Landcare Research
Manaaki Whenua

Rabbit biocontrol – what is happening with rabbit haemorrhagic disease?

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Rabbit Biocontrol Initiative

Aims

- Identify high-virulence NZ strains of rabbit haemorrhagic disease virus (RHDV)
- Clarify any protective role of pre-existing non-pathogenic rabbit caliciviruses (RCV)
- Provide information that will increase:
  - our understanding of RHDV and rabbit calicivirus
  - Identify high virulence strains adapted to temperate environments
  - maximise the cost/benefit returns from RHDV
  - reduce the financial, environmental and social stress of rabbit impacts on stakeholders
  - Result in fewer rabbits

Primary funding from:

- *Ministry of Primary Industries - Sustainable Farming Fund Project (12/055)*
- *Invasive Animal CRC, Australia*

Better RHDV Strains

- Release of pathogenic RHDV in 1997 greatly reduced rabbit numbers
- Since mid 2000's rabbit numbers have increased
  - high proportion of animals have antibodies against RHDV
- Not all factors affecting the effectiveness of RHDV in NZ are understood
- NZ rabbits have been breeding in isolation for over 150 years - their genetics and their pathogens are likely to be different
- Higher virulence strains of RHDV have been found in Europe, China Korea and Australia but no one has looked within NZ
- Not sure of the role of benign caliciviruses in RHDV pathogenicity in the NZ situation

Aim 1:

Survey rabbits throughout NZ and intensively select for the RHDV strains that kill more quickly with the aim of identifying any highly virulent strains for use by stakeholders

WANTED!

DEAD OR ~~ALIVE!~~

FOR RESEARCH TO IMPROVE RABBIT CONTROL

RHDV-KILLED RABBITS

REWARD

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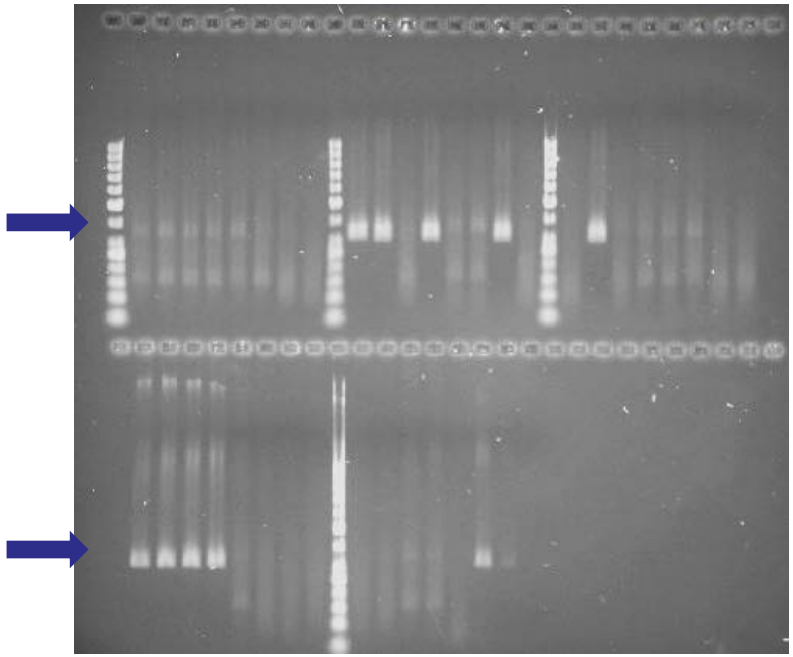
RHDV Strain Collection

RHDV sample no.	RHDV Source (RHDV positive)	Date collected
0.	RHDV inoculum (positive control)	
1. RBI 093	Taranaki- Purangi, East Taranaki	9/2/2013
2. RBI 094	Hawkes Bay- North Boundary Stream	9/3/2012
3. RBI 095	Canterbury - Eastern Mackenzie Basin	9/1/2012
4. RBI 322	Canterbury - Eastern Mackenzie Basin	20/3/2103
5. RBI 320	North Canterbury, Turuna	22/5/2013
6. RBI 515-518*	Canterbury - Lincoln	27/05/2013
7. RBI 108	Marlborough	12/11/2013
8. RBI 61	Otago Clutha	6/1/2014
9. RBI 63	Otago Wanaka	21/1/2014
10. RBI 66, 69	Otago Clutha right	24/12/2013
11. RBI 62	Otago Arrowtown	24 /1/2014
12. RBI 70, 74	Otago mid Clutha Queensberry	30/12/2014
13. RBI 67	Otago Luggate	2/1/2014
14. RBI 64, 65. 68	Otago - Alexandra	1/2/2014

RHDV Strain Collection

- From 54 samples collected 34 samples of rabbits believed to have died from RHD from 14 sites
- Sequences show genetic regional variation
- 12 RHDV samples selected for pathogenicity screening

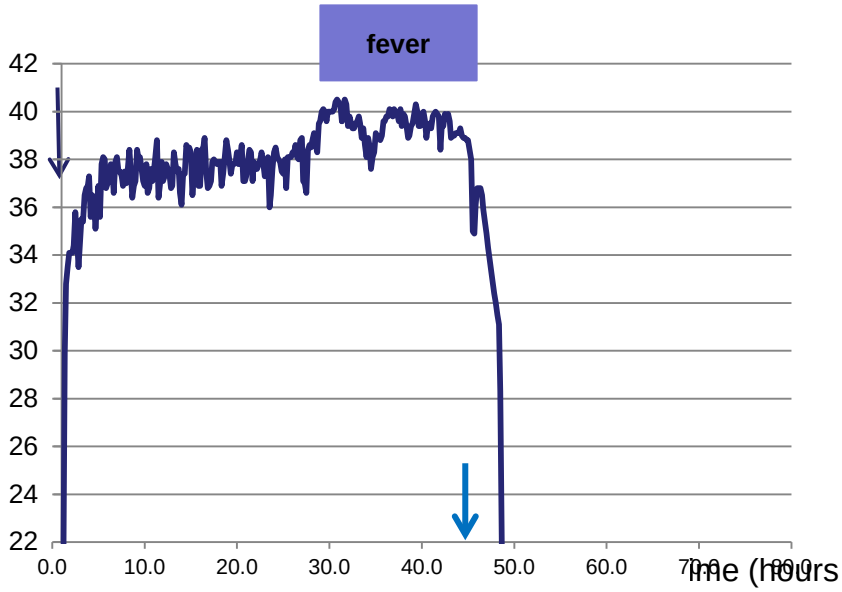


RHDV Isolates – Inoculum production

- 12 RHDV samples selected for initial screening
  - good quality inoculum for comparative testing prepared by dosing 2 rabbits orally each isolate (1 gm equivalent liver extract)
  - Assessed temperature, behaviour, feed and water intake and responsiveness
  - Time to death and viral load
  - Animal welfare assessment
  - Standardised inoculum for comparative testing

Temperature Monitoring

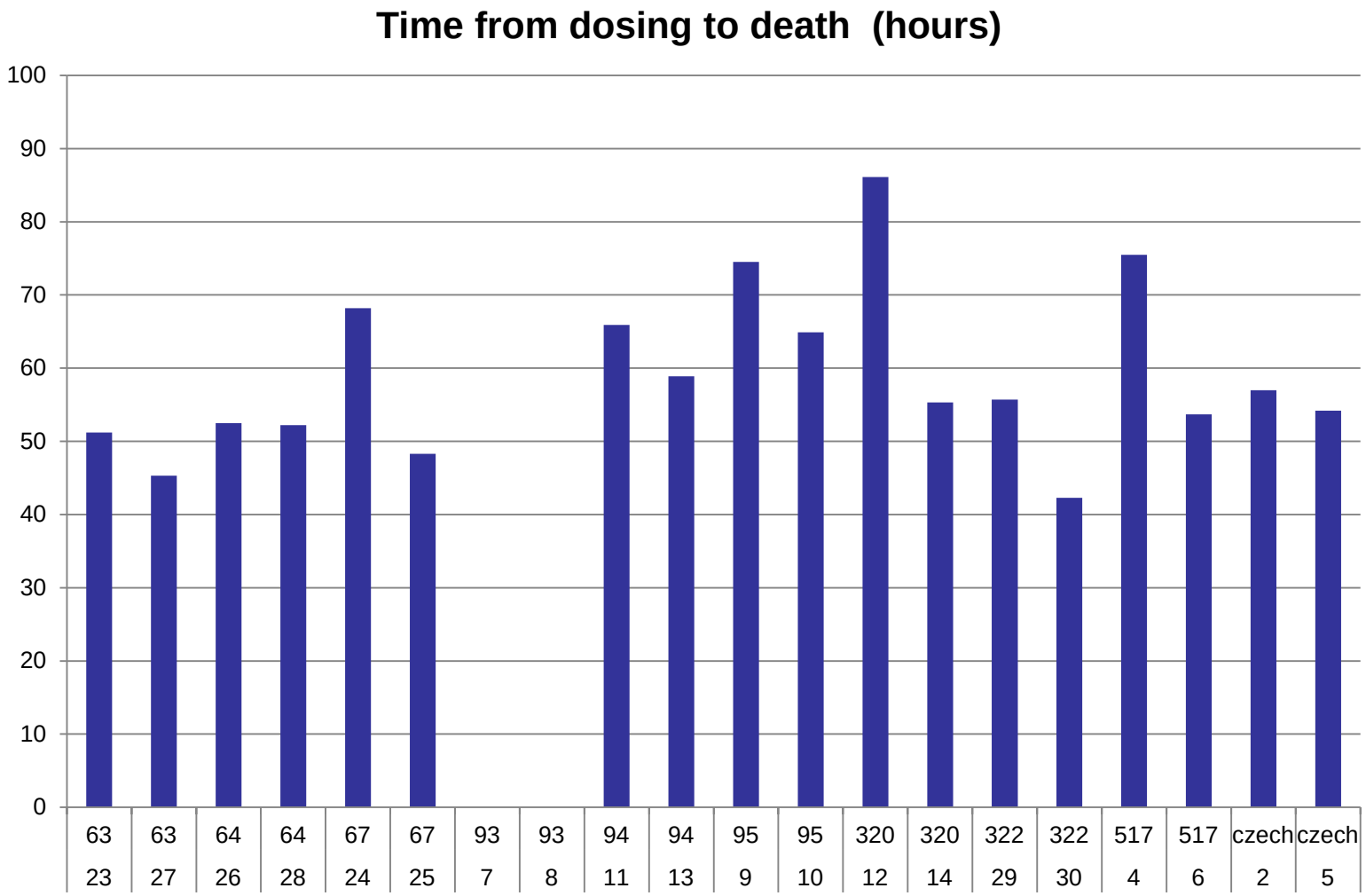
- Subcue mini temperature dataloggers
  - Fitted against skin by collar to measure relative temperature changes
  - Assess onset and duration of fever
  - Estimate time of death
  - Robust and minimal disturbance to animal



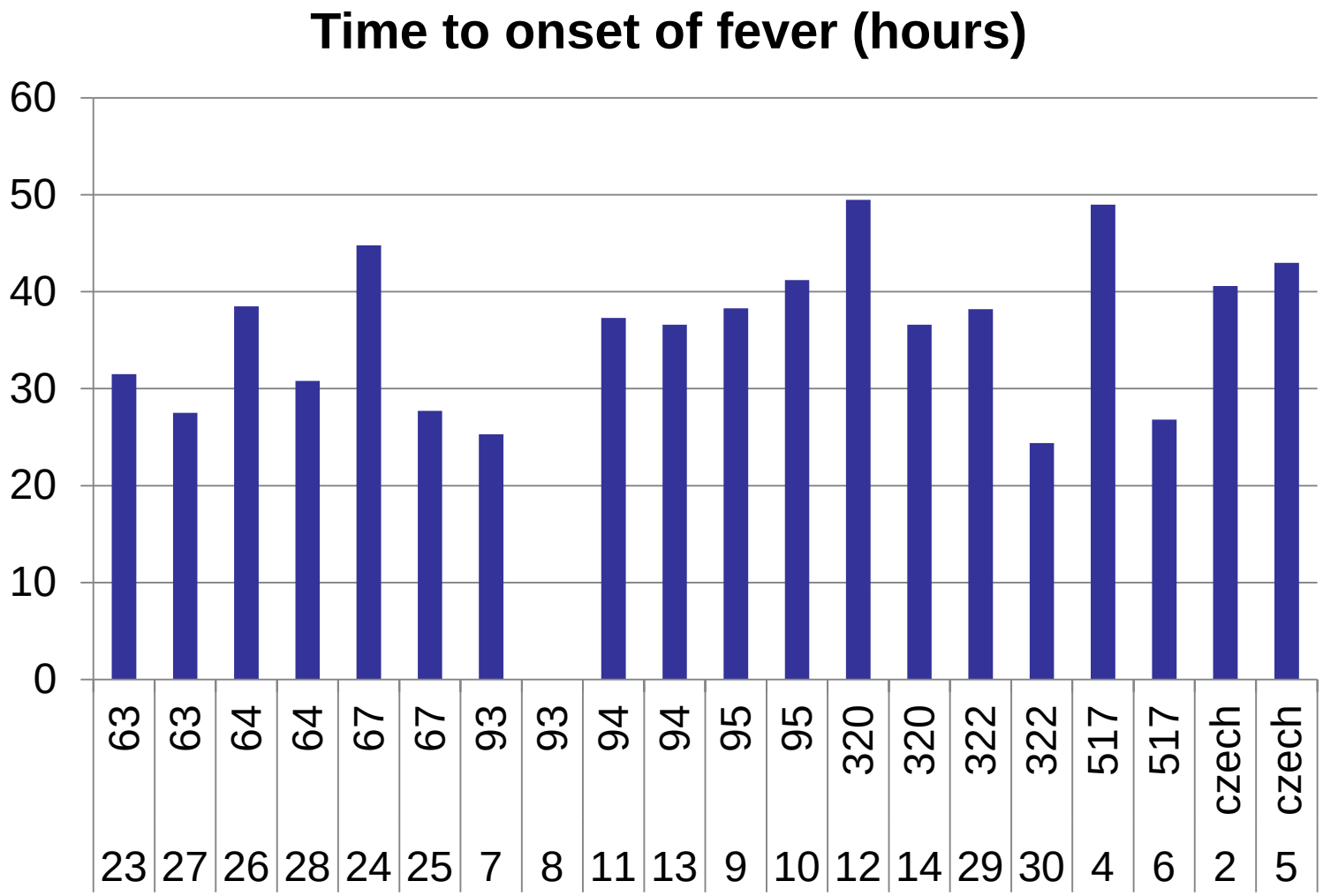
Fit collar and dose

Estimated time of death

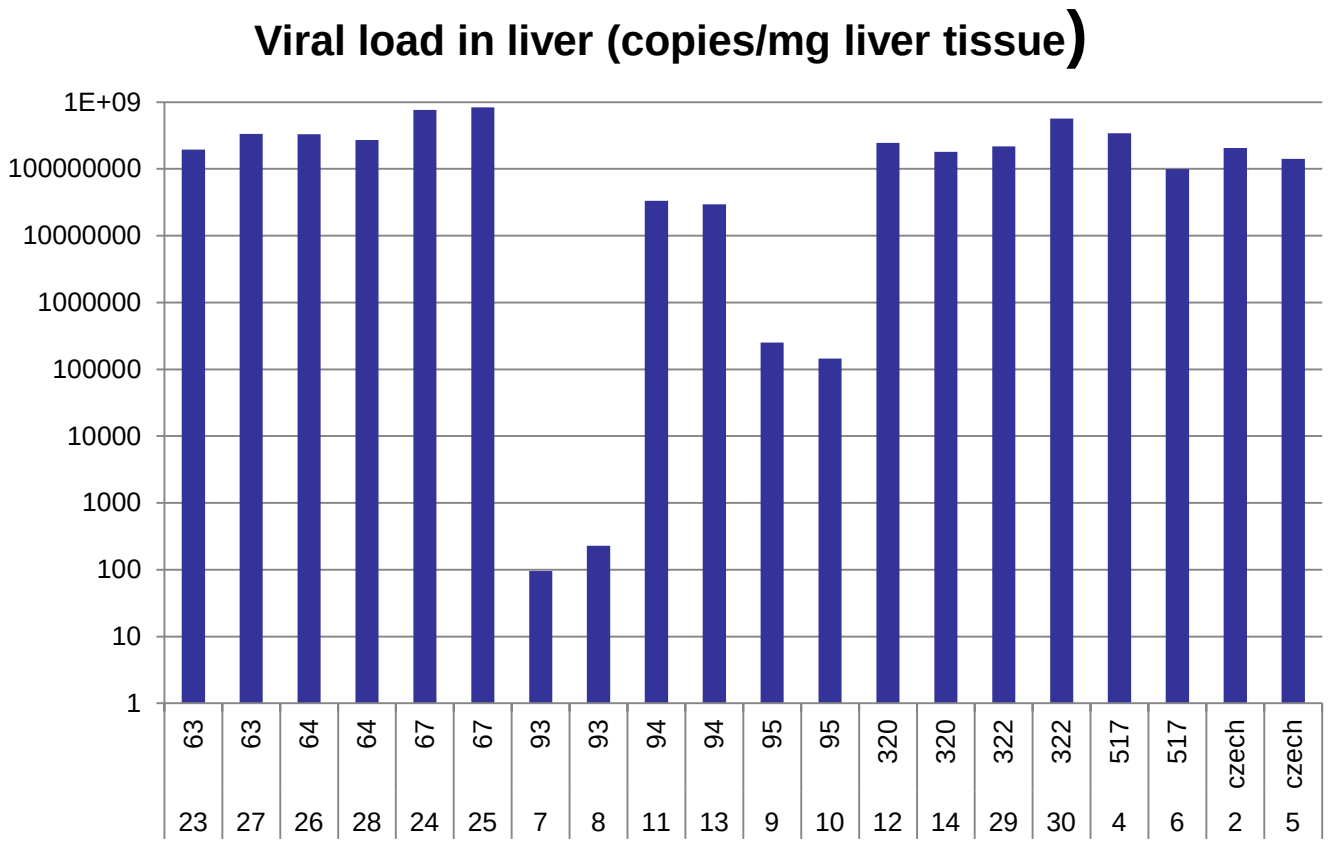
RHDV Isolates – Time to death



RHDV Isolates – onset of fever



RHDV Isolates – Viral production



- Generally viral load in liver fairly consistent 10^8 -10^9 copies per mg liver
- There was variation eg. isolates 93, 95 & 94

Pathogenicity Assessments

- 8 RHDV samples selected for intensive pathogenicity testing
  - 8 rabbits/ group minimum
  - time to death and viral load
  - routes of shedding

  (some delays due to local RHD outbreak)
- Identify regional differences
  - improved strains for biocontrol in NZ
  - part of international study identifying potential genetic indicators of virulence

Role of Benign Rabbit Calicivirus

- Antibodies present in NZ rabbits prior to the introduction of lethal RHDV
  - benign rabbit calicivirus (RCV) may be present
- In Italy a benign RCV confers ~80% protection against pathogenic RHDV infection
- In Australia a benign RCV was recently identified that protects 30-50% of rabbits against pathogenic RHDV infection
- Effect of benign RCVs on the spread and virulence of pathogenic RHDV in NZ is not known
- PhD student spent 3 months in Canberra learning latest technologies with Tanja Strive's group at CSIRO

Aim 2:

Sample rabbits throughout NZ for the presence of benign RCV and determine the potential effect of RCV on lethality of RHDV infections

Sample Survey Progress

Survey Site No.	District & Location	Sample Collection	Progress
1.	Mackenzie Country- Iron Bridge, Tekapo River	3/10/2012 n=30	complete
2	Mackenzie Country- Simon's Hills Pukaki Flat	2/10/2012 n=30	complete
3	Otago - Bendigo Station, Cromwell	13/3/2013 n=30	complete
4	Otago- Cloudy Peak, Ardgour Valley, Taras	14/3/2013 n=30	complete
5	Otago - Queensberry Hills, Wanaka	15/3/2103 n=31	complete
6	North Canterbury – Leslie Hills Station	Sept 2013 n=36	complete
7	Wellington – Orongorongo Valley	In progress n=9*	
8	Canterbury - Banks Peninsula/Selwyn	25/5/2013 n=32	complete
9	Hawkes Bay - Boundary Stream	On hold n=3	
10	Bay of Plenty - Te Puke/Pongakawa	In progress n=20*	
11	Marlborough – Isolation Flat, Molesworth Station	6/6/2013 n=31	complete
12	Southland – Wantwood Station Gore	15/5/2013 n=30	complete
13	Hawkes Bay – Cape Kidnappers	June 2013 n=30	complete
14	Wanganui - Mangapurua Valley	Sept 2013 n=10*	
		Total 342 samples	

RHDV antibody results

Survey Site No.	District & Location	RHD immune serum		
1.	Mackenzie Country- Iron Bridge, Tekapo River	Negative 13 Positive 17	57%	
2	Mackenzie Country- Simon's Hills Pukaki Flat	Negative 6 Positive 15	81%	
3	Otago - Bendigo Station, Cromwell	Negative 9 Positive 22	71%	
4	Otago- Cloudy Peak, Ardgour Valley, Taras	Negative 8 Positive 22	73%	
5	Otago - Queensberry Hills, Wanaka	Negative 12 Positive 18	60%	
7	Wellington – Orongorongo Valley	Negative 9 Positive 0	0%	
8	Canterbury - Banks Peninsula/Selwyn	Negative 31 Positive 1	3%*	
10	Bay of Plenty - Te Puke/Pongakawa	Negative 1 Positive 19	95%	
11	Marlborough – Isolation Flat, Molesworth Station	Negative 15 Positive 16	52%	
12	Southland – Wantwood Station Gore	Negative 7 Positive 23	77%	
14	Wanganui - Mangapurua Valley	Negative 1 Positive 9	90%	
	Positive dilution > 1:40			

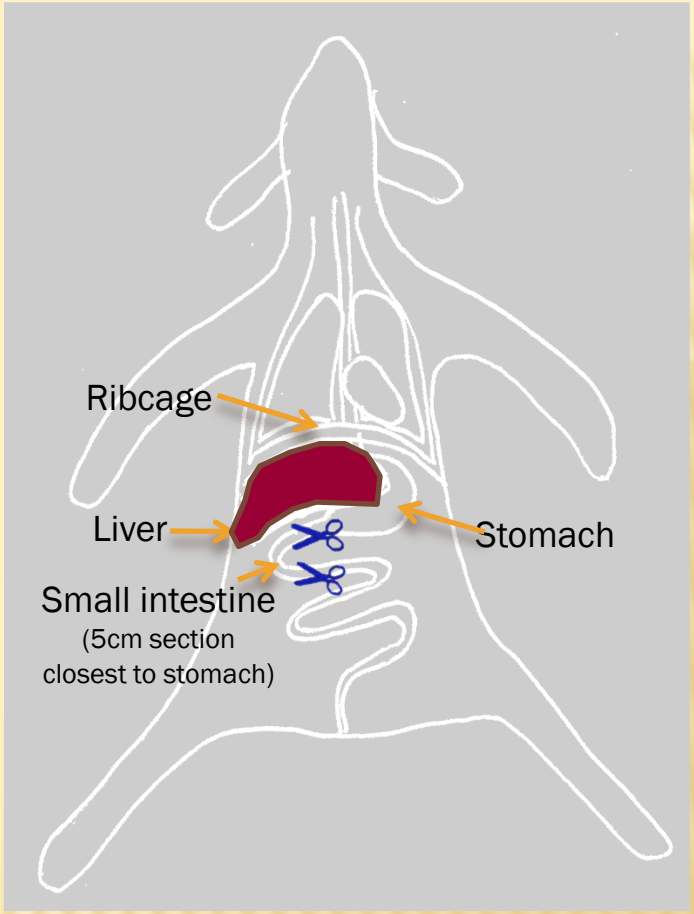
Sampling surveys complete

- Populations display a range of immunity 0 – 95%
- Sample sets currently being analysed for presence RCV

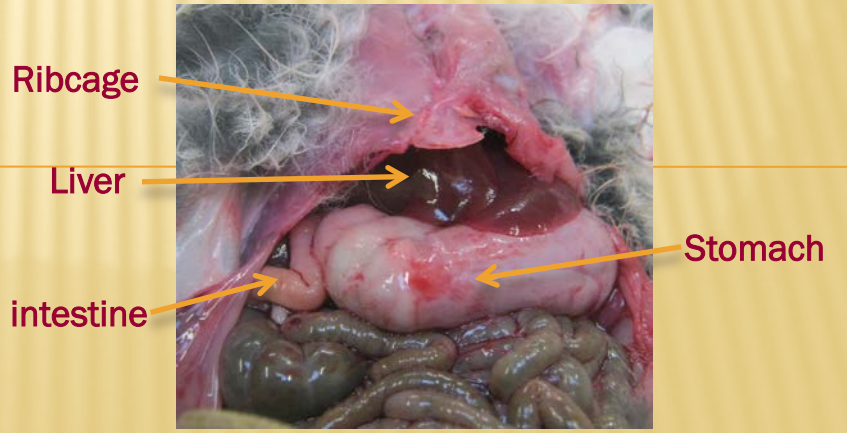
Rabbit Sample Collection

Can you please write location for each rabbit on the ziplock sample bag. On the sample sheet please also indicate if it is male/female, adult/juvenile rabbit, pregnant or lactating.
We are after the following from each animal:

1) **Blood sample** into yellow SST tube for RHD antibody analysis (Collect 3-5ml blood by syringe and needle, label tube with id no, spin in centrifuge till serum separated, then freeze).
2) **Liver:** (large white or pink top vial) (you can leave gall bladder attached)
3) **Liver RNA :** a very small piece of liver (0.5 cm cube) (small vial with RNAlater *) Approximate size
4) **Gut :** 1st 5cm of the small intestine, just after it leaves the stomach (no need to remove the gut contents) (yellow top vial)
5) **Gut:RNA :** a very small section of the small intestine (0.5cm - 1cm) (small vial with RNAlater*) Approximate size
6) **Eyeball for aging** – placed in 10% formalin* (Please label with sample number, keep at room temp and do not freeze)



Use fresh gloves and wipe down scissor/forceps with Trigene* between animals. Place liver and gut sample vials into the bag and store samples frozen until collected. Keep the eyeball samples in formalin at room temperature. Keep blood cool but not frozen until it can be centrifuged. Spin SST tube and then freeze.



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03 321 9999 or 03 3297377(hm)

* Warning: RNAlater, Trigene and 10% formalin are irritants, harmful if swallowed, cause skin and eye irritation. Handle with care.

Benign Rabbit Caliciviruses (RCV)

- Identify RCV strains present in NZ
  - regional differences in incidence
  - potential protective role against pathogenic RHD infection
  - identify RHDV strains that may overcome any such protective effects

Australian Update

- Registering new freeze-fried RHDV formulation
- Identified a new RHDV strain
  - Increased death rates particularly in rabbits protected by previous exposure to the Australian RCV strains

Acknowledgements and thanks

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- Marlborough District Council
- Land Information NZ
- Department on Conservation
- MPI Rabbit Coordination Group
- Hawkes Bay Regional Council
- Otago Regional Council
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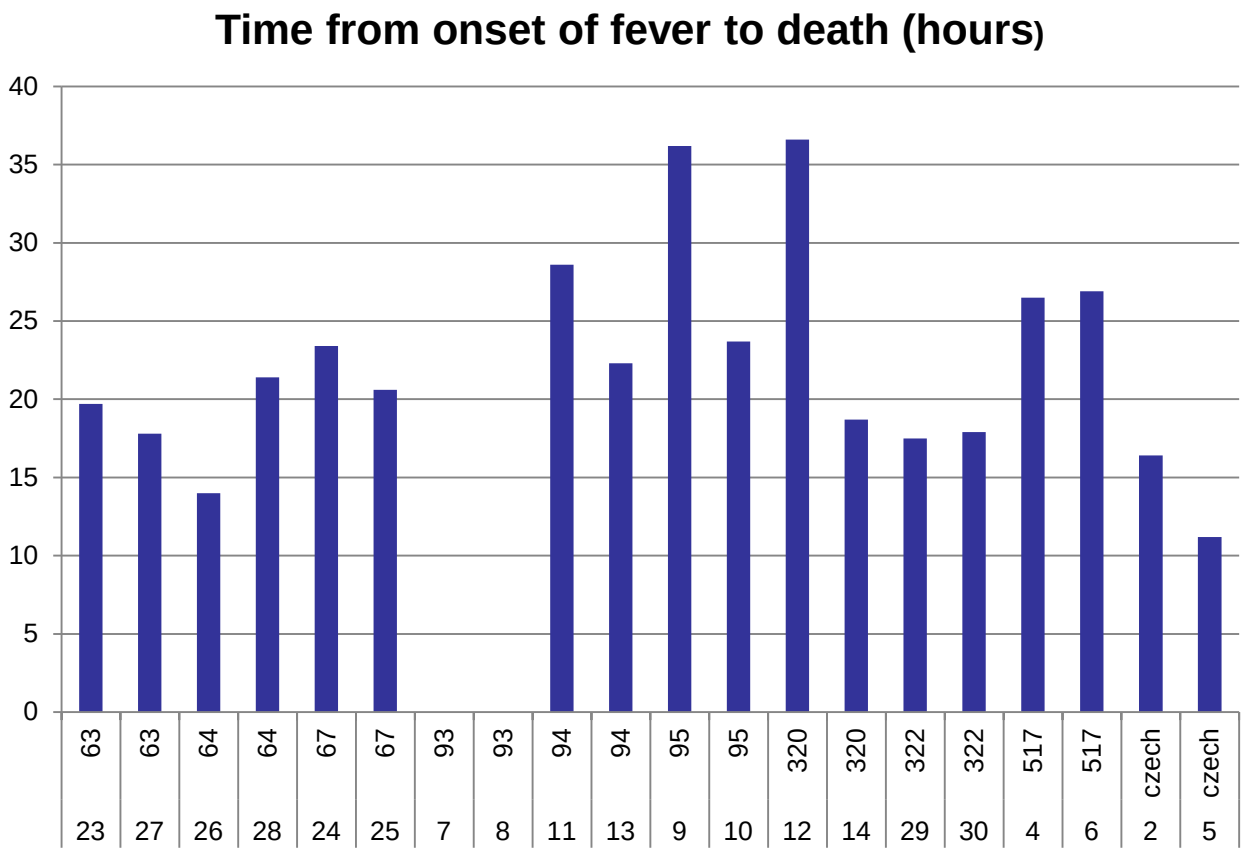
- Tao Zheng , AgResearch ……

Funding:

- *Ministry of Primary Industries - Sustainable Farming Fund Project (12/055)*
- *Invasive Animal CRC, Australia*

RHDV Isolates - Results



RHDV Isolates - Results