculation procedure.

Scruffing

- This technique should be used when removing rabbits from traps or holding cages.
- Grasp the loose skin on the scruff of the neck and press down on a flat surface initially (preferably until the animal relaxes). Pick up with one hand over the ears and nape of the neck, holding the scruff, the other hand supporting the rump; bring the rabbit in so that it is supported against the handler's body. The rabbit should be cradled in one arm with the head and eyes tucked beneath the upper arm.

Inoculation of rabbits

To avoid needle-stick injury, do not attempt to re-cap the needle after inoculation and always ensure that the rabbit is well restrained.

Surgical gloves should be worn by the operator when loading syringes and during inoculation.

- A minimum of 2 rabbits should be inoculated in each warren. It is preferable to inoculate 20 animals (one complete vial) at each release site.
- The procedure should be performed by two people. The rabbit is restrained by one operator; inoculation is performed by the other.
- Before restraining the rabbit ensure that the syringe is loaded and easily accessible to the person performing the inoculation.
- To load the syringe, invert the vial of virus, puncture rubber seal with needle, pull back plunger to fill syringe chamber to past the dose mark. With the needle still in the vial, turn the syringe so that the needle faces upwards and flick the barrel so that air bubbles rise to the top. Push plunger to expel air and to bring up to the desired dose mark. Remove the needle from the vial.
- Maintain steady restraint of the rabbit during inoculation to prevent leg damage to the rabbit and needle-stick injury to the operator.
- Whilst the head of the rabbit is still in the bag, extend one of its hind legs and allow the rabbit to relax. Locate the biceps femoris muscle at the back of the leg between the hip and the knee and hold it between the fingers so that it is raised and immobilised. Insert needle into muscle mass, taking care not to go too deep or to hit the femur. Inject the contents of syringe with constant pressure over 3-4 seconds. Remove needle and immediately discard intact needle and syringe into sharps container.

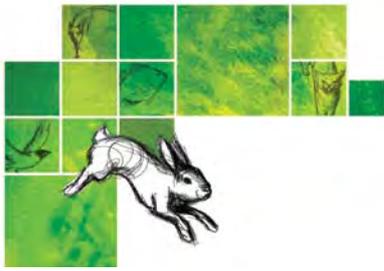
Procedural Notes

Storage of inoculum

- The virus should be stored frozen or refrigerated at or below 4°C.
- Use entire contents of the vial within 48 hours of opening.
- Store open vials at 4°C, do not refreeze.
- Unused vials of virus should be discarded into contaminated waste containers for appropriate disposal.

Disposal of contaminated waste

- All contaminated waste including sharps containers should be conveyed to a disposal facility equipped for the disposal of biomedical waste. Contact your local waste reduction and disposal services for more information.



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Image: John Kovaliski

- A new needle and syringe must be used for each rabbit.
- Release rabbit at or near the warren where it was captured. Rabbits are not to be taken away from the site where they were captured.

Further information

Contact the relevant federal, state or territory government agency from the following list of websites:

- Australian Department of Sustainability, Environment, Water, Population and Communities
<http://www.environment.gov.au/>
- Australian Department of Agriculture, Fisheries and Forestry
<http://www.daff.gov.au>
- ACT Territory and Municipal Services Directorate
http://www.tams.act.gov.au/parks-recreation/plants_and_animals/invasive_species
- NSW Department of Primary Industries
<http://www.dpi.nsw.gov.au>
- NT Department of Land Resource Management
<http://lrm.nt.gov.au/>
- Qld Department of Agriculture, Fisheries and Forestry
<http://www.daff.qld.gov.au/>
- SA Biosecurity SA, Department of Primary Industries and Regions
<http://www.pir.sa.gov.au/biosecuritysa>

- Tas Department of Primary Industries, Parks, Water and Environment
<http://www.dpiw.tas.gov.au/>
- Vic Department of Primary Industries
<http://www.dpi.vic.gov.au/>
- WA Department of Agriculture and Food
<http://www.agric.wa.gov.au>

Also refer to:

Invasive Animals Cooperative Research Centre
<http://www.invasiveanimals.com/>
or <http://www.feral.org.au>

References

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