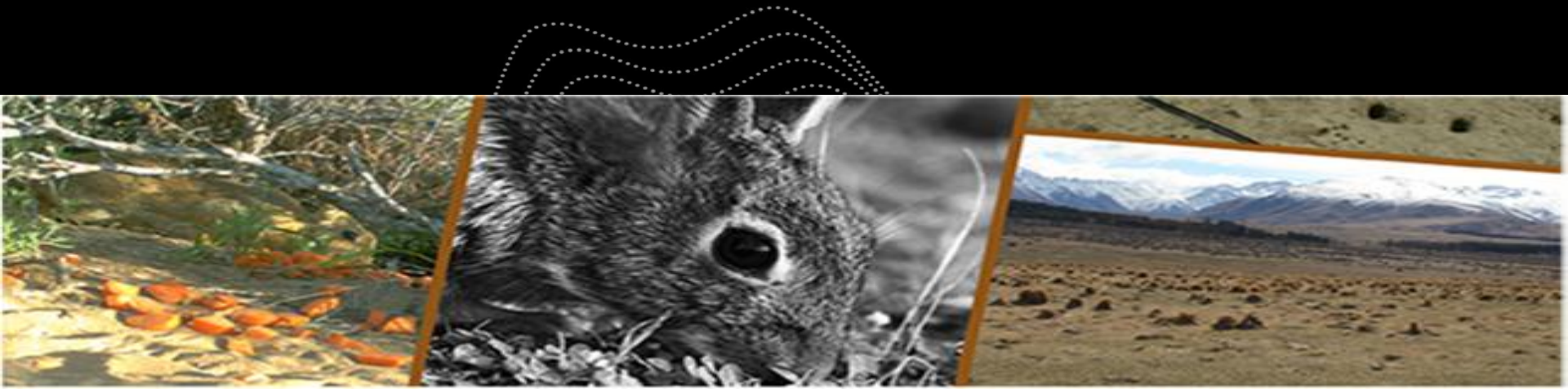




Manaaki Whenua  
Landcare Research

# Rabbit caliciviruses for biological control of rabbits in New Zealand – the good, the bad and the useful

Janine Duckworth





# Rabbits as a pest in New Zealand

- Rabbits were introduced in the 1830's and spread rapidly
- Rabbit plagues first recorded in 1870's, farms abandoned
- Periodically reach very high numbers in rabbit prone areas

## Wild rabbits:

- Compete with livestock for pasture
- Cause extensive land damage, erosion and reduce water quality
- Support high predator populations that prey on native wildlife
- Damage buildings and property, cause financial hardship

## Rabbit control:

- Shooting, poisoning, fumigation of burrows, rabbit-proof fencing
- Attempt to introduce myxomatosis was unsuccessful, lack suitable vectors
- Rabbit Haemorrhagic Disease Virus introduced in 1997



# Rabbit Caliciviruses



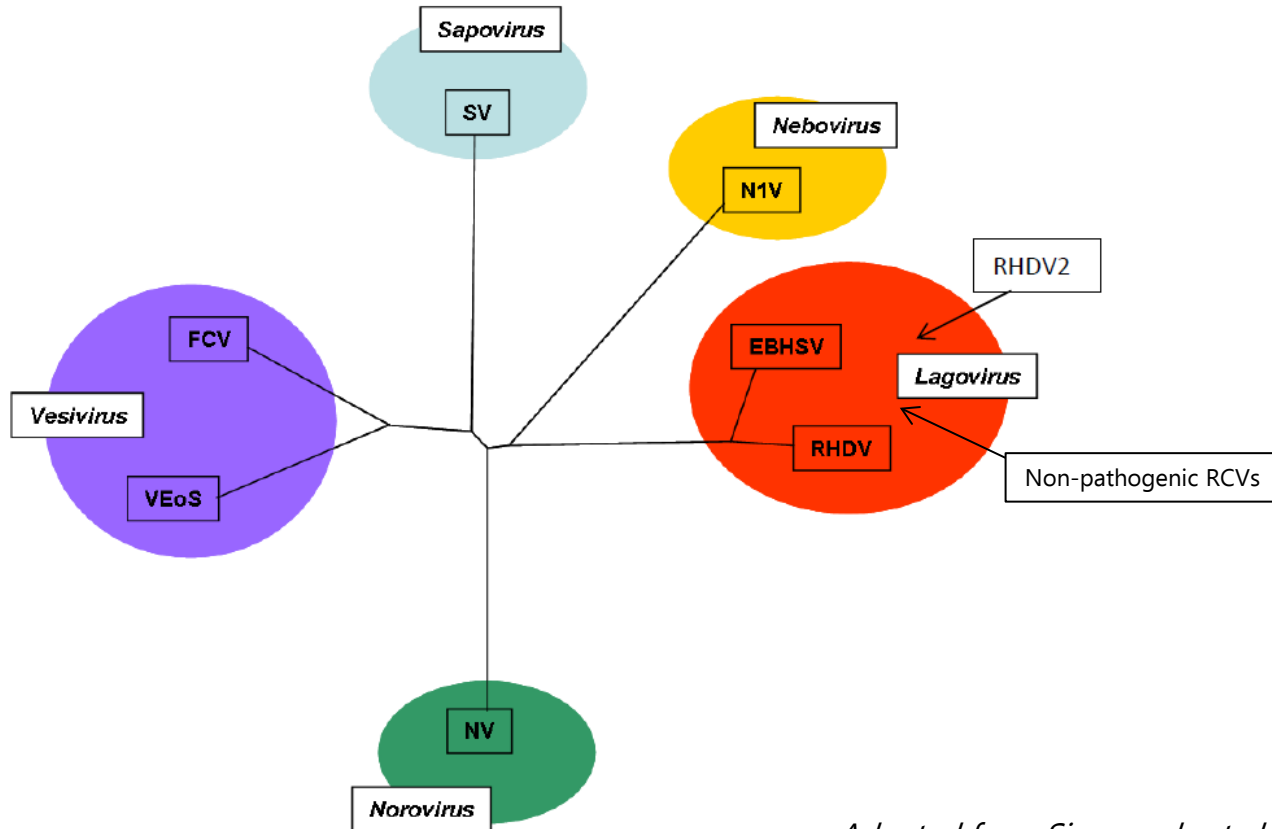
Classification:

Family: Caliciviridae

Genus: Lagovirus

Species: was 2 now 4

(includes Rabbit Haemorrhagic Disease Virus (RHDV))

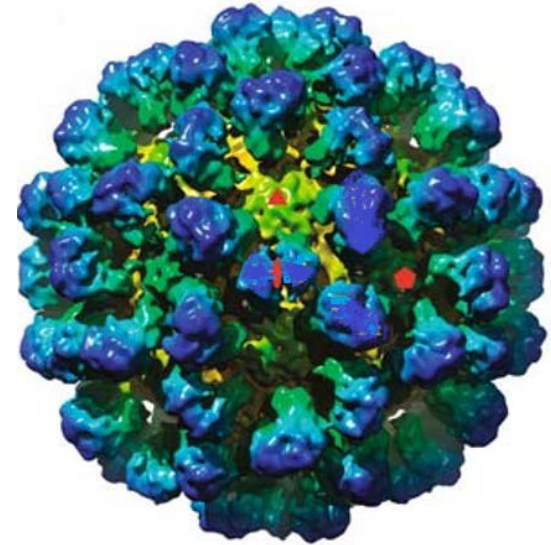


*Adapted from Simmonds et al. 2008*

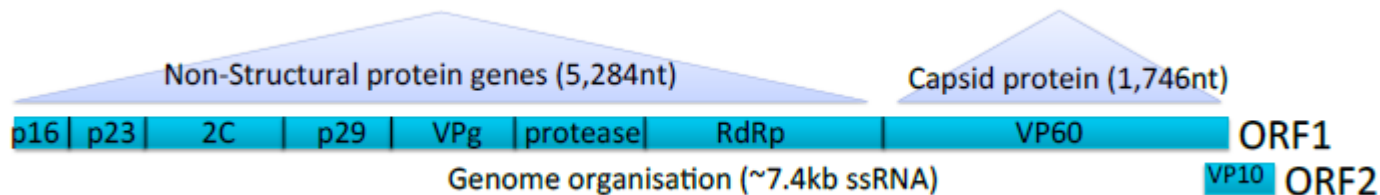


# Rabbit Haemorrhagic Disease Virus or RHDV

- Identified in China in 1984
- Kills only European rabbits
- Positive-sense, single-stranded RNA virus  
~ 7.4 kB
- Non-enveloped capsid
- Virion 35-40 nm in diameter



*from Hu et al. 2010*



*Adapted from Mahar et al. 2016*



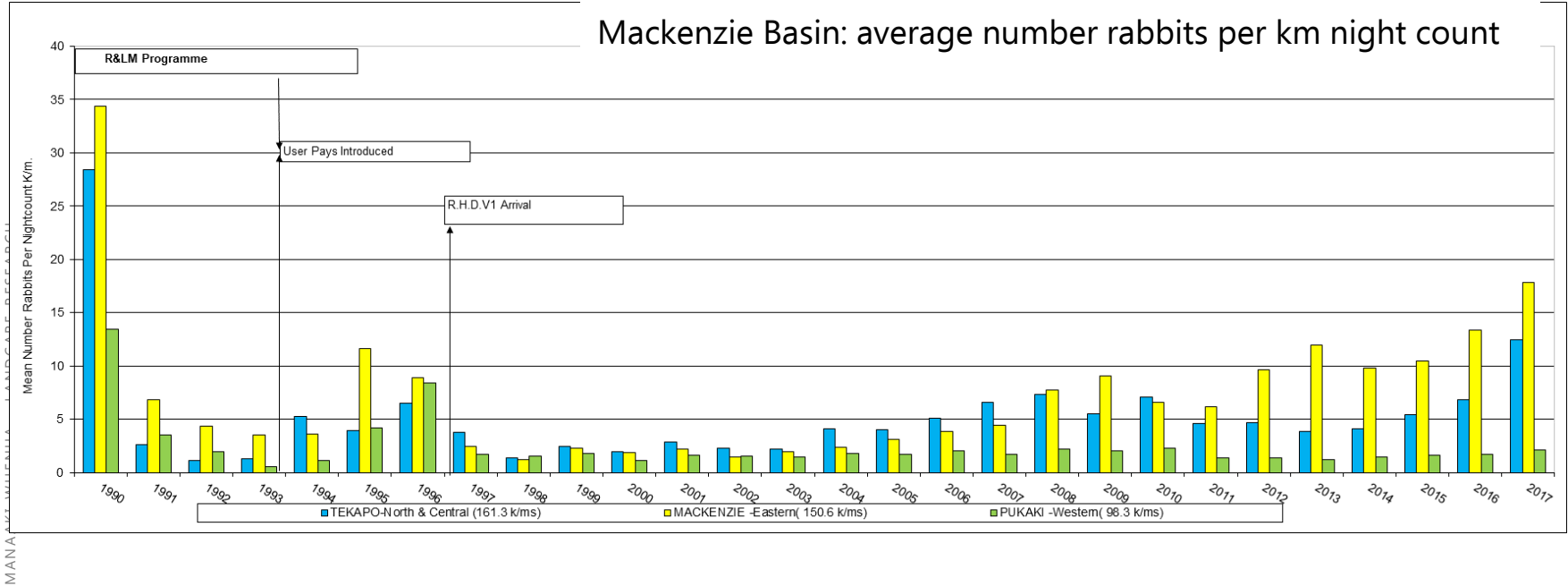
# Rabbit Haemorrhagic Disease Virus (RHDV)

- Rabbit-specific virus
  - Spread by rabbit contact with infected rabbits and carcasses, with contaminated feed, soil, faeces or bedding material or by flies or biting insects that can carry the virus
  - Naïve rabbits usually die
    - within 48-72 hours of infection
    - within 6-18 hours of onset of symptoms
    - Hepatitis and massive DIC
  - Vaccine available for domestic rabbits
  - RHDV1 Czech strain released in 1997 was initially high kill >70%
  - Cost effective, versatile
  - RHDV1 outbreaks continue to occur but overtime effectiveness has decreased





# Rabbit population trends 1990-2017



Environment Canterbury - Biosecurity



# Immunity to RHDV

Increasing proportion of rabbit populations have antibodies that protect against subsequent infection with RHDV1 Czech v351

Rabbits acquire immunity due to:

- Exposure to RHDV1 at a young age (rabbits <8-9 weeks survive exposure)
- Maternal antibodies in utero or via lactation (protect up to ~8-10 weeks)
- Surviving infection with normally lethal dose (<1-5%)
- Cross-protection from antibodies following infection with benign, non-pathogenic rabbit calicivirus (eg. RCV-A1)
- Genetic changes in the virus or the rabbit





## RHDV Research 2012-2016:

Aiming to:

1. Discover if non-pathogenic rabbit caliciviruses exist in New Zealand and potential role in immunity (Leila Nicholson, PhD thesis)
2. How has RHDV evolved since its release? Could we identify existing RHDV strains in New Zealand that might overcome immunity and improve kill rates

*In collaboration with Tanja Strive CSIRO with funding from the Invasive Animal CRC and SFF MPI Rabbit Biocontrol Initiative Project*



Ministry for Primary Industries  
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*Sustainable Farming Fund*





## Non-pathogenic rabbit caliciviruses

- Non-pathogenic RCVs have been isolated in Italy, France, England and Australia
- Found in the gut (duodenum) and transmitted by faecal-oral route
- Can elicit a cross protective immunity against RHDV – act as a vaccine
  - French benign RCV - not protective
  - Australian and Italian benign RCV cross-protective (30-80%)
- Benign RCV strain predicted to be in New Zealand as antibodies cross-reactive to RHDV antibody assay were present in sera collected before the arrival of RHDV1 Czech v351
  - 5.7% adult rabbits in June 1997 in Otago antibody positive (O’Keefe *et al.* 1999)



# 1. Finding non-pathogenic RCVs in New Zealand

## Searched wild rabbit serum for RCV antibodies

Location	Sample number	Date collected	RCV Positive serum 1:10
Mackenzie Country- Pukaki Flat , Simon's Hills	30	11/2012	17%
Mackenzie Country- Tekapo River, Iron Bridge	30	10/2012	52%
Otago- Cromwell, Bendigo Station	31	03/2013	87%
Otago- Taras, Ardgour Valley, Cloudy Peak	30	03/2013	63%
Otago- Wanaka, Queensberry Hills	31	03/2013	35%
North Canterbury- Leslie Hills Station	29	09/2013	38%
Wellington- Orongorongo Valley	9	12/2012	0%
Bay of Plenty- Te Puke/Pongakawa	20	05/2013	100%
Marlborough- Molesworth Station, Isolation Flat	30	06/2013	0%
Southland- Gore, Wantwood Station	30	05/2013	97%
Hawkes Bay- Cape Kidnappers	30	06/2013	93%
Wanganui- Mangapurua Valley	10	09/2013	0%
Otago- Cromwell, Bendigo Station	30	02/2015	80%
Canterbury- Selwyn, Motukarara	10	2013-2014	0%
Total	350		<b>54%</b>



- RCV-A1 specific ELISA (Liu *et al.*, 2010) cross-reacted with NZ RCV
- RCV antibodies widespread and show regional differences



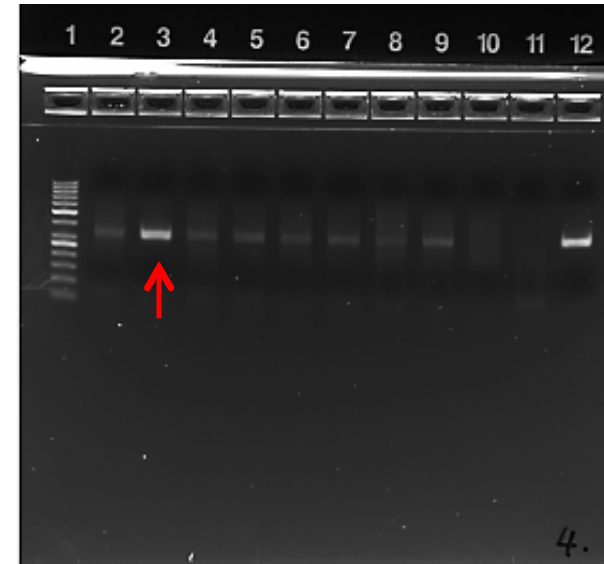
# Finding the RCV virus in New Zealand

- Identified regions where RCV Abs prevalent
- Extracted RNA from small intestine (duodenum)
- Targeted younger rabbits (<35 weeks)
- Universal Lagovirus PCR >180 samples

In 2015 identified positive sample

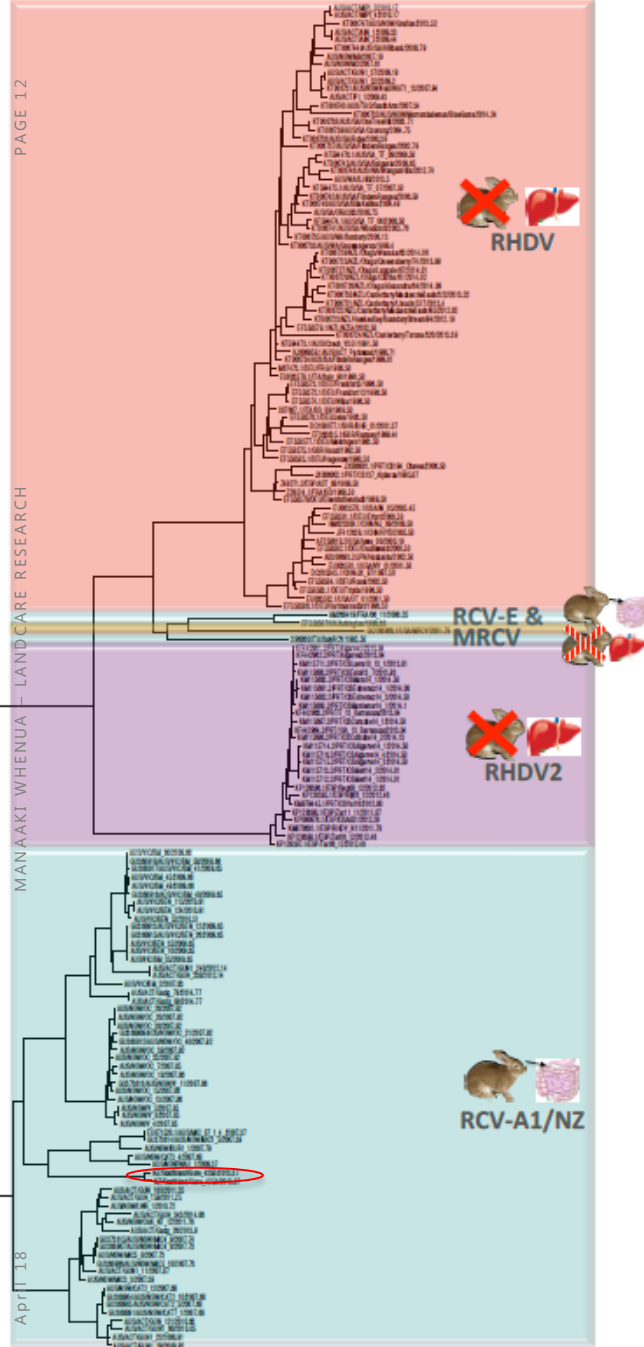
- 33 week old male rabbit from Southland
- Two NZ white rabbits dosed orally with homogenised duodenal tissue and euthanised 4 days later
- Amplified capsid region (approx 1740 nt) using two primer sets (Jahnke et al. 2010) on Illumina Miseq platform

Confirmed the existence of a new rabbit calicivirus for New Zealand (Nicholson et al. 2017)





# Phylogenetic Analysis



- New Zealand RCV is very closely related to the RCV-A1 from Australia – time to most common ancestor to late 1980s
- RCV-A1 and RHDV separated over 100 years ago
- Pathogenicity appears to be related to tissue trophism



Non-pathogenic RCV found in the gut



Pathogenic RHDV found in the liver

- Confirmed that NZ RCV is a non-pathogenic strain (Leila Nicholson, PhD thesis)



## RHDV Research 2012-2016:

Aiming to:

1. Discover if non-pathogenic rabbit caliciviruses exist in New Zealand (Leila Nicholson PhD thesis)
2. How had RHDV evolved since its release? Could we identify existing RHDV strains in New Zealand that might overcome immunity and improve kill rates?

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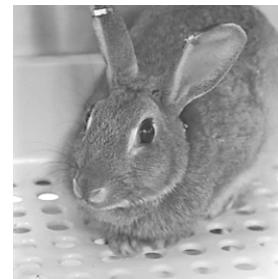
*Sustainable Farming Fund*



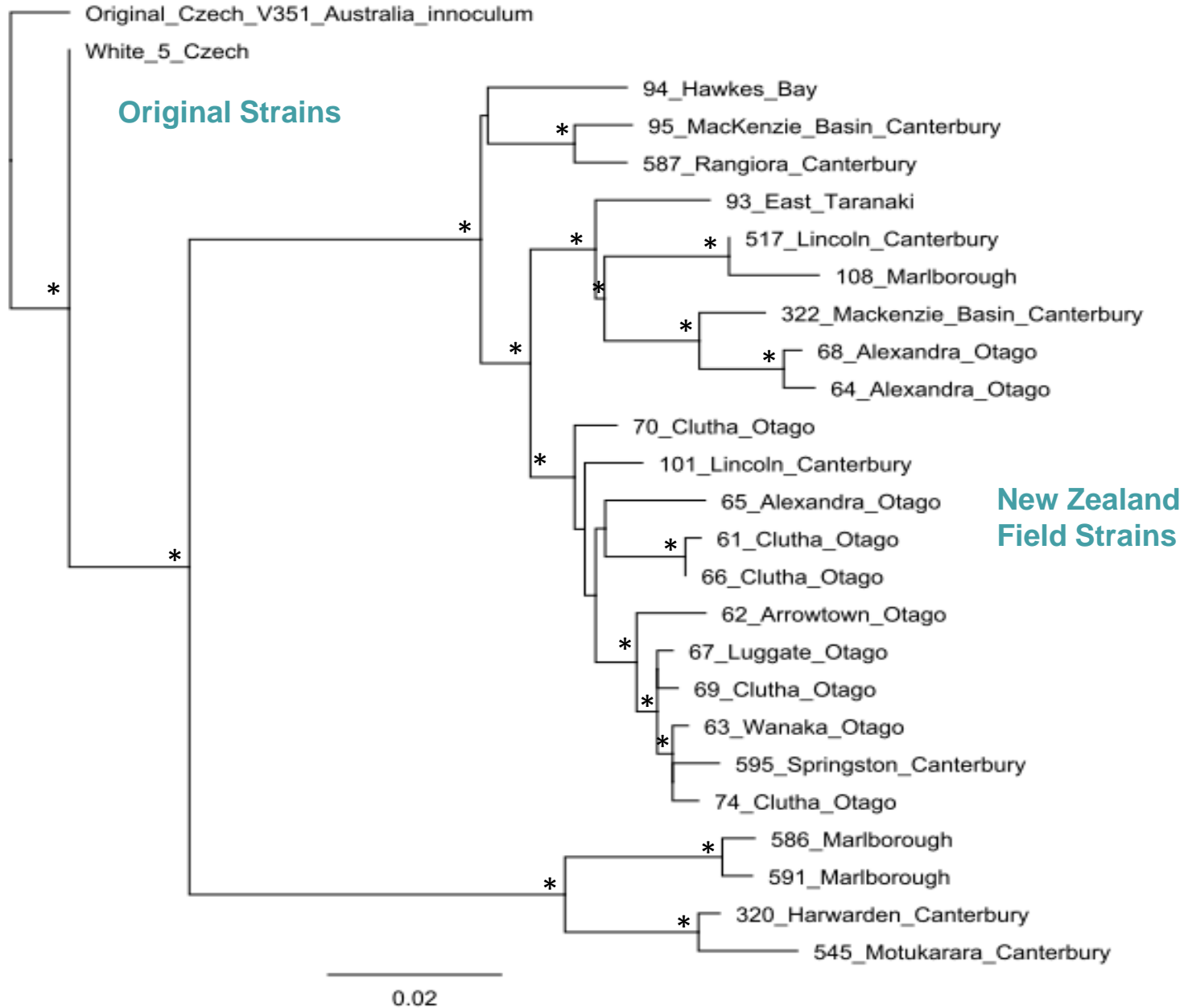
## 2. Investigating New Zealand RHDV1 Strains



- Collected 37 New Zealand RHDV1 strains
- Passaged 12 strains through rabbits to confirm viability and produce high quality inoculum
- Selected 8 strains for virulence testing



# Genotyping of NZ RHDV Strains



# Genotyping of NZ RHDV Strains



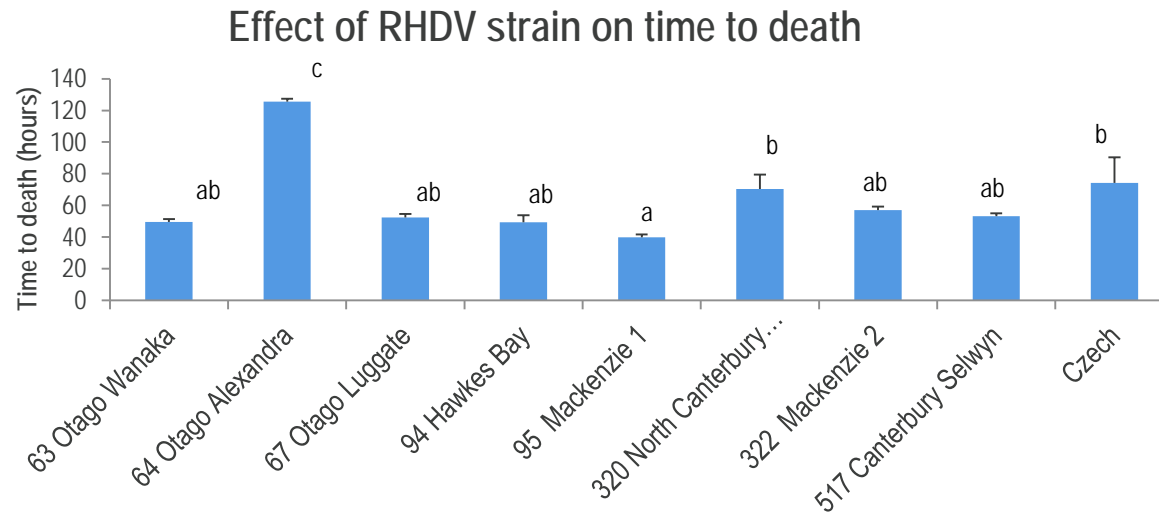




# Virulence testing of New Zealand RHDV strains

Domestic rabbits were orally dosed with 8 NZ RHDV1 field strains and compared to RHDV1 Czech strain

- Kill rates were 100% except Czech (7/8) and 64 Otago Alexandra (4/5)
- Produced  $1.1-4.5 \times 10^8$  virus copies per mg liver



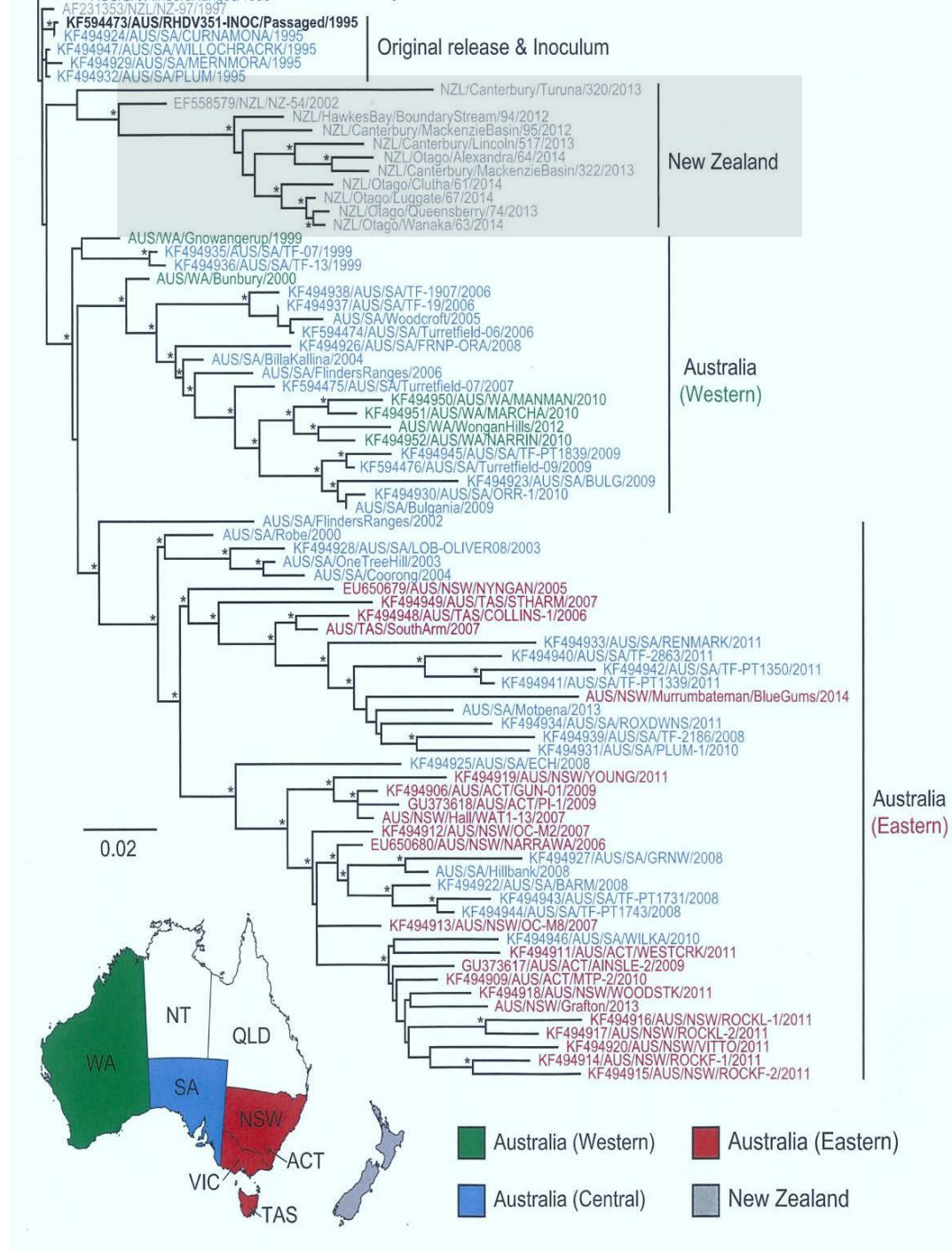
- Time to death for 95 Mackenzie was significantly faster than for Czech – potential high virulence strain

# Phylogenetics of New Zealand RHDV1 Field Strains

NZ and Australian strains:

- originated from the same strain
- have evolved over time and now genetically distinct
- no further incursions

John-Sebastian Eden *et al.* 2015





# RHDV Research 2012-2016:

Aiming to:

1. Discover if non-pathogenic rabbit caliciviruses exist in New Zealand (Leila Nicholson, PhD thesis)
2. How had RHDV evolved since its release? Could we identify existing RHDV strains in New Zealand that might overcome immunity and improve kill rates?



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# RHDV Research 2012-2016:

Aiming to:

1. Discover if non-pathogenic rabbit caliciviruses exist in New Zealand (Leila Nicholson PhD)
2. How had RHDV evolved since its release? Could we identify existing RHDV strains in New Zealand that might overcome immunity and improve kill rates?

*Australian researchers identified RHDV1 K5 as a potential improved strain for rabbit biological control and subsequently gained approval for a Australian-wide release in March 2017*



# Rabbit Caliciviruses

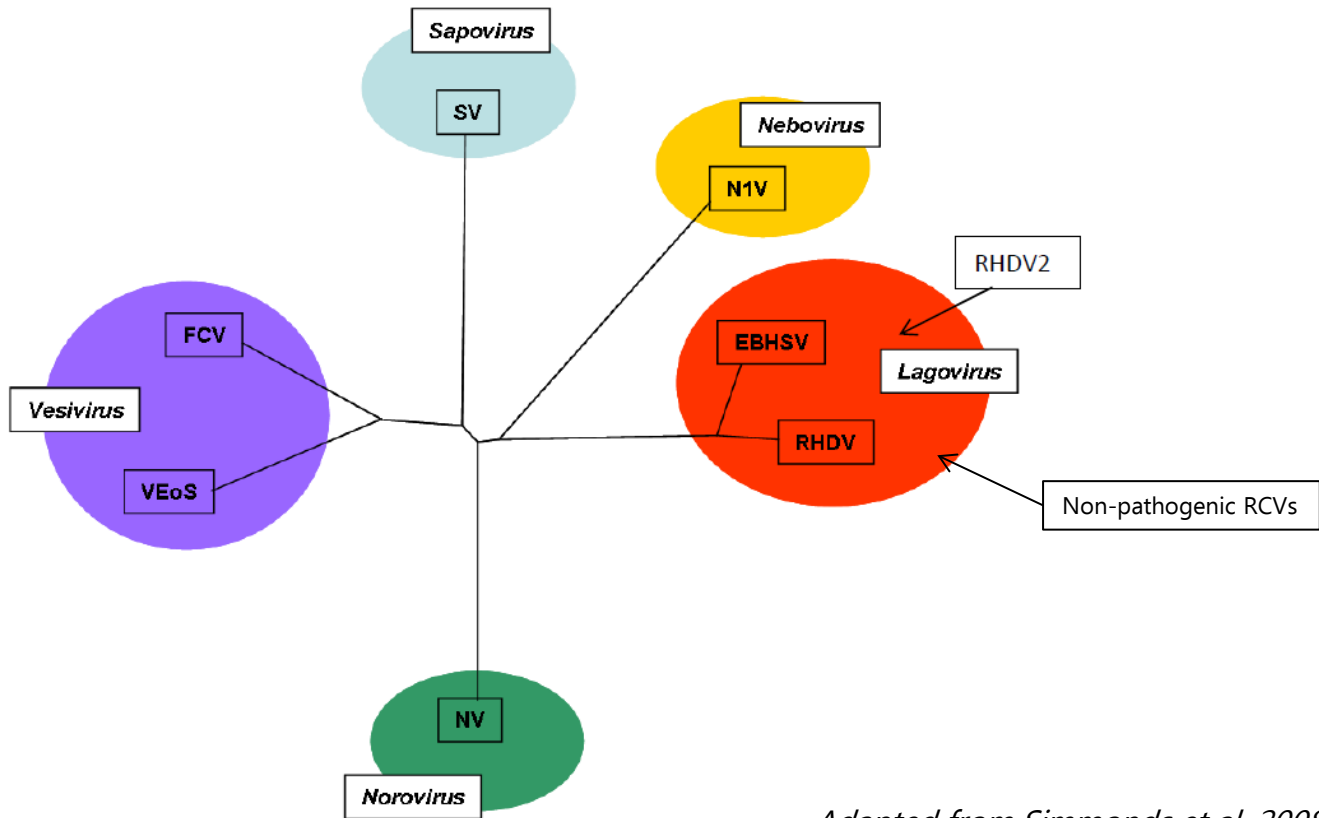


Classification:

Family: Caliciviridae

Genus: Lagovirus

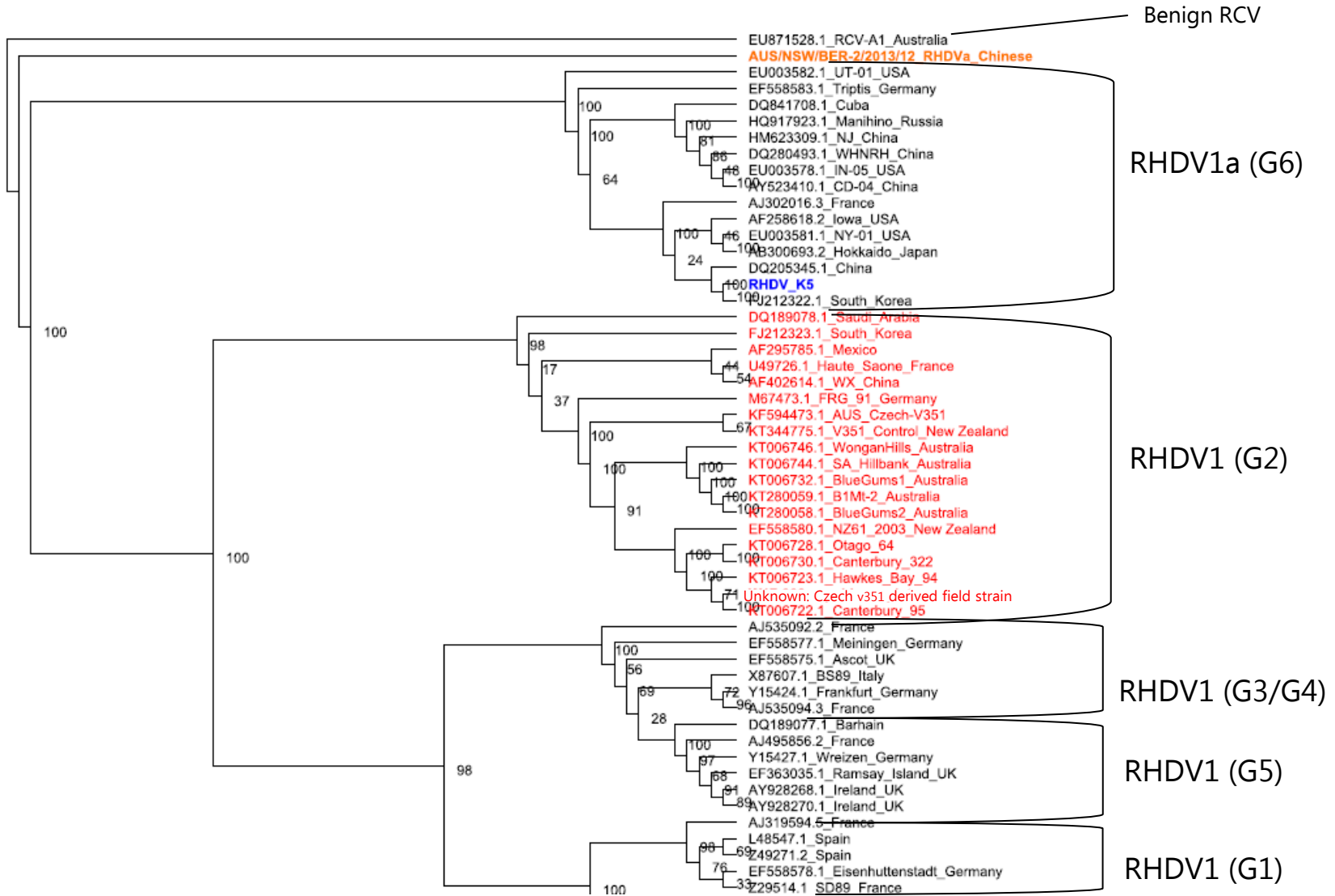
Species: includes Rabbit Haemorrhagic Disease Virus (RHDV)



*Adapted from Simmonds et al. 2008*

# RHDV1 Phylogenetic Tree

RaxML 1000 BS





# What about RHDV1 K5

- potentially significant biological control tool for pest rabbits
- not a new virus – a Korean strain of the existing RHDV1 virus already widespread in New Zealand
- selected for release in Australia because it can better overcome the protective effects of the non-pathogenic calicivirus (RCV-A1) which occurs in the feral rabbit populations in both Australia and New Zealand
- may assist in overcoming resistance of rabbits to the old virus
- expect an increased knockdown of 0 to 40% – depends on the rabbit population and the number of susceptible animals within the population
- RHDV1 K5 is not a silver bullet for rabbit eradication, and a long-term integrated approach to controlling pest rabbits is required
  - Not expecting the huge knockdown experienced in 1997
  - Rabbits no longer a naive population and virus competing with existing strain



# Why a controlled release in New Zealand

- Expected to 'boost' the effects of the existing RHDV1 strain and help slow the increase in rabbit numbers
- High quality commercially prepared product
- Release to occur at the best time of year
- Able to monitor the impacts
- Avoid biosecurity risks associated with an illegal release
- Increase the likelihood of success
- Maximise benefits to farmers and the environment

Australian data from over 200 RHDV1 K5 release sites showed average decrease in rabbits by 42%



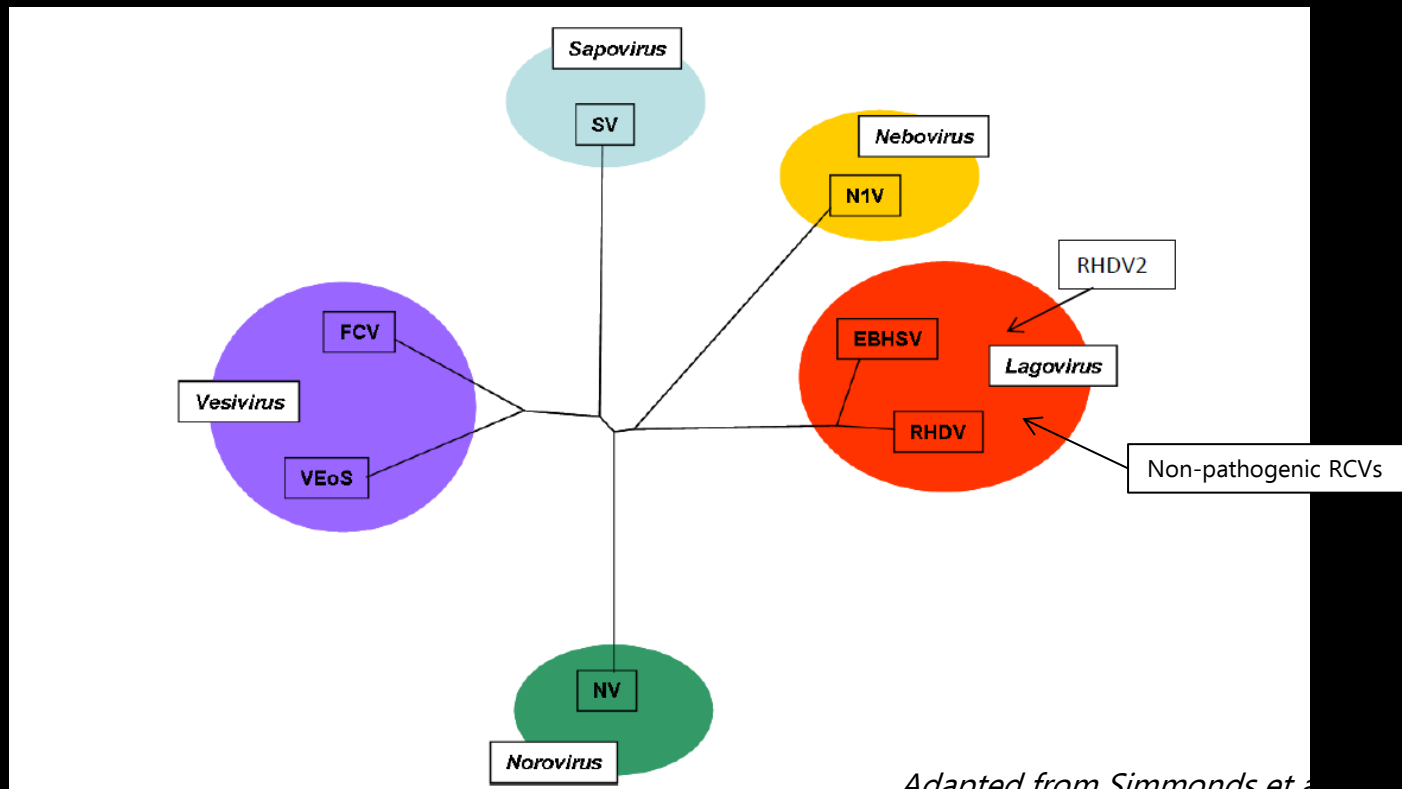




# Biosecurity Risks

Australia has pathogens not present in New Zealand

- Diseases of livestock
- Other types of rabbit caliciviruses





# Rabbit Caliciviruses in Australia

## RHDV1:

- Also known as RHDV1 to differentiate from RHDV2
- Classical (G1-5)/RHDVa (G6) serotypes
  - RHDV1 Czech v351 : original release strain present in Australia and NZ
  - RHDV1 Chinese\*: found Australia 2014 near Sydney, not present in NZ
  - RHDV1 K5 (08Q712): new strain released in Australia 2017, release currently underway in New Zealand

## RHDV2\*:

- New virus found Europe 2010 and Australia 2015, not present in NZ
- Dominant virus across Europe and China
- Not species-specific - infects several species of hares and rabbits
- Kills young rabbits (>5 weeks old)
- Low and variable death rates (25-75%)
- Less stable - tendency to form recombinants
- Not considered a suitable biological control agent
- Currently available vaccines are not very effective

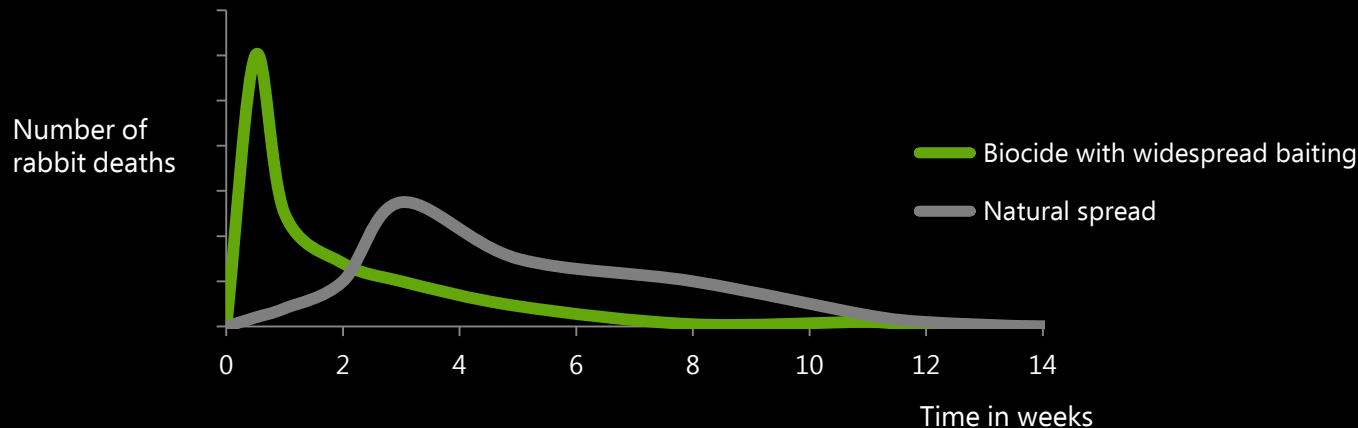
## Non-pathogenic RCV-A1:

- Numerous strains in Australia



# RHDV1 K5 Release in New Zealand

- Environment Canterbury applied for approval on behalf of members of the Rabbit Coordination Group. This group includes regional councils, Federated Farmers, the Department of Conservation and Land Information New Zealand with interest in rabbit control
- Releases at sites selected by participating local councils
- Autumn release preferred - when numbers of young rabbits are low and fly vectors are active
- Pre-feeding with untreated carrot or oat bait - essential to ensure good uptake when treated bait is applied
- Leave site undisturbed to allow the virus to establish and spread





# Ongoing

## Post-release monitoring

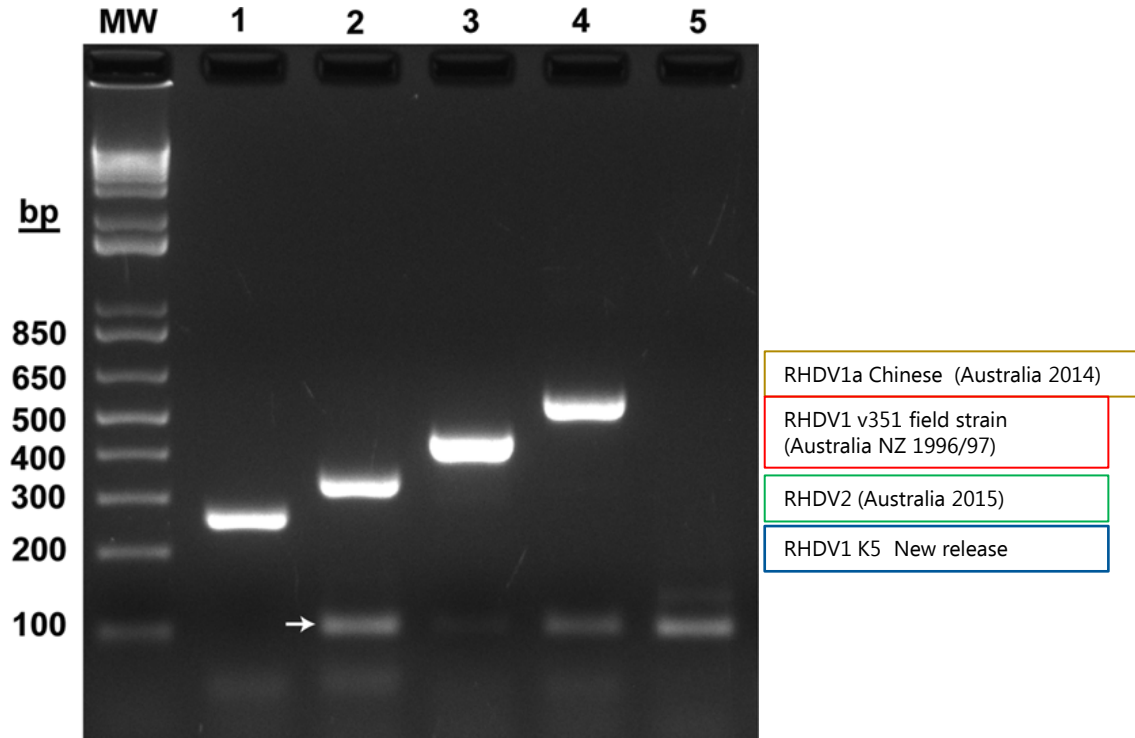
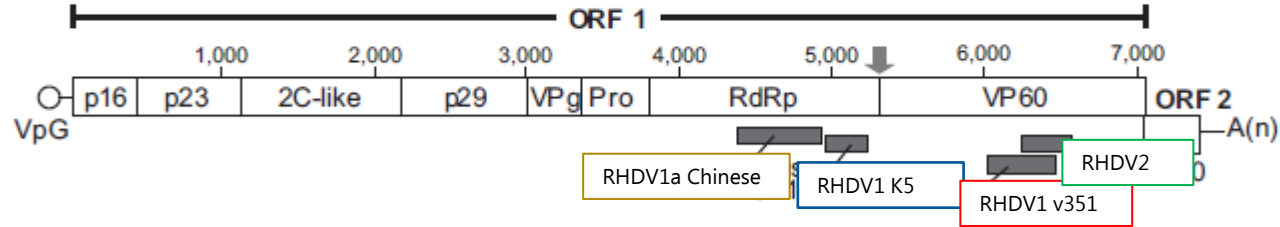
- Participating local regional councils and unitary authorities monitoring rabbits by 6-8 weeks following RHDV1 K5 release and by annual spotlight count
- Landowners and council staff have been asked to assist by recovering dead rabbits and collecting a small sample of liver to provide confirmation that RHDV1 K5 strain is killing rabbits in that area - a sampling kit and instructions are included with every bag of RHDV1-treated bait.

## Research and modelling on RHDV1 K5 movement

- Monitoring flies and virus movement from 4 science study sites
  - Measuring fly abundance, presence of virus and impact on rabbits at satellite sites up to 5km from release sites
  - Detected deaths at all sites – strain analysis pending



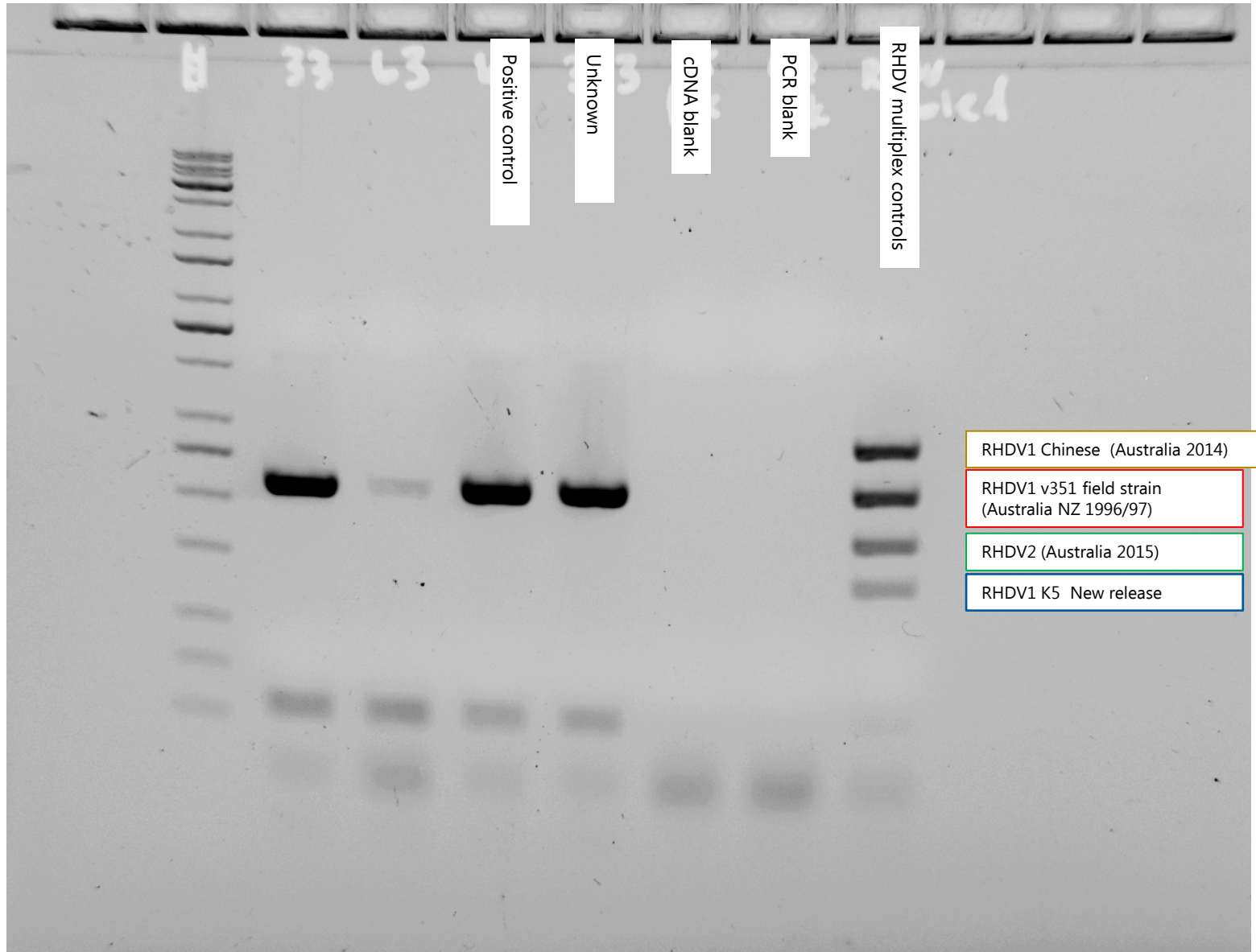
# Cool tools: RHDV strain-specific multiplex RT-PCR



*Adapted from Hall et al. 2017)*



# Example: RHDV Strain Multiplex Assay



# NZ Rabbit RHDV Tracker



## NZ Rabbit RHDV Tracker

RHDV Release Sites

Record & Submit RHDV Sample

### NZ Rabbit RHDV Tracker

Please use the tab above to submit a sample from a rabbit that is believed to have died from RHDV for RHDV strain verification. Confirmed and previous submissions are visible on the map.

The legend will provide an explanation of the colours on the map.

Supported by - [Manaaki Whenua - Landcare Research](#) and [LINZ](#)

Configured by Eagle Technology Group Ltd and powered by the [Esri ArcGIS Platform](#).



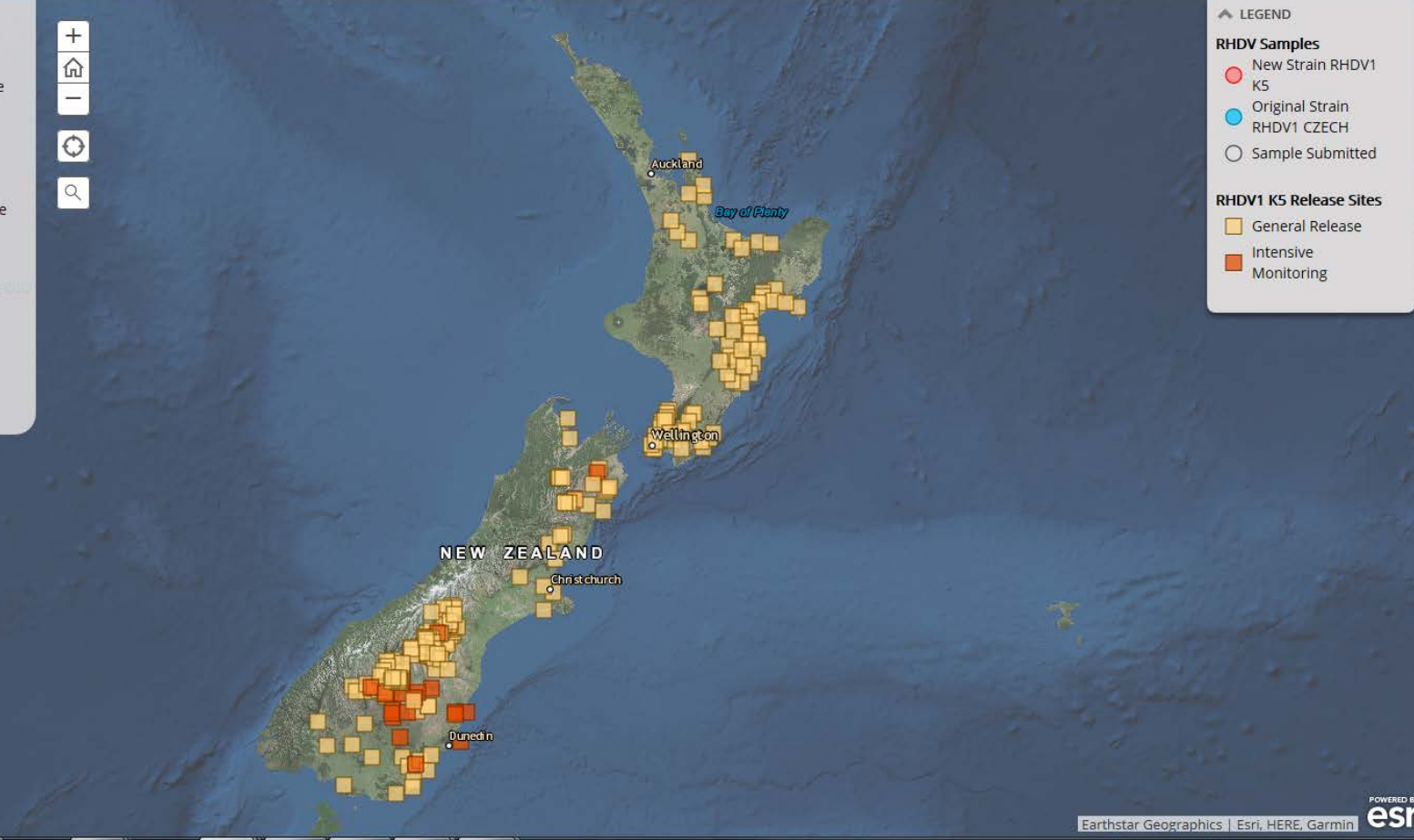
LEGEND

**RHDV Samples**

- New Strain RHDV1 K5
- Original Strain RHDV1 CZECH
- Sample Submitted

**RHDV1 K5 Release Sites**

- General Release
- Intensive Monitoring





# NZ Rabbit RHDV Tracker

## NZ Rabbit RHDV Tracker

Landcare Research

RHDV Release Sites

Record & Submit RHDV Sample

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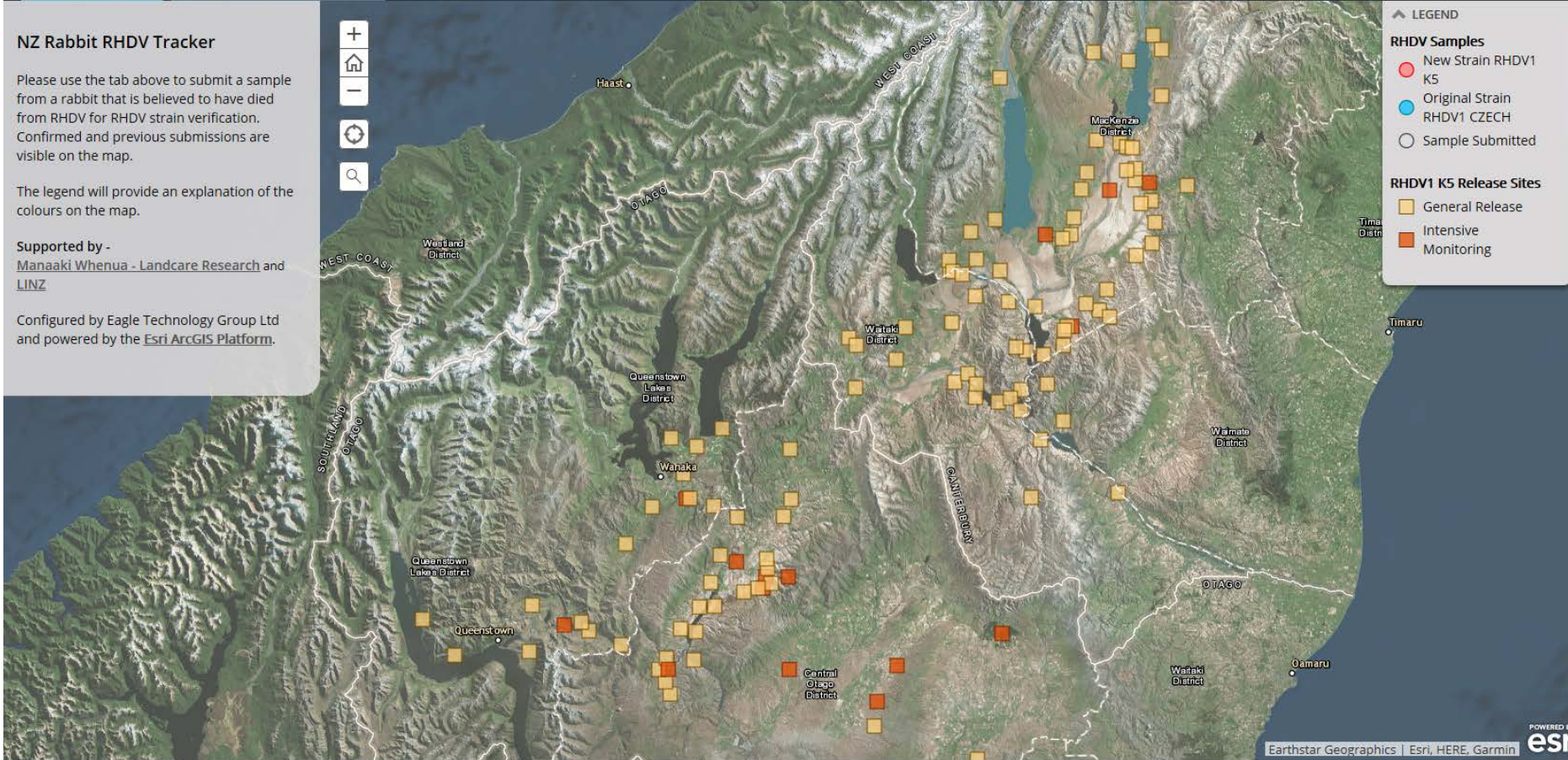
LEGEND

**RHDV Samples**

- New Strain RHDV1 K5 (Red circle)
- Original Strain RHDV1 CZECH (Blue circle)
- Sample Submitted (White circle)

**RHDV1 K5 Release Sites**

- General Release (Yellow square)
- Intensive Monitoring (Orange square)







# NZ Rabbit RHDV Tracker

## NZ Rabbit RHDV Tracker

View Contributing Organisations



RHDV Release Sites

Record & Submit RHDV Sample

### NZ Rabbit RHDV Tracker

Please use the tab above to submit a sample from a rabbit that is believed to have died from RHDV for RHDV strain verification. Confirmed and previous submissions are visible on the map.

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Supported by -  
[Manaaki Whenua - Landcare Research](#) and [Land Information New Zealand](#)

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LEGEND

No legend





# NZ Rabbit RHDV Tracker – submit samples

## NZ Rabbit RHDV Tracker

View Contributing Organisations



RHDV Release Sites

Record & Submit RHDV Sample

Testing

## NZ Rabbit RHDV Tracker - Record & Submit Sample



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Landcare Research

Please complete the form below to report and submit a liver sample from a dead rabbit that is believed to have died from RHDV. Each submission requires a corresponding liver sample to be collected using a Landcare Research liver sampling kit.

Please carefully follow all instructions included with the sample kits.

### Address for requesting OR returning a liver sample kit -

RHDV Strain Verification - Landcare Research  
54 Gerald Street  
Lincoln  
7068

Each kit contains two vials for liver samples from two dead rabbits.

If you do not have access to a kit immediately, please photograph individual rabbits before freezing the carcass (or just the rabbit livers) until one can be obtained. Email [Janine Duckworth - Landcare Research](mailto:Janine.Duckworth@landcare.co.nz) to have a liver sampling kit sent to you. This form can then be submitted retrospectively.

Confirmed RHDV submissions can also be viewed on the [New Zealand Rabbit RHDV Tracker Map \(Overview Map\)](#).



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