PESTSMART

Business case to advance the selection of new rabbit biocontrol agents

David Peacock, Biosecurity SA

Invasive Animals CRC

Government of South Australia
Primary Industries and Regions SA

Australian Government
Department of Industry and Science
Business Cooperative Research Centres Programme

Business case to advance the selection of new rabbit biocontrol agents

Endorsed by the Invasive Animals CRC
Rabbit Biocontrol Scientific Committee

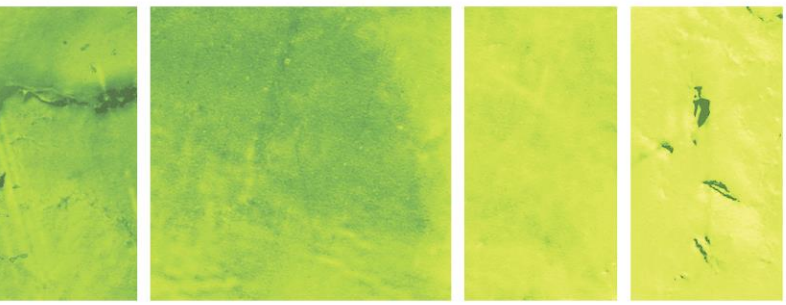
David Peacock
Biosecurity SA

October 2015
An Invasive Animals CRC Project

Invasive Animals CRC

Government of South Australia
Primary Industries and Regions SA

Australian Government
Department of Industry and Science
Business Cooperative Research Centres Programme



Business case to advance the selection of new rabbit biocontrol agents.

Disclaimer: The views and opinions expressed in this report reflect those of the author and do not necessarily reflect those of the Australian Government, Invasive Animals Ltd, or Invasive Animals Cooperative Research Centre. The material presented in this report is based on sources that are believed to be reliable. Whilst every care has been taken in the preparation of the report, it is "as is", without warranty of any kind, to the extent permitted by law.

Published by: Invasive Animals Cooperative Research Centre.

Telephone: (02) 6201 2887

Facsimile: (02) 6201 2532

Email: contact@invasiveanimals.com

Internet: http://www.invasiveanimals.com

ISBN (print): 978-0-9943800-1-2

ISBN (online): 978-0-9943800-2-9

© Invasive Animals Ltd 2015

This work is copyright. The Copyright Act 1968 permits fair dealing for study, research, information or educational purposes. Selected passages, tables or diagrams may be reproduced for such purposes provided acknowledgement of the source is included. Major extracts of the entire document may not be reproduced by any process.

The IA CRC gratefully acknowledges funding support from the Australian Government through its Cooperative Research Centres Program.

This document should be cited as: *Peacock D (2015). Business case to advance the selection of new rabbit biocontrol agents*. Invasive Animals Cooperative Research Centre, Canberra, Australia.

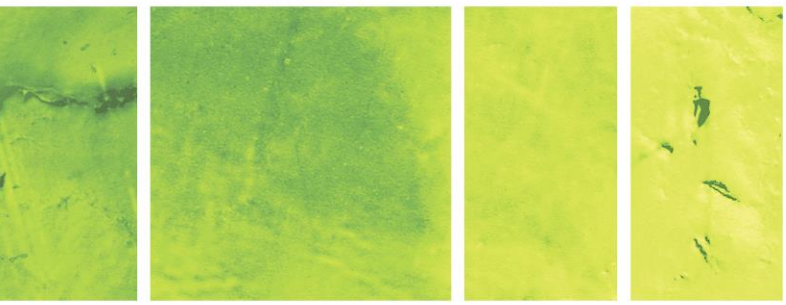
Front cover photo: Rebecca Zanker

Available online at: http://www.pestsmart.org.au/

Invasive Animals CRC Rabbit Biocontrol Scientific Committee

Members

Adj. Assoc Prof Brian Cooke	University of Canberra
Dr Tarnya Cox	NSW Department of Primary Industries
Mr John Kovaliski	Biosecurity SA
Mr Greg Mutze	Biosecurity SA
Dr David Peacock	Biosecurity SA
Dr Tony Pople	Qld Department of Agriculture and Fisheries
Dr Andrew Read	NSW Department of Primary Industries
Dr Ron Sinclair	Biosecurity SA
Dr Tanja Strive	CSIRO
Dr John Tracey	NSW Department of Primary Industries



Contents

Executive Summary v

1. Introduction 1
2. Rabbit biocontrol assessment framework and methodology 2
   - Biocontrol agent assessment criteria 3
3. Biocontrol agent candidate assessment findings 4
4. Discussion of rabbit biocontrol agent candidates recommended as 'tentatively worthwhile' 11
   1. *Eimeria intestinalis* and *E. flavescens* 11
   2. Rabbit haemorrhagic disease virus 2 (RHDV2) 12
5. Discussion of rabbit biocontrol agent candidates recommended for a watching brief 13
   3. Leporid herpesvirus-4 13
   4. California MSW strain of myxoma virus 14
6. Initial research projects for biocontrol candidtates recommended as 'tentatively worthwhile' 16
   - *Eimeria* Stage 1, 2 and 3 projects 16
   - RHDV2 Stage 1 projects 17
7. Estimated economic benefit of recommended biocontrol candidates 20
8. References 22

Appendix: Economic Evaluation of Proposed Rabbit Biocontrol Investments ... 1

Executive Summary

The current IA CRC Project 3.L.5 New Potential Rabbit Bio-control Agent Prospecting and Assessment has reviewed potential rabbit biocontrol agents for Australia that were either:

a) previously short-listed by Henzell *et al*. (2008), or

b) subsequently identified by Wildlife Health Australia (WHA) monitoring the international digital media for rabbit diseases.

The candidate agents were further reviewed by scientific experts, and then by industry representatives. As a result, seven candidate agents were rejected, two were identified for watching for possible further investigation at some future time, and two were proposed for current further investigation:

Tentatively rejected

1. Rabbit vesivirus
2. Malignant rabbit virus
3. Rabbit fibroma virus (Schope Fibroma Virus)
4. Epizootic rabbit enteropathy (ERE)
5. Disseminating genetically modified organisms (GMOs), not specified
6. Astrovirus(es)
7. Subtype VbA24 of *Cryptosporidium cuniculus*

Watching brief

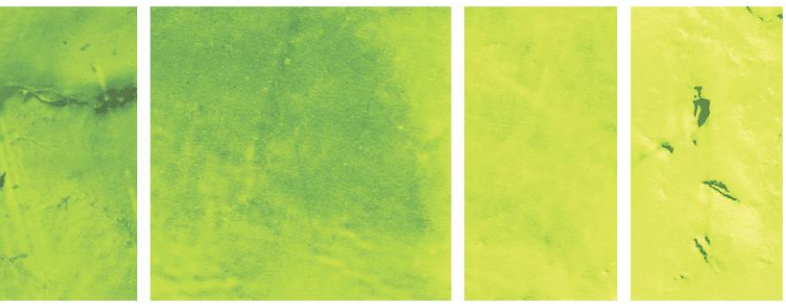
1. Leporid herpesvirus-4
2. California MSW strain of myxoma virus

Tentatively worthwhile agents for further investigation

1. *Eimeria intestinalis* and *E. flavescens*
2. Rabbit haemorrhagic disease virus 2 (RHDV2)

This business case proposes:

1. A $45,700 pilot project to
   a) Confirm that a recently published PCR methodology for all 11 *Eimeria* species (Oliveira *et al*. 2011) is effective on field samples
   b) Genetically confirm the presence of *Eimeria intestinalis* and *E. flavescens* at Wellstead (WA).
   c) Establish the legal process of translocating *Eimeria* from Western Australia to other states.



2. A 3-year project costing $571,000 for detailed investigation of the two recommended pathogens (*Eimeria* and RHDV2). In summary:
    - Metagenomic or PCR analysis of field samples to confirm the absence of *Eimeria intestinalis* and *E. flavescens* in eastern Australia, to support proposed translocations of both as biocontrol agents. Both are considered to be highly pathogenic in commercial rabbitries and wild rabbits in Europe, and could be translocated within Australia without quarantine restrictions. Benefits likely to be greatest in the cooler, wetter areas of Australia where RHDV has had less impact.
3. A 3 year project costing $1,349,082, for the thorough chracterisation of RHDV2, for its suitability to complement the selection of available Calicivirus strains for tailored rabbit control.
    - RHDV2 is a new emerging variant of RHDV that has been reported to replace other forms of RHDV in parts of Europe. It has been described to overcome immunity to other strains of RHDV, and has the ability to infect and kill up to 50% of young rabbits that are usually refractory to lethal RHDV. Different variants of RHDV2 have been described in Europe, with varying levels of virulence. One of these RHDV2 strains has now arrived in Australia, and a thorough characterization of this strain, in particular its virulence grade, is needed to gauge its impact on rabbit biocontrol. Furthermore, and depending on how and where the growing number of endemic and released RHDV strains will spread in Australia over the next few years, RHDV2 may become a useful addition to the 'RHDV-toolkit' to be used in areas where other strains are dominating. Both the RHDV2 strain now endemic to Australia as well as other RHDV2 strains currently not in Australia may be assessed.

The watching brief for Leporid herpesvirus-4 and California MSW strain of myxoma virus is briefly discussed.

An 'Economic Evaluation of Proposed Rabbit Biocontrol Investments' to evaluate the potential of *Eimeria* and RHDV2 as potential biocontrol agents has been provided under contract by Agtrans Research and is attached as Appendix 1.

Agtrans Research's key results state:

> "The investment criteria for the relatively small initial investment in *Eimeria* R&D are positive with an expected benefit-cost ratio (BCR) of 27 to 1 and an expected net present value (NPV) of $14.7 million, both estimated over a 15 year time frame from the first year of investment assumed (2017/18). If a 30 year time frame is applied, these investment criteria increase to just over 62 to 1 and $34.5 million respectively. These investment criteria take into account both risk factors as well as the expected additional costs likely to be associated with capturing the benefits from exploitation and spread of two species of *Eimeria* that are reported to exist in the SW of Western Australia but not in other locations.
>
> The investment criteria for RHDV-2 for a 15 year time frame, again with the first year of investment as 2017/18, are positive also within expected BCR of over 157 to 1 and an expected NPV of $191.3 million. If a 30 year time frame is applied, these investment criteria increase significantly to 205 to 1 and $249.2 million respectively."

1. Introduction

Australia has achieved substantial sustained success in the control of the European rabbit (*Oryctolagus cuniculus*) through the use of biological control agents. After the establishment of this pest in the 1860s and the first plagues in the 1870s and 1880s, Australia suffered catastrophic agricultural, pastoral, environmental, social and economic losses (Anon. 1890). Although numerous schemes and techniques were proposed, the introduction of the myxoma virus in 1950 was the first broad scale significant success, with mortality from myxomatosis recorded as high as 99% in some locations. Unfortunately there was rapid attenuation in the virus and development of resistance in the rabbits, with selection for virus strains of intermediate virulence which had higher chance of mosquito transmission because the infected rabbit survived for longer (Fenner and Fantini 1999). Notwithstanding this, the Australian rabbit population in general never returned to pre-myxomatosis levels. The second success in biological control was the introduction of the European rabbit flea (*Spilopsyllus cuniculi*) in 1968 enabling improved vectoring of the myxoma virus (Sobey and Conolly 1971). Poor survival of this vector in arid Australia in areas with less than 200-250mm rainfall (Cooke 1984; Foran *et al*. 1985) prompted the research and subsequent 1993 introduction of the arid adapted Spanish rabbit flea (*Xenopsylla cunicularis*) (Mutze 1996). It is unknown if this vector improved the transmission of myxomatosis in arid Australia. About 7,500 *X. cunicularis* were released in 1994-95 at 12 sites on the arid Lyndavale station, near Alice Springs (J. Kovaliski pers. comm. 2014). A report of annual myxomatosis outbreaks amongst rabbits keeping numbers low (J. Stanes, Lyndavale Station via Alice Springs, pers. comm. 2014) may offer some support for *X. cunicularis* establishing in the area and subsequently improving the efficacy of the myxoma virus biocontrol. The third very successful biological control agent was rabbit haemorrhagic disease virus (RHDV), which escaped a quarantine island trial in 1995 (Cooke 2014). In the more arid areas of Australia this agent reduced rabbit populations by greater than 90% (Bowen and Read 1998; Mutze *et al*. 1998). Not unexpectedly, the emergence of increased rabbit resistance to rabbit haemorrhagic disease (Elsworth *et al*. 2012), with varying degrees of population recovery (Mutze *et al*. 2015), has reinforced the need for additional rabbit biocontrol agents.

The search for new rabbit biocontrol agents is one of the two key objectives of the IA CRC's strategic Rabbit Biocontrol Pipeline:

1. Increasing the effectiveness of RHDV, and
2. Identifying feasible new biocontrol agents that warrant further investment (Cox *et al*. 2013).

The three IA CRC current rabbit biocontrol projects specifically aimed at improving the biological control of rabbits in Australia are:

- RHD Boost and RHD Boost Release and Performance Measurement
- RHD Accelerator
- Bioprospecting for a new potential rabbit biocontrol agent (Cox *et al*. 2013).

The likely benefits of these three rabbit biocontrol projects are indicated in Figure 1, from Cox *et al*. (2013).

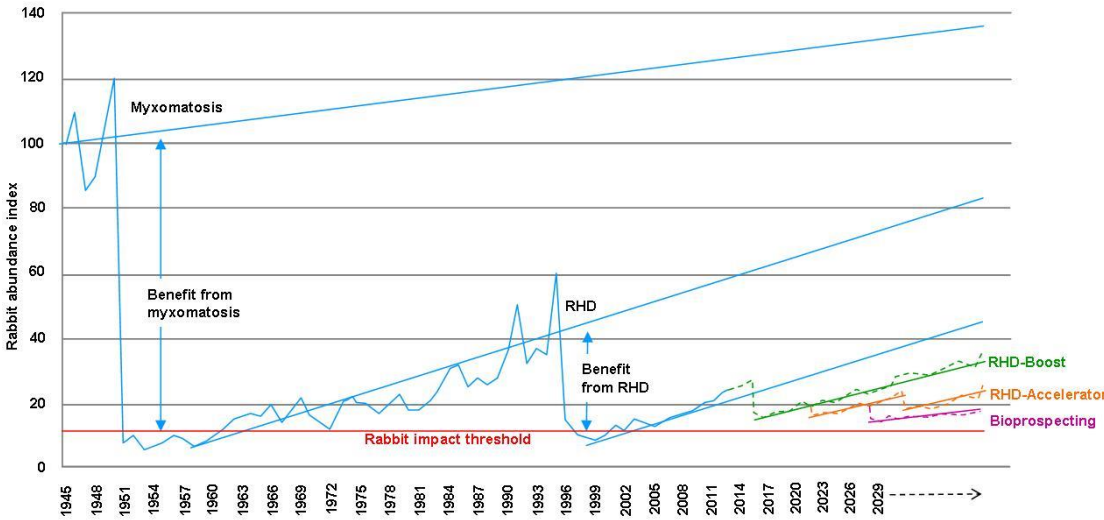


Figure 1: Benefits of Rabbit Biocontrol in Australia

2. Rabbit biocontrol assessment framework and methodology

The assessment of potential biocontrol agents was through a defined process. Original primary assessment had been made previously in a published review (Henzell *et al*. 2008). A further examination was then made of the short-listed agents from this review using the literature and consultation with researchers (Table 2 below, Appendix 2). A panel of international experts in rabbit viruses and parasites was then formed to review the assessment criteria and agent options. They were:

- Dr Peter Kerr, CSIRO Australia. Expert in rabbit viruses especially rabbit myxoma viruses
- Dr David Spratt, CSIRO Australia. Expert in rabbit parasites
- Dr Antonio Lavazza, IZLER, Brescia, Italy. Head of European RHDV reference lab and expert in RHDV
- Prof Peter Hudson, Pennsylvania State University, USA (unable to meet requirements)

The outcomes from their feedback were then provided to a panel of industry representatives for their feedback (Appendix 2).

Biocontrol agent assessment criteria

1. Appropriateness
    1.1. Species specific in Australia – the agent needs to not cause disease or inhibit reproduction and survival in a non-target species.
    1.2. Socially acceptable – the nature and biological action of the agent needs to be acceptable to the community. For example, is a vaccine available to protect farmed or companion animals?
    1.3. Humane – the agent should cause less distress, pain or suffering compared with other equivalent control agents.
2. Effectiveness
    2.1. Effectiveness in wild rabbit populations – the agent needs to provide the desired level of impact in wild rabbit populations.
    2.2. Impacts on young rabbits - young rabbits exposed to RHDV strains are more likely to seroconvert and be protected for life than are older rabbits. A change in RHDV:rabbit interaction towards increased juvenile infection has been suggested as supporting the recovery of the Australian rabbit population (Mutze *et al*. 2014; Mutze *et al*. 2015).
    2.3. Likely interactions with myxomatosis and rabbit haemorrhagic disease virus (RHDV) - the agent should have complementary or beneficial interactions with myxomatosis and RHDV and not negatively affect their effectiveness? For example, ideally, it would significantly impact young rabbits, which currently have increased infection, seroconversion and survival from RHDV (Mutze *et al*. 2014), and is likely the basis of rabbit population recoveries.
3. Efficiency
    3.1. Self-disseminating – preferably the agent would have the capacity to spread through the local, regional and national rabbit population.
    3.2. Persists in the environment – preferably the agent should persist despite death of a high proportion of hosts; but if so could be used as a biocide.
    3.3. Cost – Research and Development.
    3.4. Cost – Manufacture and Distribution - preferably, the organism(s) could be prepared and stored to allow effective distribution – this may be an issue for *Eimeria* spp. The *Eimeria* will require both being produced in the large quantities to enable distribution and stored to enable this distribution – liquid nitrogen can be used for sporozites but this is obviously not convenient for a lot of labs.
    3.5. Relative regulatory approval requirements – i.e. are there any significant differences between the options, e.g. GMO option also requires additional approval

3. Biocontrol agent candidate assessment findings

The following agents, from Henzell *et al.* (2008) and also identified through media scanning by Wildlife Health Australia and through discussions with Peter Kerr (CSIRO), were reviewed in accordance with the biocontrol assessment framework.

The *Eimeria intestinalis* and *E. flavescens* option and the RHDV2 option were considered 'tentatively worthwhile'. Leporid herpesvirus-4 and California MSW strain of myxoma virus were considered for a 'watching brief only'. The other options were 'tentatively rejected'.

Table 1: Candidate pathogens reviewed against biocontrol assessment framework. Assessment of RHDV2 based on detection in Australia on May 13th 2015.

Key: Positive Minor concerns Major concerns

	Appropriateness			Effectiveness			Efficiency				
Candidate pathogen	Species specific in Australia	Socially Acceptable	Humane	Effective in wild rabbit populations	Impacts young rabbits	Likely interactions with myxo and RHDV	Self-disseminating	Persists in the environment	Cost - research and development	Cost - manufacture / development	Relative regulatory approval requirements
RHDV2											
Eimeria intestinalis and *E. flavescens*											
Rabbit vesivirus											
Leporid herpesvirus-4											
Rabbit fibroma virus (Shope Fibroma Virus)											
Malignant rabbit virus											
California MSW strain of myxoma virus											
Epizootic rabbit enteropathy (ERE)											
Subtype VbA24 of *Cryptosporidium cuniculus*											
Astrovirus											
Disseminating genetically modified organisms (GMOs)											

Table 2: A review of pertinent information for the candidate biocontrol agents from Henzell *et al.* (2008), and also for Rabbit fibroma virus (Shope Fibroma Virus), California MSW strain of myxoma virus, astrovirus(es) and subtype VbA24 of *Cryptosporidium cuniculus*, identified through media scanning by Wildlife Health Australia and through discussions with Peter Kerr (CSIRO).

	Appropriateness			Effectiveness			Efficiency		
Candidate Pathogen	**Species Specific in Australia**	**Socially Acceptable**	**Humane**	**Effectiveness in Wild Rabbit Populations**	**Impacts Young Rabbits**	**Likely Interactions with Myxomatosis and RHDV**	**Self-Disseminating**	**Persists in the Environment**	**Discussion**
Leporid herpesvirus-4	Unknown: L. Jin (pers. comm. 2013) infected Swiss Webster mice, which had similar pathology and disease - thus mice would shed virus. Mice had few symptoms at low dose - had to give a massive dose, with no mortality. LHV-4 didn't replicate in bovine kidney or monkey tissue cell lines - had to use rabbit cell lines (Jin pers. com. 2013). Possible origin snowshoe hare (*Lepus americanus*), so introduced	Dependent on ocular symptoms which aren't consistent. "Some animals showed only signs of anorexia prior to death" (Jin *et al.* 2008). Most rabbits were found dead without observed signs of disease (Swan *et al.* 1991; Onderka *et al.* 1992).	No neurologic symptoms due to lack of that known gene. Likely to be in some distress due to non-consistent eye lesions and spleen necrosis (Jin pers. com. 2013). "Some animals showed only signs of anorexia prior to death" (Jin *et al.* 2008). "Most rabbits were found dead without" observed signs of disease (Swan *et al.* 1991; Onderka *et al.* 1992). Necrosis in spleen evident	Unknown: Alaska outbreak (domestic rabbits) had a c. 29% mortality (Jin *et al.* 2008). Herpes viruses are normally associated with sexual and oral contact so effective spread is unknown. Any impact (during summer?) on adults, especially RHDV and myxomatosis seropositive animals, and subsequent negative breeding impacts, could support the virus being an efficacious biocontrol.	Likely: "stillbirths and neonatal deaths occurred in a litter from a doe that had recovered following clinical illness the previous year" (Jin *et al.* 2008).	LHV-4 impacts lymph nodes so likely that the suppression of the immune system would influence the pathogenicity of subsequent myxo, & possibly RHDV, infection and vice versa. Stress from a myxo or RHDV infection could cause latent LHV-4 to reactivate and if it doesn't kill the rabbit it may suppress the immune system enough for the myxo or RHDV to cause mortality (Jin pers. com. 2013). Benefits likely with any removal of RHDV	Probably. Suspect primarily rabbit to rabbit (sexually) and possibly mother to young. Due to the observed respiratory symptoms, transmission may occur via aerosols or direct contact. Most cases associated with rabbit to rabbit contact, except Alaska case which may have been associated with insect activity "animals were individually housed in outside open-sided hutches … mosquito … and biting fly …	Probably: It has LAT gene. Becomes latent in the trigeminal ganglion (Jin pers. com. 2013), but virus can still be shed (M. Szpara pers. comm. 2012). Sickness and stress can reactivate latent virus, but dry weather and UV can kill it (M. Szpara pers. comm. 2012). You can't get rid of it from a population once it is present (Jin pers. com. 2013). "Clinical disease in this rabbitry the following year suggests	LHV-4 is an alphaherpesvirus 125kb in size. LHV-4 outbreaks in Canada: NE Alberta, North British Columbia, Saskatchewan, Ontario; and Wasilla, Alaska. Alaska 2006 outbreak affected c. 30 (of 55) rabbits "including adults and nursing young" and c. 16 "died or were euthanized" (only c. 29% mortality) (Jin *et al.* 2008). Ocular symptoms when occurred similar to myxo, but death more rapid (3-7 days) (Jin *et al.* 2008; Sunohara-Neilson *et al.* 2013). "This and prior outbreaks occurred during summer months" (Jin *et al.*

	European hare (*L. europaeus*) may also be able to be infected.		and one of the most severe symptoms of LHV-4 (Jin pers. com. 2013).			& myxomatosis seropositive rabbits (Mutze *et al*. 2002; Mutze *et al*. 2014).	activity was high" (Jin *et al*. 2008).	viral reactivation from latent infection" (Jin *et al*. 2008).	2008) – in Australia this would impact populations at their lowest level/time of highest impact. Brash *et al*. (2010) report death of 1.5 yr old doe. Two adult rabbits "challenged … via intranasal inoculation" required euthanasia at 5.5 dpi (Sunohara-Neilson *et al*. 2013).
Eimeria intestinalis and *E. flavescens*	Yes: no "cross-transmission" known (Duszynski and Couch 2013).	Yes? Flavescens causes severe enteritis with progressive weight loss up to 50% of body weight....' '....Heavily infected animals produce watery faeces with strips of intestinal epithelium and varying amounts of blood...' (Norton *et al*. 1979).	Yes	Yes – these are the two most pathogenic species (Duszynski and Couch 2013 - citing others), but bioclimatic suitability is required and persistence unknown. Likely best in cooler, wetter areas. *E. intestinalis* may cause diarrhoea and increased mortality of adults (von Holst *et al*. 1999).	Yes	Yes if they kill myxomatosis or RHDV seropositive young or reduce fitness and make animals more susceptible to these biocontrols as per Lello *et al*. (2005) and Boag *et al*. (2013). Selection for resistance may be rapid (Osipovskiy 1955).	Yes. Rabbit to rabbit and possibly bird dispersed as per believed for *E. stiedae* and *E. piriformis* (Mykytowycz 1962).	Yes: has persisted in SW WA. Very unlikely to establish and persist in semi-arid and arid areas (outside bioclimatic suitability) (Stodart 1968a).	Both species reported present in south-west Western Australia (Hobbs and Twigg 1998).

Rabbit vesivirus	Yes?	Yes?	Yes?	Unknown, but unlikely. Only known from an Oregon, USA rabbit farm in 1995, with perhaps only 1 of the 5 tested rabbits infected (Martin-Alonso *et al*. 2005).	?Yes –"isolated from juvenile feeder European rabbits … showing symptoms of diarrhea" (Martin-Alonso *et al*. 2005).	Unlikely, but possible if it impacts myxomatosis or RHDV seropositive young	Likely rabbit to rabbit	Unlikely	"The virus was isolated from one rabbit … and also from a pool of five fecal samples including feces from this animal … All five animals displayed intestinal pathology, while the livers of three of them had rare white linear foci. Two dead rabbits were parasitized by coccidia and there was heavy growth of *Escherichia coli* evident in pooled intestinal contents of dead animals. *E. coli* was isolated from liver tissue derived from the same animals. Given the presence of multiple possible disease agents, disease etiology was difficult to ascertain" (Martin-Alonso *et al*. 2005).
Malignant rabbit virus	Yes?	No – as per myxomatosis	No – as per myxomatosis	Unknown, but unlikely.	Probably – as per myxomatosis	Unlikely for myxomatosis as is almost identical.	Assume yes, by mosquitoes as per myxomatosis	Probably – as per myxomatosis	A recombinant between myxoma virus and rabbit fibroma virus – the majority of the virus genome is myxoma virus with a small segment of rabbit fibroma virus

									replacing the equivalent genes in myxoma virus. It is essentially myxoma virus and would have no obvious advantage to existing virulent field strains and there would be complete cross protection between acquired immunity to myxoma virus and malignant rabbit virus (Kerr pers. comm. 2014).
Rabbit fibroma virus (Shope Fibroma Virus)	Yes?	Yes? – only causes cutaneous fibromas	No – as per myxomatosis	Unknown, but unlikely.	"In suckling rabbits, more generalized disease & death usually occur … In all but suckling or experimentally immunosuppressed rabbits, the disease is not normally significant." (Kerr and Donnelly 2013)	Unlikely for myxomatosis as "is used as a live virus heterologous vaccine against myxomatosis" (Kerr and Donnelly 2013). Unknown for RHDV but possible if it impacts RHDV seropositive young.	Yes by mosquitoes (Kerr and Donnelly 2013)	Probably – as per myxomatosis	
California MSW strain of myxoma virus	Yes?	No – as per myxomatosis	No – as per myxomatosis	Unknown, but unlikely as “titres of virus in the skin were relatively low suggesting poor mosquito transmissibility. The	Probably – as per myxomatosis	In experiments done some years ago, infection with the MSW strain of Californian myxoma virus	Assume yes, by mosquitoes as per myxomatosis	Probably not due to likely being outcompeted by myxomatosis (Kerr pers. comm.2014)	

				rapid death of the rabbits meant it would likely be outcompeted by existing well adapted field strains [of myxomatosis]" (Kerr pers. comm. 2014)		completely overcame genetic resistance in Australian wild rabbits (Silvers *et al*. 2006).			
Epizootic rabbit enteropathy (ERE)	Yes	Yes?	Yes	No – multi-factorial (Kerr and Donnelly 2013); only known from farming rabbits (A. Lavazza pers. comm. 2012).	Yes	Unlikely, but possible if it impacts myxomatosis or RHDV seropositive young	No	Unlikely	
Disseminating genetically modified organisms (GMOs)	None available	No	None available	None available	None available	None available	None avail. *Trypanosoma nabiasi* proposed as vector (Hamilton *et al*. 2005).	None available	Previous IA CRC's research provided no effective outcome.
Astrovirus	Unknown, but likely	Probably	Yes	Unknown, but unlikely. Reported impacts likely to be associated with ERE syndrome and farming rabbits (Martella *et al*. 2011; Stenglein *et al*. 2012).	Yes	Unknown, but possible if it impacts myxomatosis or RHDV seropositive young	Likely rabbit to rabbit	Unknown, but unlikely	Stenglein *et al*. (2012) couldn't culture virus. Virus impact believed due to rabbit farming processes (A. Lavazza pers. comm. 2012)
Subtype VbA24 of *Cryptosporidium cuniculus*	No. Human cryptosporidiosis is rarely caused by *Cryptosporidium cuniculus* (Chalmers *et al*. 2011; Hadfield	Probably for impact on rabbit but no for possible impact on people and other species.	Yes	Probably only an issue in farmed rabbits and part of the ERE syndrome	Yes. Highly pathogenic: "first symptoms of infection seen in 53 day old animals ... All the sick rabbits	Unknown, but possible if it impacts myxomatosis or RHDV seropositive young	Likely rabbit to rabbit	Unknown, but unlikely	Species (but not subtype?) reportedly already in Australia (Nolan *et al*. 2010). Genotype VbA26 has been identified from an Eastern grey kangaroo (*Macropus*

	and Chalmers 2012).				demonstrated apathy, anorexia and diarrhoea. The rabbits that developed the clinical symptoms died between 5 and 10 days after onset. At that time, about 300 of the post-weaned rabbits died. (Kaupke *et al*. 2014)"				*giganteus*) (Koehler *et al*. 2014).

4. Discussion of rabbit biocontrol agent candidates recommended as 'tentatively worthwhile'

1. *Eimeria intestinalis* and *E. flavescens*

Eimeria intestinalis and *E. flavescens* are considered the two most pathogenic species of coccidia with an "LD50=3,000 to 5,000 oocysts" (Coudert *et al*. 1995). Mortality was high [and positively correlated with dose] in 6-week-old Dutch rabbits which received 104 or more [*E. flavescens*] oocysts" (Norton *et al*. 1979). "In *E. intestinalis* … inoculation of immunologically naive rabbits may result in the production of 3-5 x 10^8 oocysts per animal and 50% mortality" (Oliveira *et al*. 2011 citing Coudert *et al*. 1995). In spite of this reported mortality and Coudert *et al*. (1995) stating "no correlation between oocyst excretion and the severity of the disease … [with] the excretion peak … about 48 h", Hobbs *et al*. (1999), using such a correlation, report coccidia to not be an important mortality factor. The efficacy of *E. intestinalis* and *E. flavescens* is likely to be highly spatially and temporally variable with regard to rabbit age, environmental conditions and rabbit abundance, and also influenced by concurrent infections (Coudert *et al*. 1995), including myxomatosis (Boag *et al*. 2013). Mortality from *E. intestinalis* and *E. flavescens* occurs "between the 9th and the 12th day post inoculation", with any recovery by 14 days (Coudert *et al*. 1995) and resulting in "a strong immunity" (Licois and Coudert 1980; Coudert *et al*. 1993).

On mainland Australia *Eimeria intestinalis* and *E. flavescens* are only known from Wellstead, south-west Western Australia (Hobbs and Twigg 1998), with *E. flavescens* also reported from Macquarie Island (Bull 1960). They were not detected at four locations in the ACT or at Merricumbene, NSW (Mykytowycz 1956) or near Moruya, NSW (Stodart 1971) or Snowy Plains, Urana or Tero Creek (NSW), or Mitchell (Qld) (Stodart 1968b). Hobbs and Twigg (1998) suggest the technique and identification images used by Stodart (1968b; 1971) may have precluded identification of *E. intestinalis* and *E. flavescens*. The detection of *Eimeria* species in fecal samples can now be done with high sensitivity and accuracy using molecular assays (Oliveira *et al*. 2011).

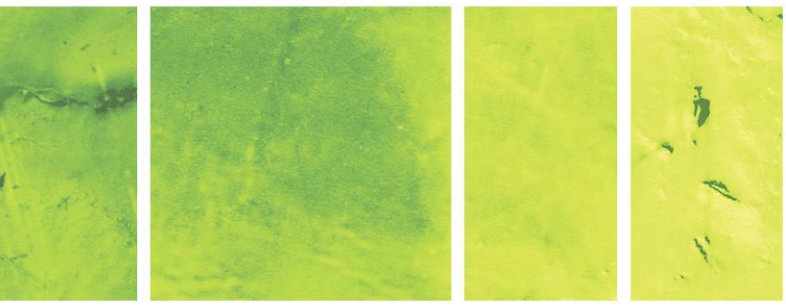
Another option for the *Eimeria* is as a biocide, including in the cooler wetter seasons in more arid areas. Dosing of 10^4 or more oocysts using a bait material could effect a 50% reduction in young rabbits (Norton *et al*. 1979; Coudert *et al*. 1995). Such impact would be during the growing season when productivity of pastures is highest.

Potential benefits:

- Efficacy will be greatest in wetter regions of Australia, which tend to be the higher productivity areas where RHDV has had less effect due to cross-protection from the non-pathogenic RCV-A1 (Strive *et al*. 2010).

Potential issues:

- Selection for resistance may be rapid (Osipovskiy 1955).
- Will be unsuited to hot, dry regions (Stodart 1968b).



- Geographical spread may be limited if not aided by people and vectors.
- Preparation and storage to allow effective distribution may be an issue – potassium dichromate and liquid nitrogen can be used for oocysts but this adds to costs of transport and use.

2. Rabbit haemorrhagic disease virus 2 (RHDV2)

RHDV2 is a new variant of RHDV that was first recognized in France in 2010 (Le Gall-Recule *et al*. 2011) and reported to cause higher infection rates in young rabbits, and to infect and kill vaccinated rabbits (Dalton *et al*. 2012), but causing low and highly variable mortality rates in challenge studies with fully susceptible domestic rabbits (Le Gall-Recule *et al*. 2013). More recent reports describe a highly virulent form of RHDV2 (Schirrmeier *et al*. 2015). Unlike all other known forms of RHDV, RHDV2 is not strictly species specific, but has been reported to infect three species of hares (Puggioni *et al*. 2013; Camarda *et al*. 2014), recently also the European Brown hare (Lavazza and Le Gall-Recule pers. comm. 2015), the only hare species present in Australia (Stott *et al*. 2015). RHDV2 was imported and reviewed as part of the 'RHD Boost' project and although unlike any other strains tested it "did show a non-dose dependent ability to infect and kill some rabbits with RHDV antibodies" (Invasive Animals Cooperative Research Centre 2014 p. 15), it was initially not proposed for release due to its lack of strict species specificity. Recent research found that RHDV2 has evolved further though viral recombination as it has spread through Europe (Lopes *et al*. 2015a), where it appears to be replacing other forms of RHDV (Lopes *et al*. 2015b), with three variants recognised: RHDV2 and the two recombinants G1/RHDV2 and NP/RHDV2. It has reportedly caused high mortality in wild rabbits in Spain, Portugal and Scotland in the past 5 years. RHDV2 has now also arrived in Australia, where it was first reported in a wild rabbit in the ACT in May 2015 and identified as the G1/RHDV2 variant from Portugal/Spain (Hall *et al*. 2015).

Potential benefits

- Demonstrated capacity to kill rabbits immune to RHDV and RHDVa, and potentially those with cross-immunity from RCV-A1 (major declines reported in Scotland rabbit populations with high prevalence of benign virus - Trout *et al*. 1997; B. Boag pers. comm. 2015).
- Potential capacity to overcome genetic resistance in Australian wild rabbits to RHDV and RHDVa
- RHDV2 is now endemic in Australia and hence research is possible outside of quarantine
- Reported capacity to infect and kill a high proportion of young rabbits.

Potential issues

- The genetics of RHDV2 in wild rabbits appears to be changing rapidly and the relative virulence of different strains is currently being tested in Portugal. It is therefore necessary to experimentally determine the virulence grade of the RHDV2 isolate present in Australia.

- Infection of younger rabbits may be an issue if not accompanied by increased mortality rates. Younger rabbits exposed to other RHDV strains are more likely to seroconvert and be protected for life than are older rabbits. A change towards increased juvenile infection has been suggested as supporting the recovery of the Australian rabbit population (Mutze *et al*. 2014; Mutze *et al*. 2015). Mortality of RHDV2 in young rabbits will need to be exactly quantified.
- RHDV2 lacks complete host-specificity, having been shown to infect and kill three hare species, *Lepus capensis mediterraneus* (Puggioni *et al*. 2013), *Lepus corsicanus* (Camarda *et al*. 2014), and now also *Lepus europaeus* (Lavazza and Le Gal pers. comm. 2015), the hare species introduced to Australia. Although now present in Australia, additional species specificity testing may be required by the Australian Pesticides and Veterinary Medicines Authority (APVMA) for any use as the freeze dried virus preparation.

5. Discussion of rabbit biocontrol agent candidates recommended for a watching brief

3. Leporid herpesvirus-4

Leporid herpesvirus-4 (LHV-4) is an alphaherpesvirus. LHV-4 was identified after rabbits died in outbreaks in Canada (north-eastern Alberta, northern British Columbia (Swan *et al*. 1991), and Saskatchewan, Ontario in 1990), at Wasilla, Alaska in 2006 (Jin *et al*. 2008) and in Canada in 2010 (Brash *et al*. 2010).

Potential Benefits:

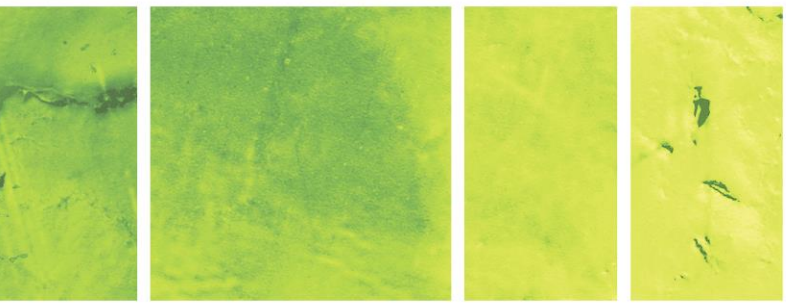
Amongst the current potential biocontrols, LHV-4 perhaps offers the greatest capacity for achieving the broadest spatial and demographic additional control of rabbits. It is reported to:

- Have negatively impacted all age classes of rabbits.
- Have caused stillbirths and neonatal deaths, and hence could impact rabbit recruitment; including in infected but surviving females in which the virus becomes latent.
- Go latent in rabbits and subsequently reactivate, perhaps under the stress of a myxomatosis or RHDV infection, potentially increasing the efficacy of all agents.
- Possibly be insect vectored (deaths have occurred in caged and farmed rabbits).

It does however also come with the greatest, albeit unknown, potential issues.

Potential issues:

- Humaneness and social acceptance – in some rabbits disease has caused purulent conjunctivitis (in others no observed signs). Ling Jin (Oregon State University) suggested some of the ocular symptoms could relate to her ocular mode of dosing animals. Some of the illustrations of infected rabbits are very difficult to tell apart from myxomatosis with very swollen heads and blepharoconjunctivitis.



- Species specificity - L. Jin (pers. comm. 2013) infected Swiss Webster mice with no mortality. Also, it is most closely related to "bovine herpesvirus 2 (BHV-2) … and two closely related viruses of marsupials (wallabies), macropodid herpesvirus 1 and 2 (MaHV-1, -2)" (Babra *et al*. 2012).
- In the one detailed pseudo-field example it only caused 50% morbidity and 29% mortality in rabbits.
- As with most herpesviruses, transmission could be primarily animal to animal, and hence LHV-4 may only be effective in higher density areas with contiguous populations.

Proposed course of action

- Continue to monitor the scientific media through the WHA search protocols, and continue to liaise with Ling Jin and any other researchers of this virus. However, without some further basic research being undertaken (specificity, humaneness, efficacy) our decision making on this virus cannot become more educated.

Moving this candidate pathogen from a 'watching brief' would involve:

- Developing a collaborative research arrangement with Ling Jin (Oregon State University)
- Importation of the virus into the Australian Animal Health Laboratories (AAHL). This could be the strain held by Ling Jin, as unfortunately Patricia Turner at University of Guelph has stated no interest in assisting Australia in this research.
    - Testing on Australian cattle, marsupials and rodents would relatively quickly provide an insight into specificity of the provided strain.
    - Testing on wild Australian rabbits would relatively quickly provide an insight into humaneness and give an insight into efficacy. This would then guide the value of any further broadscale specificity testing.

4. California MSW strain of myxoma virus

A Californian strain of myxoma virus, a type of leporipoxvirus found in *S. bachmani* in Western North America and the Baja peninsula of Mexico, is the most lethal of the leporipoxviruses for European rabbits. In experimental infections with the MSW strain of Californian myxoma virus it completely overcame genetic resistance in Australian wild rabbits (Silvers *et al*. 2006). However, titres of virus in the skin were relatively low suggesting poor mosquito transmissibility and also the rapid death of the rabbits meant that it would likely be outcompeted by existing well adapted field strains. The complete genome sequence of this virus has recently been published (Kerr *et al*. 2013).

Potential benefits

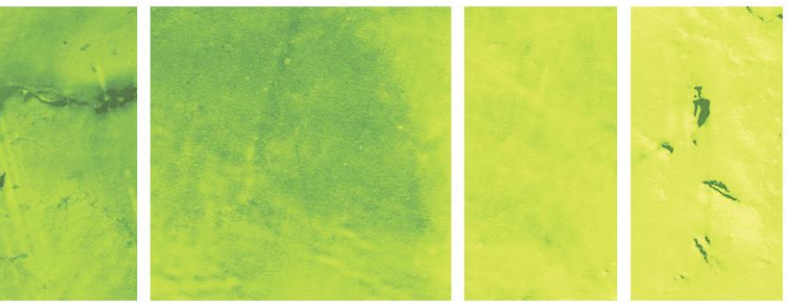
- Capacity to overcome genetic resistance in Australian wild rabbits deemed it worthy of not being immediately rejected

Potential issues

- Likely to have poor transmission.
- May not persist in competition with existing field strains (Berman *et al*. 2006)

Proposed course of action

- Continue to monitor the scientific media through the WHA search protocols. However, without some further basic research being undertaken (specificity, humaneness, efficacy) our decision making on this virus cannot become more educated. For such a virulent myxoma virus, where medium virulence strains have been found to persist best in Australia, the low likelihood that the cost of such research would be returned in agricultural and ecological benefits isn't considered to warrant significant progression of action on this potential pathogen.



6. Initial research projects for biocontrol candidates recommended as 'tentatively worthwhile'

Eimeria Stages 1-7 projects

Stage 1: Pilot project to confirm PCR methodology is effective on field samples and then genetically confirm the presence of *E. intestinalis* and *E. flavescens* at Wellstead (WA)

Prior to implementing a national *Eimeria intestinalis* and *E. flavescens* testing and translocation project, confirm the presence of these two most pathogenic *Eimeria* species at Wellstead (WA) and that the published PCR method (Oliveira *et al*. 2011) is effective at identifying these species in field samples.

This six month sub-project costed at $45,700.

Stage 2: Metagenomic or PCR analysis of field samples to confirm absence of *Eimeria* in Eastern Australia and confirmation of efficacy

Research suggests the Australian rabbit population had a number of primary and secondary origins (e.g. Rolls 1969; Stodart and Parer 1988; Phillips *et al*. 2002; Peacock and Abbott 2013). This is likely to influence the distribution of rabbit parasites, particularly those that occur primarily in cool-wet areas and may not be easily transmitted through the arid region between the southwest and southeast of Australia. This could underly parasites such as the pathogenic *Eimeria intestinalis* and *E. flavescens* only being known from Wellstead, south-west WA (Hobbs and Twigg 1998). A national project is proposed, utilizing both archived and freshly collected tissue and faecal samples to establish the Australian distribution of *Eimeria intestinalis* and *E. flavescens*. The survey should primarily focus on unsampled locations with historical accounts of successful rabbit releases (Peacock and Abbott 2013) and utilise metagenomics or the PCR *Eimeria* identification methodology of Oliveira *et al*. (2011). This research will answer the question of the true Australian distribution of *Eimeria intestinalis* and *E. flavescens*, and therefore establish much of the validity of any translocations of these parasites as biocontrol agents.

Should the relatively new field of metagenomics be confirmed as feasible for this project, then the archived tissue and freshly collected faecal and tissue samples could be analysed to provide an Australian distribution of both *Eimeria intestinalis* and *E. flavescens* as well as other gut parasites and diseases. This will provide the critical baseline knowledge of the distribution (and environmental tolerance) of rabbit gut parasites and diseases using projective modelling techniques outlined in Liu *et al*. (2014).

Part of a three year project costed at $571,000.

Stage 3: Pilot trials and preparation for translocation and spread of parasite (sporozites).

To be able to effectively disseminate the *Eimeria* will require collection and preparation of the sporulated oocysts and utilization of the McMaster method for quantification of *Eimeria* into effective doses (Coudert *et al*. 1995). In addition the survival of the sporulated oocysts will need to be monitored and a distribution and inoculation (see Coudert *et al*. 1995 p. 62) process

established.

Part of a 5 year project costed at $2m.

Stage 4: Efficacy testing and registration with APVMA for *Eimeria* based biocontrol

Eimeria intestinalis and *E. flavescens* are recognised as the two most pathogenic of the 11 coccidia species known to infect *Oryctolagus cuniculus* (Duszynski and Couch 2013). Their virulence is dose dependent (Coudert *et al*. 1995; 1993) and as with *E. stiedae* is likely also influenced by whether the rabbit's immune system has been compromised by myxomatosis (Boag *et al*. 2013). Efficacy testing will assess the virulence of these parasites on rabbits in Australia and provide critical data for the associated registration of the parasites with the Australian Pesticides and Veterinary Medicines Authority (APVMA) for release as additional biocontrol agents for the rabbit.

Part of a 5 year project costed at $2m.

Stage 5: Nationally coordinated release of the two *Eimeria* species

With confirmation of the presence of *Eimeria intestinalis* and *E. flavescens* at Wellstead (WA), their absence at other Australian sites, and the value of their introduction as additional biocontrol agents, they will need to be translocated from Wellstead to other sites. This will require utilising specific culturing and transportation methods for the *Eimeria* sporulated oocysts and their release during cool and moist conditions, which are preferential for their survival and establishment. This application will be primarily for the Australian High Rainfall Zone (see Figure 2 in Appendix).

Project has been costed at $600,000 in 2025/26 (Agtrans Research – see Appendix).

The fully staged project is outlined below in the Appendix with each stage costs.

Stage 6: Ongoing spread to maintain impact

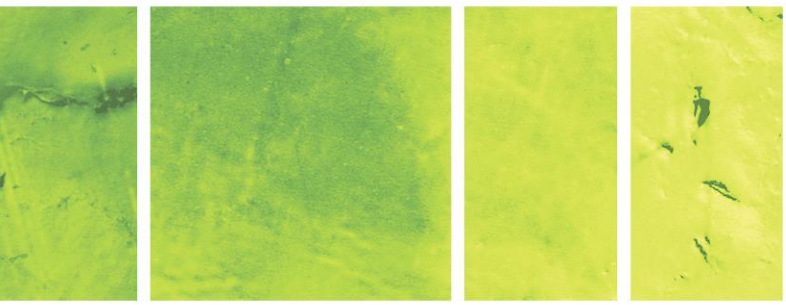
Additional to the nationally coordinated release are further releases to areas of high rainfall and value where it has not been logistically possible to translocate the *Eimeria* through the nationally coordinated release program.

Project has been costed at $100,000 per annum from 2026/27 (Agtrans Research – see Appendix).

Stage 7: Tactical use (akin to a biocide) of *Eimeria* outside of HRZ when and where applicable

Coudert *et al*. (1995 p. 64) state that "doses > 1,000 oocysts always cause clinical pathology irrespective of the species" but that *Eimeria* virulence is dose dependent. Hence if susceptible rabbits could be dosed with enough sporulated oocysts they are likely to be immunologically and physiologically compromised, potentially causing death. Therefore, with APVMA approvals, *Eimeria* could be used tactically, species specifically and safely in areas where rabbits are a problem and conventional control methods aren't feasible.

Project has been costed at $150,000 per annum from 2026/27 (Agtrans Research – see Appendix).



RHDV2 Stage 1, 2 and 3 projects

1. **RHDV Boost Reloaded – assessing the suitability of RHDV2 as an additional biocontrol tool in Australia**

Reports from Europe show that the virulence of RHDV2 varies greatly regarding its levels of virulence (Le Gall Recule *et al*. 2013; Schirrmeier *et al*. 2015). For any virus isolate to be useful as a biocontrol agent in Australia, high levels of virulence are needed. Furthermore, the ability to infect and kill young rabbits is potentially very advantageous (see also section 2.2.2), unless increased infection rates are not accompanies by increased mortality rates. A first, essential step is therefore to characterize the properties of the RHDV2 strain now in Australia. Virulence in adult (and ideally also wild rabbits) needs to be assessed, as well as virulence in young rabbits of various ages. Should the Australian isolate be of reduced virulence, other isolates of RHDV2 from Europe may also be imported and investigated. Furthermore, the ability of the Australian RHDV2 to overcome immunity to all RHDV strains circulating in Australia needs to be determined, as well as the ability to overcome immunity to the Australian RCV-A1.

Part of a 3 year project (EMAI/CSIRO) costed at $1,349,082.

2. **Species specificity testing**

If the stage 1 characterisation reveals that one of the RHDV2 strains assessed has the desired traits, an application will be prepared and submitted to the APVMA to approve RHDV2 as a biocide/biocontrol agent. As RHDV2 has been described to be able to infect and kill certain hare species, it is likely that additional species specificity testing will be required.

Part of a 2 year project with an estimated cost of $2,000,000 per annum.

3. **Production and release of RHDV2**

Depending on the epidemiology of circulating endemic and released RHDV strains in Australia, RHDV2 may be proposed as an additional virus for controlled release.

Due to partial immunological cross protection between RHDV2 and other RHDV strains, it may be useful to have a 'toolkit' of different RHDV strains available, to use the strain for which any given rabbit population has the lowest level of population immunity. For example:

a) Should RHDV2 not spread naturally to all wild rabbits populations, it can be experimentally released. If sufficient ability of RHDV2 to infect and kill young rabbits is confirmed, it can be released when young rabbits are present in the population, i.e. before field strains are usually starting to spread.

b) Should RHDV2 completely replace previous strains of RHDV in some rabbit populations, as has been reported from certain parts of Europe, such populations may then be targeted with other strains of RHDV developed through the 'RHD Boost' (K5) or 'RHD-Accelerator' project. In subsequent years, RHDV2 may be re-used in these populations.

Estimated costs for production and release are $1,550,000 (1yr), followed by $256,000 p.a. for ongoing releases (including monitoring and surveillance) to maintain effectiveness.

Stage 3 relies heavily on the outcomes of the epidemiological monitoring and surveillance efforts of the current 'RHD-Boost Rollout' project.

7. Other activities

Maintaining professional engagement with International researchers studying rabbit pathogens.

It is worth noting that Australian authorities were completely unaware of the discovery of Leporid herpesvirus-4 for almost 20 years after it was first isolated in Canada, partially characterized, then lost before it was fortuitously isolated again from Alaskan rabbits. Although the benefits are hard to quantify, it is critical that Australia maintains a watching brief for further lagoviruses that may be of use in controlling Australian rabbit populations so that other opportunities do not slip past.

Funding Wildlife Health Australia = $6,500 per year. Project manager receives and assesses media monitoring reports.

8. Estimated economic benefit of recommended biocontrol candidates

Rabbit damage in Australian agricultural areas is estimated to cost ca. $200 million per year (Gong *et al*. 2009), with approximately $100 million of this in higher rainfall/livestock areas. Estimating project benefits is however very difficult due to the lack of available information on the current Australian distribution of *Eimeria intestinalis* and *E. flavescens* and hence the likely impact of these pathogens on rabbit populations, and probable longevity of this impact. In addition, parasites such as *E. intestinalis* and *E. flavescens* generally have greatest impact on susceptible young rabbits. As these rabbits can have innate resistance to RHDV and are more likely to seroconvert than die when challenged by this established biocontrol (Mutze *et al*. 2014; Mutze *et al*. 2015), increased mortality of young rabbits by *E. intestinalis and E. flavescens* would likely alter the interaction and mortality from RHD, and myxomatosis (Boag *et al*. 2013), likely resulting in a combined greater impact of all biocontrols on rabbit populations.

An 'Economic Evaluation of Proposed Rabbit Biocontrol Investments' has been provided under contract by Agtrans Research and is attached as Appendix 1. This review has examined the potential economic returns on investments made in the *Eimeria* and RHDV2 biocontrol options. Agtrans Research's key results state:

> "The investment criteria for the relatively small initial investment in *Eimeria* R&D are positive with an expected benefit-cost ratio (BCR) of 27 to 1 and an expected net present value (NPV) of $14.7 million, both estimated over a 15 year time frame from the first year of investment assumed (2017/18). If a 30 year time frame is applied, these investment criteria increase to just over 62 to 1 and $34.5 million respectively. These investment criteria take into account both risk factors as well as the expected additional costs likely to be associated with capturing the benefits from exploitation and spread of two species of *Eimeria* that are reported to exist in the SW of Western Australia but not in other locations.

The investment criteria for RHDV-2 for a 15 year time frame, again with the first year of investment as 2017/18, are positive also within expected BCR of over 157 to 1 and an expected NPV of $191.3 million. If a 30 year time frame is applied, these investment criteria increase significantly to 205 to 1 and $249.2 million respectively."

9. References

Anon (1890). Royal Commission of Inquiry into Schemes for Extermination of Rabbits in Australasia. Progress Report, Minutes of Proceedings, Minutes of Evidence, and Appendices. Charles Potter, Government Printer: Sydney.

Babra B, Watson G, Xu W, Jeffrey BM, Xu JR, Rockey DD, Rohrmann GF and Jin L (2012). Analysis of the genome of leporid herpesvirus 4. *Virology* 433, 183-91.

Berman D, Kerr PJ, Stagg R, van Leeuwen BH and Gonzalez T (2006). Should the 40-year-old practice of releasing virulent myxoma virus to control rabbits (*Oryctolagus cuniculus*) be continued? *Wildlife Research* 33, 549-556.

Boag B, Hernandez AD and Cattadori IM (2013). Observations on the epidemiology and interactions between myxomatosis, coccidiosis and helminth parasites in a wild rabbit population in Scotland. *European Journal of Wildlife Research* 59, 557-562.

Bowen Z and Read J (1998). Population and demographic patterns of rabbits (*Oryctolagus cuniculus*) at Roxby Downs in arid South Australia and the influence of rabbit haemorrhagic disease. *Wildlife Research* 25, 655-662.

Brash ML, Nagy É, Pei Y, Carman S, Emery S, Smith AE. and Turner PV (2010). Acute hemorrhagic and necrotizing pneumonia, splenitis, and dermatitis in a pet rabbit caused by a novel herpesvirus (leporid herpesvirus-4). *Canadian Veterinary Journal* 51, 1383-1386.

Bull PC (1960). Parasites of the European rabbit, *Oryctolagus cuniculus* (L.), on some subantarctic islands. *New Zealand Journal of Science* 3, 258-273.

Camarda A, Pugliese N, Cavadini P, Circella E, Capucci L, Caroli A, Legretto M, Mallia E and Lavazza A (2014). Detection of the new emerging rabbit haemorrhagic disease type 2 virus (RHDV2) in Sicily from rabbit (*Oryctolagus cuniculus*) and Italian hare (*Lepus corsicanus*). *Research in Veterinary Science* 97, 642-5.

Chalmers RM, Elwin K, Hadfield SJ and Robinson G (2011). Sporadic human cryptosporidiosis caused by *Cryptosporidium cuniculus*, United Kingdom, 2007-2008. *Emerging Infectious Diseases* 17, 536-8.

Cooke BD (1984). Factors limiting the distribution of the European rabbit flea, *Spilopsyllus cuniculi* (Dale) (Siphonaptera), in inland South Australia. *Australian Journal of Zoology* 32, 493-506.

Cooke BD (2014). Australia's War Against Rabbits. The Story of Rabbit Haemorrhagic Disease. CSIRO Publishing Collingwood, Victoria.

Coudert P, Licois D and Drouet-Viard F (1995). *Eimeria* species and strains of rabbit. Office for Official Publications of the European Communities. Luxembourg.

Coudert P, Licois D, Provôt F and Drouet-Viard F (1993). *Eimeria* sp. from the rabbit (*Oryctolagus cuniculus*): Pathogenicity and immunogenicity of *Eimeria intestinalis*. *Parasitology Research* 79, 186-190.

Cox TE, Strive T, Mutze G, West P and Saunders G (2013). Benefits of Rabbit Biocontrol in Australia. PestSmart Toolkit publication. Invasive Animals Cooperative Research Centre. Canberra, Australia.

Dalton, K. P., Nicieza, I., Balseiro, A., Muguerza, M. A., Rosell, J. M., Casais, R., Alvarez, A. L. and Parra, F. (2012). Variant rabbit hemorrhagic disease virus in young rabbits, Spain. *Emerging Infectious Diseases* **18**(12), 2009-2012.

Duszynski DW and Couch L (2013). The Biology and Identification of the Coccidia (Apicomplexa) of Rabbits of the World. Academic Press: San Diego, USA.

Elsworth PG, Kovaliski J and Cooke BD (2012). Rabbit haemorrhagic disease: are Australian rabbits (*Oryctolagus cuniculus*) evolving resistance to infection with Czech CAPM 351 RHDV? *Epidemiology and Infection* 140, 1972-81.

Fenner F and Fantini B (1999). Biological Control of Vertebrate Pests. The History of Myxomatosis, an Experiment in Evolution. CABI Publishing: New York.

Foran BD, Low WA and Strong BW (1985). The response of rabbit populations and vegetation to rabbit control on a calcareous shrubby grassland in central Australia. *Australian Wildlife Research* 12, 237-248.

Gong W, Sinden J, Braysher M and Jones R (2009). The economic impacts of vertebrate pests in Australia. Invasive Animals Cooperative Research Centre. Canberra.

Hadfield SJ and Chalmers RM (2012). Detection and characterization of *Cryptosporidium cuniculus* by real-time PCR. *Parasitology Research* 111, 1385-1390.

Hamilton PB, Stevens JR, Holz P, Boag B, Cooke B and Gibson WC (2005). The inadvertent introduction into Australia of *Trypanosoma nabiasi*, the trypanosome of the European rabbit (*Oryctolagus cuniculus*), and its potential for biocontrol. *Molecular Ecology* 14, 3167-3175.

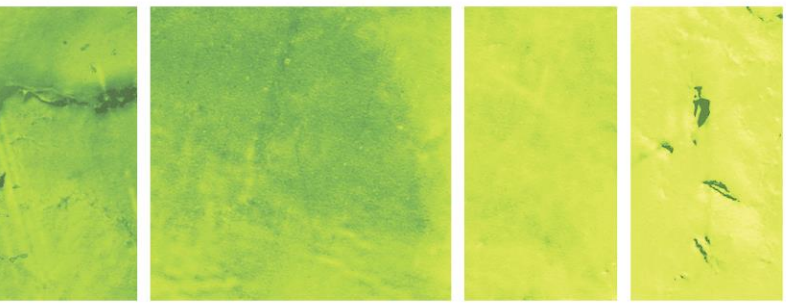
Henzell RP, Cooke BD and Mutze GJ (2008). The future biological control of pest populations of European rabbits, *Oryctolagus cuniculus*. *Wildlife Research* 35, 633-650.

Hobbs RP and Twigg LE (1998). Coccidia (*Eimeria* spp.) of wild rabbits in southwestern Australia. *Australian Veterinary Journal* 76, 209–210.

Hobbs RP, Twigg LE, Elliot AD and Wheeler AG (1999). Evaluation of the association of parasitism with mortality of wild European rabbits *Oryctolagus cuniculus* (L.) in southwestern Australia. *The Journal of Parasitology* 85, 803-808.

Invasive Animals Cooperative Research Centre. (2014). RHD-Boost. Import and evaluate new rabbit haemorrhagic disease virus (RHDV) variants to strengthen rabbit biocontrol. Report to the Vertebrate Pests Committee. PestSmart Toolkit publication. Invasive Animals Cooperative Research Centre. Canberra, Australia.

Jin L, Valentine BA, Baker RJ, Lohr CV, Gerlach RF, Bildfell RJ and Moerdyk-Schauwecker M (2008). An outbreak of fatal herpesvirus infection in domestic rabbits in Alaska. *Veterinary Pathology* 45, 369-74.



Kaupke A, Kwit E, Chalmers RM, Michalski MM and Rzeżutka A (2014). An outbreak of massive mortality among farm rabbits associated with *Cryptosporidium* infection. *Research in Veterinary Science*.

Kerr PJ and Donnelly TM (2013). Viral infections of rabbits. *Veterinary Clinics of North America: Exotic Animal Practice* 16, 437-68.

Kerr PJ, Rogers MB, Fitch A, Depasse JV, Cattadori IM, Hudson PJ, Tscharke DC, Holmes EC and Ghedin E (2013). Comparative analysis of the complete genome sequence of the California MSW strain of myxoma virus reveals potential host adaptations. *Journal of Virology* 87, 12080-9.

Koehler AV, Whipp MJ, Haydon SR and Gasser RB (2014). *Cryptosporidium cuniculus*-new records in human and kangaroo in Australia. *Parasites & Vectors* 7, 492-.

Le Gall-Reculé, G., Zwingelstein, F., Boucher, S., Le Normand, B., Plassiart, G., Portejoie, Y., Decors, A., Bertagnoli, S., Guérin, J.-L. and Marchandeau, S. (2011). Detection of a new variant of rabbit haemorrhagic disease virus in France. *Veterinary Record* **168**, 137-138.

Le Gall-Reculé, G., Lavazza, A., Marchandeau, S., Bertagnoli, S., Zwingelstein, F., Cavadini, P., Martinelli, N., Lombardi, G., Guérin, J.-L., Lemaitre, E., Decors, A., Boucher, S., Le Normand, B. and Capucci, L. (2013). Emergence of a new lagovirus related to rabbit haemorrhagic disease virus. *Veterinary Research* **44**, 1-13.

Lello J, Boag B and Hudson PJ (2005). The effect of single and concomitant pathogen infections on condition and fecundity of the wild rabbit (*Oryctolagus cuniculus*). *International Journal for Parasitology* 35, 1509-1515.

Licois D and Coudert P (1980). Attempt to suppress immunity in rabbits immunized against *Eimeria intestinalis*. *Annales de Recherches Vétérinaires* 11, 273-278.

Liu J, Fordham DA, Cooke BD, Cox T, Mutze G and Strive T (2014). Distribution and prevalence of the Australian non-pathogenic rabbit calicivirus is correlated with rainfall and temperature. *PLoS One* 9, e113976.

Lopes, A. M., Dalton, K. P., Magalhaes, M. J., Parra, F., Esteves, P. J., Holmes, E. C. and Abrantes, J. (2015a). Full genomic analysis of new variant rabbit hemorrhagic disease virus revealed multiple recombination events. *Journal of General Virology* **96**, 1309-19.

Lopes, A., Correia, J., Abrantes, J., Melo, P., Ramada, M., Magalhães, M., Alves, P. and Esteves, P. (2014). Is the new variant RHDV replacing genogroup 1 in Portuguese wild rabbit populations? *Viruses* **7**, 27.

Martella V, Moschidou P, Pinto P, Catella C, Desario C, Larocca V, Circella E, Banyai K, Lavazza A, Magistrali C, Decaro N and Buonavoglia C (2011). Astroviruses in rabbits. *Emerging Infectious Diseases* 17, 2287-93.

Martin-Alonso JM, Skilling DE, Gonzalez-Molleda L, del Barrio G, Machin A, Keefer NK, Matson DO, Iversen PL, Smith AW and Parra F (2005). Isolation and characterization of a new Vesivirus from rabbits. *Virology* 337, 373-83.

Mutze G, Bird P, Kovaliski J, Peacock D, Jennings S and Cooke B (2002). Emerging epidemiological patterns in rabbit haemorrhagic disease, its interaction with myxomatosis, and their effects on rabbit populations in South Australia. *Wildlife Research* 29, 577-590.

Mutze G, Cooke B and Alexander P (1998). The initial impact of rabbit hemorrhagic disease on European rabbit populations in South Australia. *Journal of Wildlife Diseases* 34, 221-227.

Mutze GJ (1996). Release of Spanish Rabbit Fleas as Vectors of Myxomatosis in Inland Australia. Australian Nature Conservation Agency, Final Report of Project 24d, Feral Pests Program. Canberra.

Mutze GJ, Bird P, Jennings S, Peacock D, de Preu N, Kovaliski J, Cooke B and Capucci L (2015). Recovery of South Australian rabbit populations from the impact of rabbit haemorrhagic disease. *Wildlife Research* 41, 552–559.

Mutze GJ, Sinclair RG, Peacock DE, Capucci L and Kovaliski J (2014). Is increased juvenile infection the key to recovery of wild rabbit populations from the impact of rabbit haemorrhagic disease? *European Journal of Wildlife Research* 60, 489-499.

Mykytowycz R (1956). A survey of endoparasites of the wild rabbit, *Oryctolagus cuniculus* (L.) in Australia. *CSIRO Wildlife Research* 1, 19-25.

Mykytowycz R (1962). Epidemiology of coccidiosis (*Eimeria* spp.) in an experimental population of the Australian wild rabbit, *Oryctolagus cuniculus* (L.). *Parasitology* 52, 375-395.

Nolan MJ, Jex AR, Haydon SR, Stevens MA and Gasser RB (2010). Molecular detection of *Cryptosporidium cuniculus* in rabbits in Australia. *Infection, Genetics and Evolution* 10, 1179-1187.

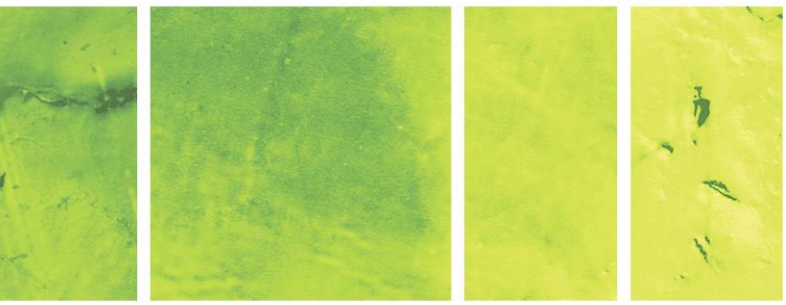
Norton CC, Catchpole J and Joyner LP (1979). Redescriptions of *Eimeria irresidua* Kessel & Jankiewicz, 1931 and *E. flavescens* Marotel & Guilhon, 1941 from the domestic rabbit. *Parasitology* 79, 231-48.

Oliveira UC, Fraga JS, Licois D, Pakandl M and Gruber A (2011). Development of molecular assays for the identification of the 11 *Eimeria* species of the domestic rabbit (*Oryctolagus cuniculus*). *Veterinary Parasitology* 176, 275-280.

Onderka DK, Papp-Vid G and Perry AW (1992). Fatal herpesvirus infection in commercial rabbits. *Canadian Veterinary Journal* 33, 539-543.

Osipovskiy AI (1955). Inheritance of resistance to coccidiosis by rabbits [in Russian]. *Zhurnal Obshchey Biologii* 16, 64-68.

Peacock D and Abbott I (2013). The role of quoll (*Dasyurus*) predation in the outcome of pre-1900 introductions of rabbits (*Oryctolagus cuniculus*) to the mainland and islands of Australia. *Australian Journal of Zoology* 61, 206-280.



Phillips S, Zenger K and Richardson BJ (2002). Are Sydney rabbits different? *Australian Zoologist* 32, 49-55.

Puggioni G, Cavadini P, Maestrale C, Scivoli R, Botti G, Ligios C, Le Gall-Recule G, Lavazza A and Capucci L (2013). The new French 2010 Rabbit Hemorrhagic Disease Virus causes an RHD-like disease in the Sardinian Cape hare (*Lepus capensis mediterraneus*). *Veterinary Research* 44, 96.

Rolls E (1969). They All Ran Wild. Angus and Robertson: Australia.

Schirrmeier, H. (2015). Zum Auftreten von RHDV-2 in Deutschland – aktuelle Herausforderungen in der Diagnostik und Bekaempfung. *Der LabLoeffler News* 2015; 1:10, p4-7. [In German.]

Silvers L, Inglis B, Labudovic A, Janssens PA, van Leeuwen BH and Kerr PJ (2006). Virulence and pathogenesis of the MSW and MSD strains of Californian myxoma virus in European rabbits with genetic resistance to myxomatosis compared to rabbits with no genetic resistance. *Virology* 348, 72-83.

Sobey WR and Conolly D (1971). Myxomatosis: the introduction of the European rabbit flea *Spilopsyllus cuniculi* (Dale) into wild rabbit populations in Australia. *Journal of Hygiene* 69, 331-346.

Stenglein MD, Velazquez E, Greenacre C, Wilkes RP, Ruby JG, Lankton JS, Ganem D, Kennedy MA and DeRisi JL (2012). Complete genome sequence of an astrovirus identified in a domestic rabbit (*Oryctolagus cuniculus*) with gastroenteritis. *Virology Journal* 9, 216.

Stodart E (1968a). Coccidiosis in wild rabbits, *Oryctolagus cuniculus* (L.), at four sites in different climatic regions in eastern Australia 11. The relationship of oocyst output to climate and some aspects of the rabbits' physiology. *Australian Journal of Zoology* 16, 619-628.

Stodart E (1968b). Coccidiosis in wild rabbits, *Oryctolagus cuniculus* (L.), at four sites in different climatic regions in eastern Australia I. Relationship with the age of the rabbit. *Australian Journal of Zoology* 16, 69-85.

Stodart E (1971). Coccidiosis in wild rabbits, *Oryctolagus cuniculus* (L.) at a site on the coastal plain in eastern Australia. *Australian Journal of Zoology* 19, 287-292.

Stodart E and Parer I (1988). Colonisation of Australia by the rabbit, *Oryctolagus cuniculus* (L). CSIRO Division of Wildlife and Ecology.

Stott P (2015). Factors influencing the importation and establishment in Australia of the European hare (*Lepus europaeus*). *Australian Journal of Zoology* 63, 46-75.

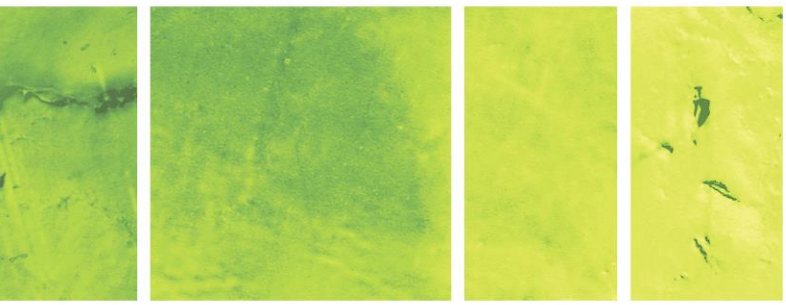
Strive T, Wright J, Kovaliski J, Botti G and Capucci L (2010). The non-pathogenic Australian lagovirus RCV-A1 causes a prolonged infection and elicits partial cross-protection to rabbit haemorrhagic disease virus. *Virology* 398, 125-134.

Sunohara-Neilson JR, Brash M, Carman S, Nagy E and Turner PV (2013). Experimental infection of New Zealand white rabbits (*Oryctolagus cuniculi*) with Leporid herpesvirus 4. *Comparative Medicine* 63, 422-31.

Swan C, Perry AW and Papp-Vid G (1991). Herpesvirus-like viral infection in a rabbit. *Canadian Veterinary Journal* 32, 627-628.

Trout, R. C., Chasey, D. and Sharp, G. (1997). Seroepidemiology of Rabbit Haemorrhagic Disease (RHD) in wild rabbits (*Oryctolagus cuniculus*) in the United Kingdom. *Journal of Zoology* **243**, 846-853.

von Holst D, Hutzelmeyer HD, Kaetzke P, Khaschei M and Schönheiter R (1999). Social rank, stress, fitness, and life expectancy in wild rabbits. *Naturwissenschaften* 86, 338–393.



Researchers consulted

- Dr Ling Jin, University of Oregon
- Professor Peter Hudson, Pennsylvania State University
- Dr Moriah Szpara, Pennsylvania State University
- Dr Isabella Cattadori, Pennsylvania State University
- Dr Andrew Read, NSW Elizabeth Macarthur Agricultural Institute
- Professor Joseph DeRisi, University of California San Francisco
- Dr Mark Stenglein, University of California San Francisco
- Dr Antonio Lavazza, IZLER, Brescia, Italy
- Dr Mario Chiari, IZLER, Brescia, Italy
- Dr Tanja Strive, CSIRO

Expert panel

- Dr Peter Kerr, CSIRO, Australia
- Dr David Spratt, CSIRO, Australia
- Dr Antonio Lavazza, IZLER, Brescia, Italy
- Professor Peter Hudson, Pennsylvania State University, USA (unable to meet requirements)

Industry representatives

- Mr Cameron Allan, Meat and Livestock Australia
- Mr Ian Evans, Australian Wool Innovation
- Mr David Lord, Australian Wool Innovation
- Ms Susan Campbell, Australian Wool Innovation
- Ms Joanne Nathan, Department of the Environment, Canberra
- Mr Nicholas Newland, Foundation for Rabbit-Free Australia Inc.
- Mr Peter Michelmore, Rangeland NRM Alliance
- Dr Brian Cooke, international rabbit expert (retired)
- Dr Tanja Strive, rabbit researcher and virus expert, CSIRO
- Mr Greg Mutze, rabbit researcher, Biosecurity SA
- Dr Tony Pople, Qld Department of Agriculture and Fisheries
- Dr John Tracey, NSW Department of Primary Industries
- Mr Steve McPhee, rabbit control and research authority (semi-retired; ex-Victorian state government)
- Mr Andreas Glanznig, Invasive Animals CRC

Appendix: Economic Evaluation of Proposed Rabbit Biocontrol Investments

Economic Evaluation of

Proposed Rabbit Biocontrol Investments

Final Report

By

Talia Hardaker and Peter Chudleigh

Agtrans Research

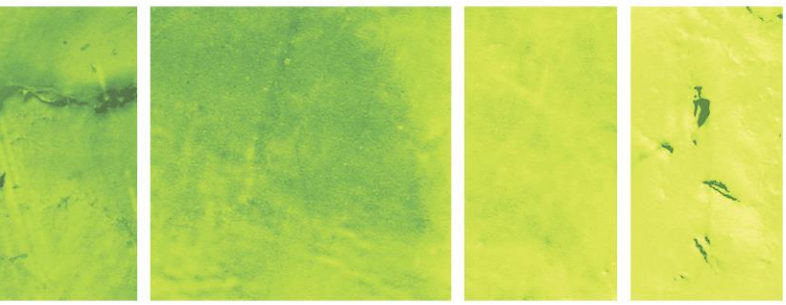
To

Biosecurity SA

16 September 2015

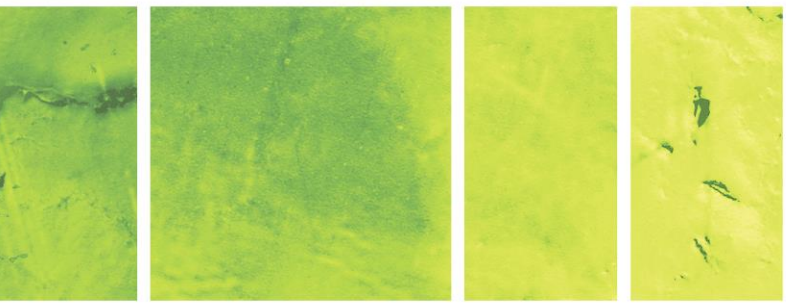
Please address all correspondence in relation to this proposal to:

AGTRANS RESEARCH
Suite 36, Benson House, 2 Benson Street,
Toowong, Brisbane, Australia
PO Box 385, Toowong Qld 4066
Telephone: (07) 3870 4047 or (07) 3870 9564
Facsimile: (07) 3371 3381
Email: info@agtrans.com.au
ABN 18 010 605 964 ACN 010 605 964



Contents

Executive Summary 4
1. Introduction 5
2. Terms of Reference 6
3. Background 6
4. Impact Assessment Approach 7
5. Review of Current and Projected Rabbit Costs to Industry and the Counterfactual 7
6. The Logical Framework for the Investment 8
7. The Initial Investments 10
8. Impacts and their Valuation 12
Triple bottom line impacts 12
Impacts not valued 13
Impacts valued 13
Eimeria 14
RHDV-2 19
Cost-benefit analysis process 22
9. The Cost-Benefit Analysis Results 23
Base Results 23
Sensitivity analyses 26
EIMERIA 26
RHDV-2 28
10. Discussion 30
11. Conclusions 31
Acknowledgments 31
References 31
Additional Bibliography 32
Researchers consulted 28
Expert panel 28
Industry representatives 28



Executive Summary

This economic impact analysis has been generated to support the case for a new investment in research and development of two biocontrol agents to assist with the future control of Australian rabbits. The agents are two species of *Eimeria*, a gut parasite, and RHDV-2, a new serotype of the RHD virus.

The approach undertaken in this report is to identify and describe the investment in each biocontrol agent and then to analyse the costs and benefits of each investment. The impact analysis uses a logical framework to describe the pathway from investment to impact followed by a cost-benefit analysis (CBA). The CBA uses estimates of investment and other costs associated with introducing each biocontrol agent as well as estimates of the potential impacts of the agents on economic loss reductions to industry including control costs.

The key objective is to assess whether the investment (the costs of the R&D addressing the two biocontrol agents and the associated costs of using and managing the agents) will be paid for by the impact of the agents in rabbit cost reduction.

The investment criteria estimated from the base set of assumptions for the initial investment in *Eimeria* are all positive from a period of 15 years after the first year of investment, and are all positive from 10 years for the investment in RHDV-2. The positive investment criteria suggest that the initial investments would be worthwhile given the estimates made of future likely pathways, the additional investment and associated timelines required, the risks involved, and the expected sequencing and level of rabbit impacts.

Furthermore, the proposed investment can be staged conditionally so that as the investment proceeds along a particular pathway, directions can be changed according to any past success and any new information so avoiding or minimising losses and maximising the chances of significant impacts being delivered.

The investment criteria for the relatively small initial investment in *Eimeria* R&D are positive with an expected benefit-cost ratio (BCR) of 27 to 1 and an expected net present value (NPV) of $14.7 million, both estimated over a 15 year time frame from the first year of investment assumed (2017/18). If a 30 year time frame is applied, these investment criteria increase to just over 62 to 1 and $34.5 million respectively. These investment criteria take into account both risk factors as well as the expected additional costs likely to be associated with capturing the benefits from exploitation and spread of two species of *Eimeria* that are reported to exist in the SW of Western Australia but not in other locations.

The investment criteria for RHDV-2 for a 15 year time frame, again with the first year of investment as 2017/18, are positive also with in expected BCR of over 157 to 1 and an expected NPV of $191.3 million. If a 30 year time frame is applied, these investment criteria increase significantly to 205 to 1 and $249.2 million respectively.

Some care should be taken in interpreting the different investment criteria. Apart from the

initial R&D investment, any ongoing investment assumed to be required in any year is subtracted from the estimated benefits from the corresponding year. The benefit-cost ratios (BCRs) for both agents appear quite large (62 to 1 for *Eimeria* and 205 to 1 for RHDV-2). One of the main reasons for this is the small magnitude of the size of the initial committed investments represented by the present values of costs (PVC). The same issue also must be taken into account when considering the return on investment (ROI) figures for both proposed biocontrol investments. Calculated by the ratio of the NPV (PVB - PVC) to the PVC, at 61 to 1 for *Eimeria* and 204 to 1 for RHDV-2, the ROI results are similar to those for the BCRs and are high as a result of the small PVCs for each agent.

Perhaps the most meaningful criteria in interpreting the results as the time period increases is the net present value (NPV) as it captures the flow of values of both expected benefits and costs including the original project investment costs. At 15 years the *Eimeria* NPV is $14.7 million and that for RHDV-2 is $191.3 million. By year 30 the RHDV-2 NPV has grown to $249.2 m (1.3 times) but *Eimeria* has increased by 2.3 times its 15 year NPV, but still only to $34.5 m. This demonstrates the earlier impact of RHDV-2 and the assumed greater pathogenicity and more widespread impact of RHDV-2.

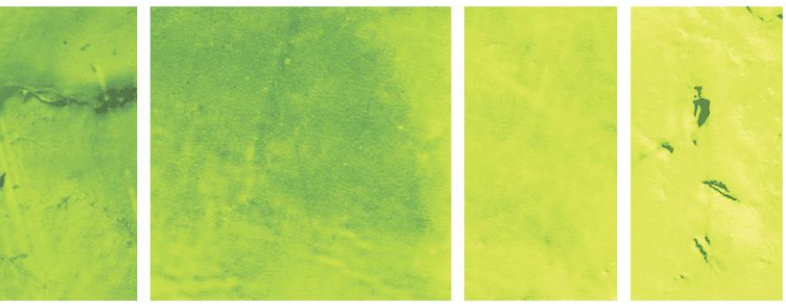
The investment criteria for RHDV-2 are all significantly higher than those for *Eimeria*. The principal reason for the higher expected investment criteria for RHDV-2 over 30 years is the higher level of impact of RHDV-2 on rabbit costs (25% compared to *Eimeria* at 10%) and its potential application to all zones compared with *Eimeria* impacts being restricted to the High Rainfall Zone (HRZ).

An unspecified strain of RHDV-2 has been detected in the ACT. Should this strain prove virulent and become widespread throughout Australia there may be consequences to the development of the recombinant strain of RHDV-2 as described in this report. This analysis does not report on the impacts and benefits of investing in the recombinant RHDV-2 should the existing virus spread leading to some effect on the Australian rabbit population. As more information about the distribution and virulence of existing strains of RHDV-2 in Australia becomes available, in the future it would be possible to update the model in order to accommodate other potential scenarios and/or the registration of the recombinant strain of RHDV-2 for the more limited purpose of tactical biocontrol only.

The investment criteria estimated from the base set of assumptions for the initial investment in *Eimeria* are all positive from a period of 15 years after the first year of investment, and are all positive from 10 years for the investment in RHDV-2. The positive investment criteria suggest that the initial investments would be worthwhile given the estimates made of future likely pathways, the additional investment and associated timelines required, the risks involved, and the expected sequencing and level of rabbit impacts.

1. Introduction

This report provides an independent assessment of a prospective investment in rabbit bio-control. The report has the purpose of supporting the business case for further research investment in delivering new rabbit biocontrol options following the successful investment in



rabbit control (including rabbit biocontrol) by both phases of the Invasive Animals Cooperative Research Centre (IA CRC) from 2006 to date.

2. Terms of Reference

The terms of reference for this assessment include:

1 Review and discuss previous and existing rabbit biocontrol initiatives (including RHD, RHD-Boost and RHD-Accelerator), together with likely scenarios for future rabbit impact costs and control costs.

2 Value the expected net economic benefits of the proposed biocontrol investments, taking into account the projected investment costs, timelines and risk factors.

3 Carry out sensitivity analyses that show the change in investment criteria with changes in key assumptions.

4 Provide conclusions on the economic merits of the potential project investment.

3. Background

Biosecurity South Australia (Biosecurity SA) required an economic impact study carried out on a proposed project involving further research investment to advance the potential use of two proposed rabbit biocontrol agents. The agents are:

- two species of gut parasites of rabbits (*Eimeria intestinalis* and *Eimeria flavescens*), identified as being highly pathogenic and target species specific; these two pathogenic species have been reported as existing in SW Western Australia but have not been reported in other regions of Australia.
- A recombinant strain of RHDV-2; this agent is a variant of RHDV and is phylogenetically distinct from other lagoviruses and presents a unique antigenic profile (Le Gall-Recule *et al*. 2013).The recombinant strain is the current field strain of RHDV-2 circulating in Europe (T. Strive and A. Read, 2015).

The intended investment follows the success of both myxomatosis (Myxoma virus) in the 1950s and, more recently, Rabbit Haemorrhagic Disease Virus (RHDV), also known as rabbit calicivirus. Despite the past success of these two agents resulting in significant reductions of the wild rabbit population, there is some uncertainty of continued control by these agents into the future due to the potential threat of an increasing development of genetic resistance in rabbits.

Given the past success of Australian rabbit biocontrol agents and, as the process of identifying, testing, and introducing new biocontrol agents can be protracted, the IA CRC has thought it

prudent to commence identifying and testing new agents to guard against the risk of development of resistance and an associated reduction in the effectiveness of rabbit control.

A scanning and prioritisation exercise for potential biocontrol agents has been undertaken by Australian scientists and industry. This exercise has provided the basis for the current business plan and the selection of the two biocontrol agents shortlisted for further research. These two agents are the focus of this impact assessment.

4. Impact Assessment Approach

The impact assessment uses cost-benefit analysis (CBA) set within a staged risk management framework of investment. The approach is to identify and describe the investment and its objectives, expected outputs and outcomes and the associated expected economic impacts from the prospective investment.

The key objective is to assess whether the investment (the costs of the R&D addressing the two biocontrol agents) will be paid for by the impact of the agents in reduction of the costs of rabbits to the Australian economy.

It is axiomatic that successful biocontrol investment can often take long periods and can be considered risky. One of the principal considerations in analysing and valuing impacts from biocontrol is how the counterfactual is specified. A second principal consideration that has to be accommodated in the analysis is how riskiness is represented. A description of the assumed counterfactual, and how it is used as a baseline enabling measurement of the impacts due to the investment, is provided in a later section of this report.

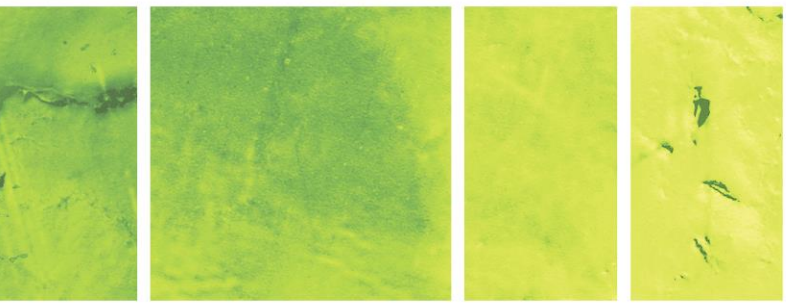
It is anticipated that the total investment costs will be staged (go/no go decisions at particular stages of the investment, depending on progress) in order to minimise investment risk. Also, risk factors are built into the analysis in order to ensure output, outcome and impact risks are taken into account so that the likely benefits from the investment are not overestimated.

The CBA focuses on identifying and valuing economic benefits to industry with some consideration given to identifying environmental and social benefits where impacts exist and where reasonable assumptions can be made.

5. Review of Current and Projected Rabbit Costs to Industry and the Counterfactual

There are a number of past studies that have addressed the cost of rabbits to Australian rural industries. Other analyses have updated these expected costs given the recent impacts of the original RHD strain released and the potential cost reductions due to the pending release of RHD-Boost. Studies examining the impact of the IA CRC's RHD-Accelerator investment (also associated with the rabbit calicivirus) also exist. All of these studies and their likely impacts on rabbits have been reviewed and have provided rabbit damage and control cost levels likely to exist in the future thus providing a baseline for costs in the "without new investment in biocontrol agents" that represents the counterfactual scenario.

The counterfactual assumed can be represented by the situation for annual rabbit damage and



control costs into the future in current $ terms and commencing in the year ended 30th June 2018. These annual cost data take into account existing knowledge regarding the impact on costs of RHD-Boost being released in calendar 2016 and any new RHDV strains expected to be released as a result of the IA CRC's RHD-Accelerator project with new cell culture systems available from 2020/21 expected to be released after 2024/25 when the effects of RHD-Boost begin to wane.

6. The Logical Framework for the Investment

A logical framework for analysing the investment and its impact was developed. This required an understanding of the intended investments and their likely outputs, outcomes and impacts. Sources of information for building this understanding included:

- A face-to-face meeting held between the Agtrans Research personnel and the Principal Investigator for the project (David Peacock).
- Information extracted from the discussion paper and business case prepared by Biosecurity SA.
- Scientific contacts identified by David Peacock (referred to in the acknowledgements section).

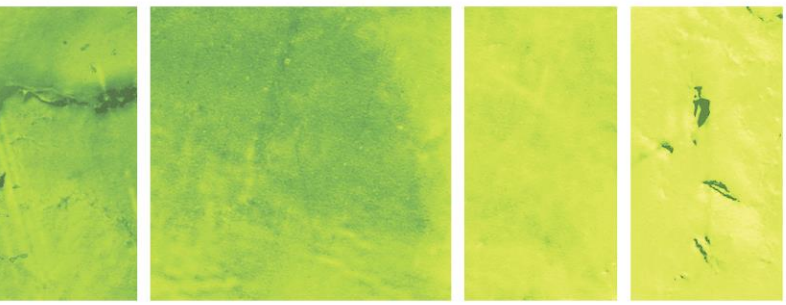
Initial logical frameworks for the impact assessment of the prospective investment in each biocontrol agent were developed. These frameworks related investment costs (including any other industry and in-kind research provider inputs), to likely activities and expected outputs, outcomes, and impacts. Each outcome or expected outcome was described in terms of its expected impact on the value of rabbit damage as well as on control costs themselves.

Draft descriptions of these initial logical frameworks, together with some initial questions to clarify some aspects, were then circulated to Biosecurity SA personnel for comment. After feedback, the logical frameworks and their pathways to impact diagrams were enhanced and the diagrammatic representations of the pathways to impact in a decision framework were finalised and provided for each biocontrol agent in Figures 1 and 3 in section 8.

In addition:

- Any positive and negative impacts on the environment were identified as well as any social impacts.
- Although each of the two prospective biocontrol agents were treated independently of the other, the framework allowed representation of any potential positive natural synergies between each agent and biocontrol agents already affecting Australian rabbit populations (existing strains of RHDV and the Myxoma virus).
- Apart from these potential natural synergies, the framework also captured additional potential impacts from tactical use of each single agent in particular areas and seasonal

conditions. When used in this fashion the agent will be referred to as a ‘biocide’ in this report.



7. The Initial Investments

The initial investments required for each of the biocontrol agents are shown in Tables 1 and 2. As Figures 1 and 3 show, there is likely to be further investment required at different stages along the pathway to impact in order to capture the final impacts. These investment costs are provided in later tables.

Table 1: Initial Investment for *Eimeria* Biocontrol based on the proposed project "Distribution of the Genetics, Gut Diseases and Parasites (Primarily *Eimeria*) of the Australian Rabbit Population" (2013/2014 $)

Year ended 30th June	2018 ($)	2019 ($)	2020 ($)	Total ($)
Funding Organisation	45,700[a] + 190,333[b]	190,333[b]	190,333[b]	616,700

[a]Stage 1: Pilot project to confirm PCR methodology is effective on field samples and then genetically confirm the presence of *E. intestinalis* and *E. flavescens* at Wellstead (WA).

[b]Stage 2: Metagenomic or PCR analysis of field samples to confirm absence of *Eimeria* in Eastern Australia and confirmation of efficacy.

Table 2: Initial Investment for RHDV-2 Biocontrol based on the proposed project "RHDV Boost Reloaded – New European RHDV-2 Strains for Rabbit Biocontrol" (2013/2014 $)[a]

Year ended 30th June	2018 ($)	2019 ($)	2020 ($)	Total ($)
Funding Organisation	240,792	226,801	293,090	760,683
CSIRO/NSW DPI	162,203	208,263	217,933	588,399
Total	402,995	435,064	511,023	1,349,082

[a]A strain of RHDV-2 has recently been detected in the ACT, it is assumed that its spread and presence elsewhere will be monitored within the existing IA CRC biocontrol project for the years ending June 2016 and June 2017. The investment considered in this analysis is for the importation of a particular recombinant strain of RHDV-2 identified as the predominant field strain that is currently circulating in Europe (Strive, T. and Read, A., 2015) and is treated as a separate biocontrol agent to the strain of RHDV-2 already detected in Australia.

If the investments in Table 1 and 2 are funded, for purposes of the current analysis they are considered sunk costs whatever the subsequent outputs and outcomes. However, the findings of these initial investments will direct the less certain pathways and their associated costs and impacts that subsequently occur.

8. Impacts and their Valuation

Triple bottom line impacts

Table 3 summarises, in a triple bottom line framework, the broad impacts that may be delivered from the investment. The principal economic impact will be lowered cost of rabbit damage and control costs incurred by agricultural industries compared to the situation where the new biocontrol investment is not made.

Positive environmental impacts may be delivered also from reduced rabbit populations in the form of:

- enhanced biodiversity of native vegetation from reduced impacts of rabbits on native tree and shrub regeneration,
- reduced greenhouse gas emissions from increased regeneration of young trees and shrubs, and
- reduced landscape damage and soil erosion from reduced impact of rabbits due to overgrazing and burrowing.

Social impacts will include the regional community impacts from maintained or increased farm incomes from increased grazing opportunities for livestock

Table 3: Principal Impacts in a Triple Bottom Line Framework

Economic	Environmental	Social
Reduced cost of damage caused by rabbits to the agricultural sector and associated increase in farm profits due to improved productivity because of increased grazing resource.	Enhanced vegetation biodiversity	Regional community impacts from maintained or increased grower incomes.
	Reduced greenhouse gas emissions from increased regeneration of young trees and shrubs	
Reduced rabbit control costs.	Reduced land and soil damage from overgrazing and burrowing	Producer impacts from less anxiety and income related stress.

Impacts not valued

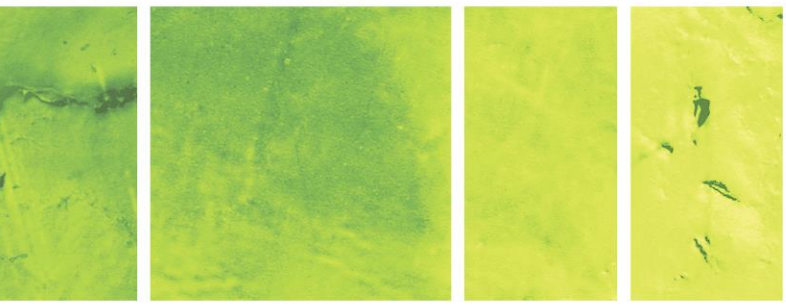
The impacts identified but not valued include the impact on natural resources, landscapes, vegetation, soils and greenhouse gas emissions and the community impacts from a healthier farm economy.

Impacts valued

The economic impacts valued in the quantitative analysis are the expected impact of the new biocontrol agents on rabbit damage costs and rabbit control costs to industry. These cost reductions are based on the counterfactual scenario as described earlier (section 5). The counterfactual scenario is the projected costs of rabbit damage and control that are likely to be present if the proposed investment in new biocontrol agents does not proceed. The counterfactual is based on the baseline rabbit costs to agriculture in the Gong report (Gong et al, 2009) reduced by expected subsequent impacts on rabbits (mostly attributed to IA CRC funding). The subsequent impacts used to estimate reduced impact figures based on the Gong Report refer to the anticipated damage reductions resulting from RHD-Boost, RHD-Accelerator, and the smaller reductions due to the Rabbit Decision Support System and the National Rabbit Facilitator. This process provided a new expected baseline of current and future costs relevant to any new biocontrol initiatives.

The expected potential impacts from each of *Eimeria* and RHDV-2 are estimated separately. For example, the estimates of impacts for *Eimeria* assume that the Australia-wide spread of the recombinant strain of RHDV-2 does not occur. Each of these primary analyses accommodate the assumptions made regarding additional investment required, timelines, impacts and risk factors for the single agent and do not assume any competitive or complementary/additive impacts with the other proposed biocontrol agent. The investment in and impact of each of the potential biocontrol agents therefore is assessed independently.

Many of the assumptions required to value the impacts for each biocontrol investment are in the future and are therefore uncertain. While reasonable and conservative assumptions have been made in the analyses, the resulting investment criteria should be viewed with some caution.



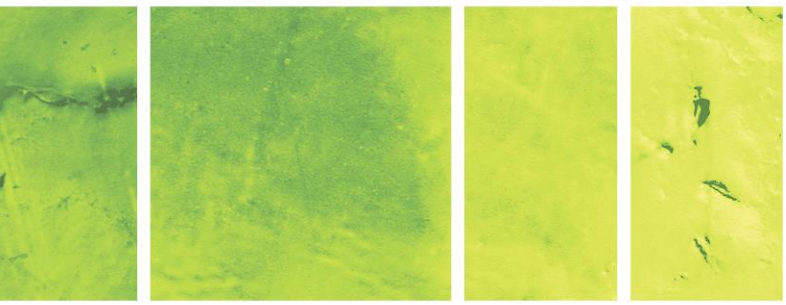
Eimeria

The assumptions made to estimate the expected costs additional to the investment costs outlined in Table 1 and expected benefits of the investment in *Eimeria* are provided in Table 4.

Table 4: Summary of Assumptions for Valuation of Additional Investment Costs and Benefits for *Eimeria* (2013/2014 $ terms)

Counterfactual – without *Eimeria* investment		
Rabbit damage costs and control costs to Australia assuming RHD-Boost, RHD-Accelerator, and the current investment in rabbit decision support systems and the National Rabbit Facilitator are all successful. The damage and control costs of rabbits from 2020/21 are assumed to be approximately $304.6 m per annum. (Agtrans Research)		
With *Eimeria* investment		
Variable	**Value**	**Source**
Stage 1: Pilot project to confirm PCR methodology is effective on field samples and then genetically confirm the presence of E. intestinalis and E. flavescens at Wellstead (WA).		
Cost	$45,700 (6 months project) in 2017/18	Peacock (2015b)
Probability of success	90% (Stage 1 and Stage 2 combined)	Agtrans Research[(a)]
Stage 2: Metagenomic or PCR analysis of field samples to confirm absence of Eimeria in Eastern Australia and confirmation of efficacy.		
Cost	$571,000 over 3 years from 2017/18	Peacock (2015b)
Probability of success	90% (Stage 1 and Stage 2 combined)	Agtrans Research[(a)]
Stage 3: Pilot trials and preparation for translocation and spread of parasite (sporozites).		
Cost	$2,000,000 p.a. over 5 years from 2020/21 (Stage 3 and Stage 4 combined)	Based on investment costs for RHD-Boost, Agtrans Research (2011) and R&D timeline estimates outlined in the Eimeria business case document, Peacock (2015b)
Probability of success	75% (Stage 3 and Stage 4 combined)	Agtrans Research
Stage 4: Efficacy testing and registration with APVMA for Eimeria based biocontrol.		
Cost	$2,000,000 p.a. over 5 years from 2020/21 (Stage 3 and Stage 4 combined)	Based on investment costs for RHD-Boost, Agtrans Research

		(2011) and R&D timeline estimates outlined in the Eimeria business case document, Peacock (2015b)
Variable	**Value**	**Source**
Probability of success	75% (Stage 3 and Stage 4 combined)	Agtrans Research
Stage 5: Nationally coordinated release of the two Eimeria species.		
Area applicable for *Eimeria* use as a general rabbit biocontrol agent	Australian High Rainfall Zone (HRZ) – see Figure 2	D. Peacock (pers. comm.)
Cost	$600,000 in 2025/26	Agtrans Research[(b)]
Probability of Success	80% (Stage 5 and Stage 6 combined)	Agtrans Research
Stage 6: Ongoing spread to maintain impact		
Cost	$100,000 per annum from 2026/27	Agtrans Research[(b)]
Probability of Success	80% (Stage 5 and Stage 6 combined)	Agtrans Research
Impacts of Investment in Stages 1 to 6		
Probability of impact given successful coordinated translocation across HRZ.	90%	Agtrans Research
Cost of rabbits in HRZ as proportion of total Australian rabbit costs.	39%	Agtrans Research adapted from Gong *et al.* (2009)
Overall reduction in rabbit impact and rabbit control costs.	10% in the HRZ commencing in 2026/27	D. Peacock (pers. comm.)
Period of stable impact assuming naïve rabbit population in the HRZ.	8 years from 2026/27 to 2033/34	Agtrans Research[(b)]
Rate of increase of rabbit costs assuming resistance becomes evident after stable period.	2% per annum from 2034/35	Based on research indicating that resistance to Eimeria may be rapid (Osipovskiy 1955)
Maximum level of rabbit costs reached given resistance build and assuming positive interactions (Cox *et al.*, 2013) between Myxomatosis, RHDV (Czech-351 and K5), and *Eimeria* in some seasons.	95% of 2025/26 (pre-release) levels	Agtrans Research[(a)]
Stage 7: Tactical use (akin to a biocide) of Eimeria outside of HRZ when and where applicable.		
Cost	$150,000 per annum from 2026/27 onwards	Agtrans Research[(b)]



Impacts of Investment in Stage 7		
Variable	**Value**	**Source**
Overall cost reduction factor applied for impact valuation. This represents the cost reduction through the localised use of *Eimeria* in specifically applicable areas outside of the HRZ and only in applicable seasons.	0.3% (calculated by 25% x 10% x 61% x 20% - see following rows for individual component sources).	Agtrans Research
Reduction in total rabbit costs (damage and control)	25% in applicable areas and seasons	Based on Eimeria baits effecting up to a 50% reduction in young rabbits (dose dependent). (Norton *et al*. 1979; Coudert *et al*. 1995)
Proportion of Australian rabbit area (excluding the HRZ) applicable for biocide use in a given season.	10% (applies only to non-HRZ use represented by the remaining 61% rabbit damage area)	Agtrans Research
Applicable 'cooler/wetter' seasons estimated to occur once every 5 years.	1/5 (20%)	Agtrans Research
Probability of impact occurring	90%	Agtrans Research

(a) Based on discussions with David Peacock of Biosecurity SA and/or correspondence with personnel of the Invasive Animals CRC.

(b) Derived from estimated additional costs expected for the initial release and ongoing upkeep from RHD-Boost (IA CRC Impact Assessment, *submitted draft*, Agtrans Research, 2015)

Figure 1 is a simple, generalised diagrammatic representation of the pathway to impact most likely for the proposed investment in *Eimeria* as a rabbit biocontrol agent.

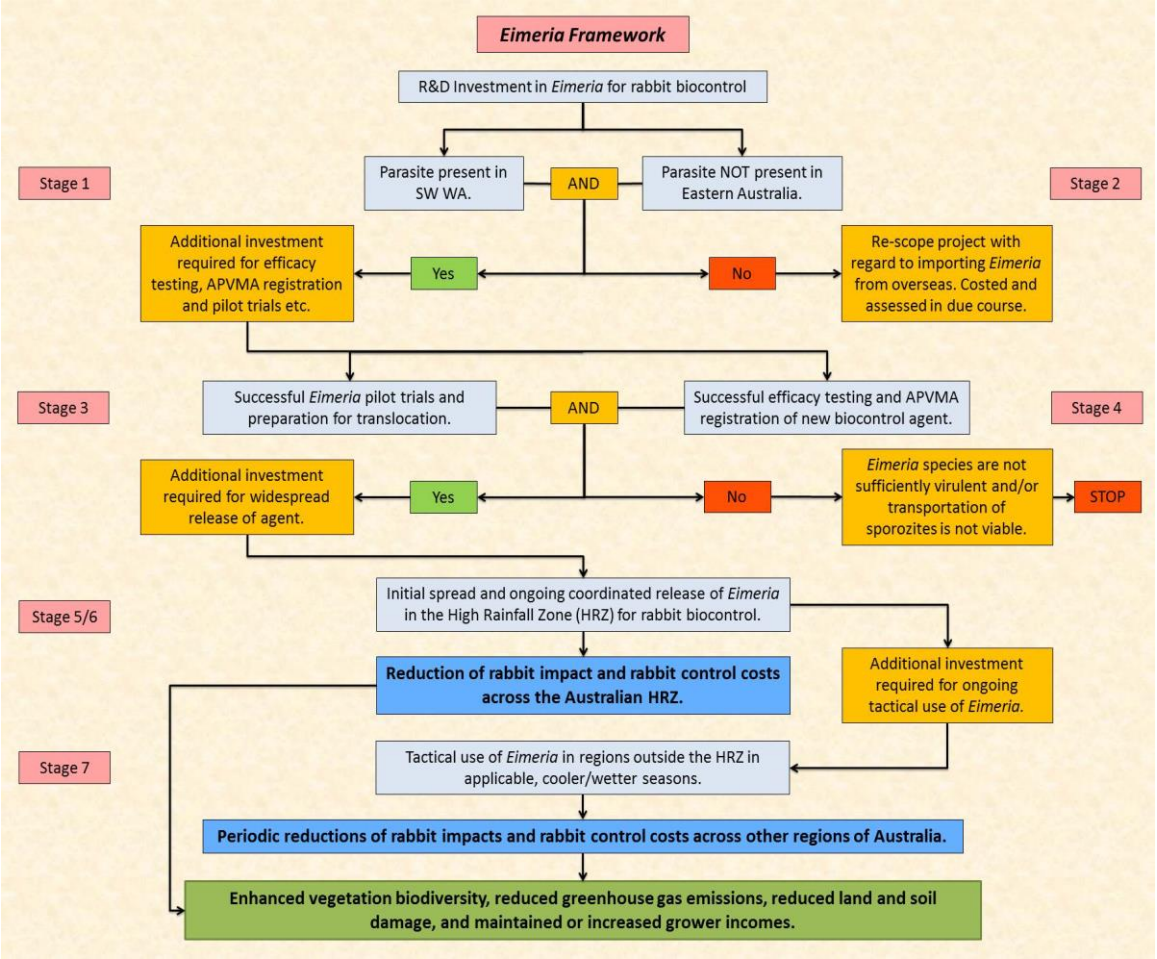


Figure 1: Diagrammatic Representation of the Pathway to Impact for *Eimeria* Biocontrol

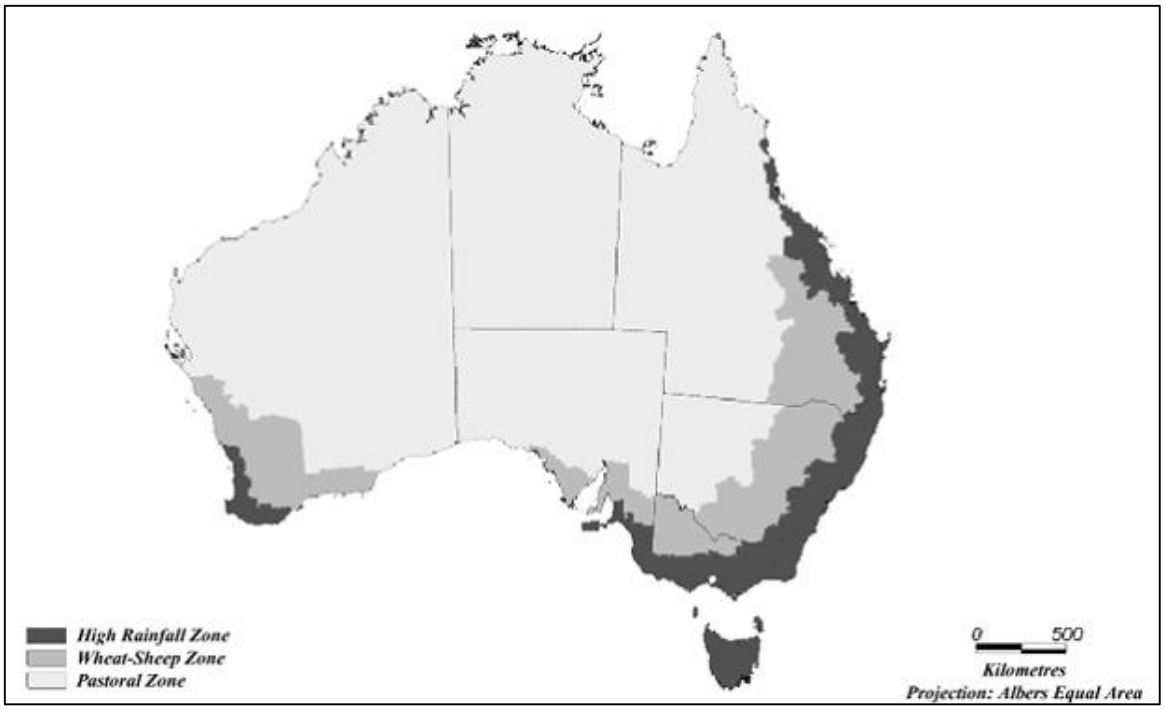


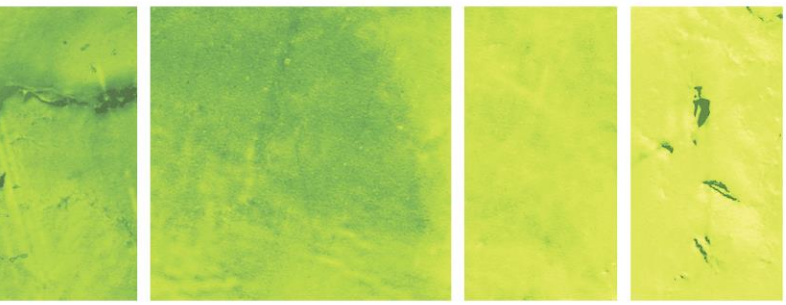
Figure 2: Map of Australia Showing the Three Agricultural Zones (Ewing, M. and Flugge, F., 2005)

RHDV-2

The assumptions made to estimate the expected costs additional to Table 2 and expected benefits of the investment in the recombinant strain of RHDV-2 are provided in Table 5.

Table 5: Summary of Assumptions for Valuation of Additional Investment Costs and Benefits for RHDV-2 (2013/2014 $ terms)

Counterfactual – without RHDV-2 investment		
Rabbit damage costs and control costs to Australia assuming RHD-Boost, RHD-Accelerator, and the current investment in rabbit decision support systems and the National Rabbit Facilitator are all successful. The damage and control costs of rabbits from 2020/21 are assumed to be $304.6 m per annum (Agtrans Research).		
With RHDV-2 investment		
Variable	**Value**	**Source**
Stage 1: RHDV Boost Reloaded – importation of desired recombinant strain of RHDV-2 and efficacy testing.		
Cost	$1,349,082 over 3 years from 2017/18	Strive and Read (2015)
Probability of success	75%	Agtrans Research
Stage 2: Investment for limited species specificity testing and registration of RHDV-2 with APVMA		
Cost	$2,000,000 p.a. for 2 years from 2020/21 to 2021/22	Agtrans Research[(a)(b)]
Probability of success	90%	Agtrans Research
Stage 3: Nationally coordinated release of RHDV-2 as a biocontrol agent.		
Cost	$1,550,000 in 2022/23	Agtrans Research[(a)]
Probability of success	80% (Stage 3 and Stage 4 combined)	Agtrans Research
Stage 4: Ongoing release of RHDV-2 to maintain impact.		
Cost	$256,000 per annum commencing 2023/24	Agtrans Research[(a)]
Probability of success	80% (Stage 3 and Stage 4 combined)	Agtrans Research
Impact of investment in Stages 1 to 4		
Reduction in rabbit costs (damage and control costs)	25% commencing in 2023/24	Agtrans Research[(c)]
Probability of impact occurring	90%	Agtrans Research
Period of stable impact assuming naïve rabbit population.	8 years from 2023/24 to 2030/31	Agtrans Research[(a)]



Variable	**Value**	**Source**
Rate of increase of rabbit costs assuming resistance becomes evident after stable period.	5% p.a. from 2031/32 onward	Agtrans Research[(a)]
Maximum level of rabbit costs reached given resistance and assuming positive interactions (Cox *et al.*, 2013) between Myxomatosis, RHDV (Czech-351 and K5), and RHDV-2.	95% of 2022/23 (pre-release) level	Agtrans Research
Stage 5: Tactical use (akin to a biocide) of RHDV-2 in applicable areas and seasons.		
Cost	$150,000 per annum from 2031/32 onwards	Agtrans Research[(a)]
Impact of Investment in Stage 5		
Overall cost reduction factor applied for impact valuation. This represents the cost reduction through the localised use of RHDV-2 in specifically applicable areas and only in applicable seasons.	0.825% (calculated by 25% x 10% x 33% - see following rows for individual component sources).	Agtrans Research
Reduction in total rabbit costs (damage and control)	25% in applicable areas and seasons	Agtrans Research
Proportion of Australia applicable for biocide use in a given season.	10%	Agtrans Research
Applicable seasons estimated to occur once every 3 years.	1/3 (33%)	Agtrans Research
Probability of impact occurring	90%	Agtrans Research

a) Derived from estimated additional costs expected for the initial release and ongoing upkeep of RHD-Boost (IA CRC Impact Assessment, submitted draft, Agtrans Research, 2015).

b) The RHDV-2 additional evaluation project costs have been developed based on best knowledge as of August 2015. The IA CRC assumes that some additional non-target testing will be required, though not as extensive as that required for the original evaluation of the RHDV-1 Czech strain. As there is more extensive knowledge about lagoviruses (virus family that affects rabbits and hares, which includes RHDV-1 and RHDV-2 viruses) and their host-preferences, it is considered reasonable to assume that only a subset of species would be required (e.g. hares and rodents for example). Also, considering the RHDV-2 virus is already endemic to Australia, the APVMA may require no target testing at all (Tanja Strive, pers. comm.).

c) Based on discussions with David Peacock of Biosecurity SA and/or correspondence with personnel of the Invasive Animals CRC.

Figure 3 is a simple, generalised diagrammatic representation of the pathway to impact most likely for the proposed investment in RHDV-2 as a rabbit biocontrol agent.

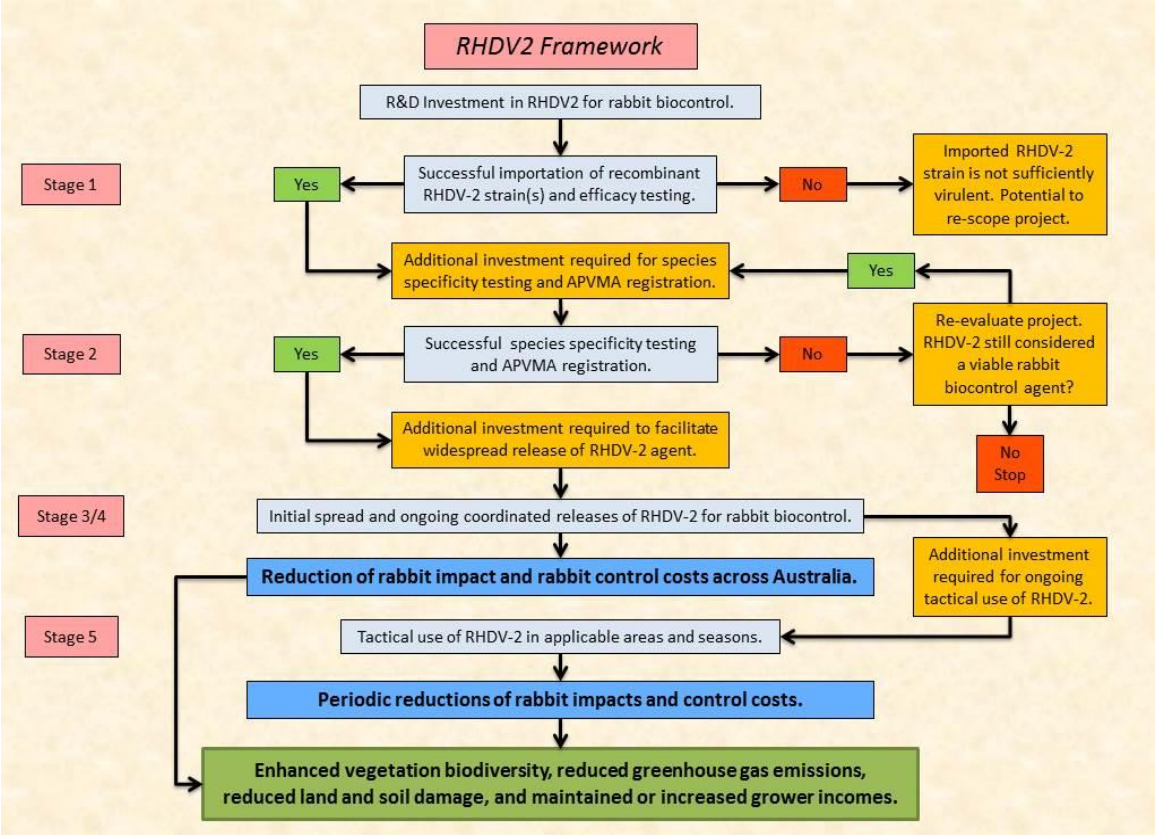


Figure 3: Diagrammatic Representation of the Pathway to Impact for RHDV-2 Biocontrol

Cost-benefit analysis process

All costs and benefits were expressed in 2013/14 dollar terms. All costs and benefits were discounted to 2017/18 (the first year of investment) using a discount rate of 5%. Investment criteria estimated included the net present value, the benefit-cost ratio, return on investment and the internal rate of return.

The basic analysis used assumptions for the best estimates of each variable, notwithstanding a high level of uncertainty for many of the estimates. All analyses ran for the length of the investment period plus 30 years from the first year of investment (2017/18). Each set of investment criteria were estimated for different periods measured from the first year of

investment.

9. The Cost-Benefit Analysis Results

Base Results

All investment costs and associated benefits were expressed in 2013/14 dollar terms. All costs and benefits were discounted to year one (2017/18) using a discount rate of 5%. The present value of costs (PVC) refers to the initial R&D investment committed. Other costs incurred along the pathway to impact were subtracted from the value of the benefits and therefore are incorporated in the present value of benefits (PVB).

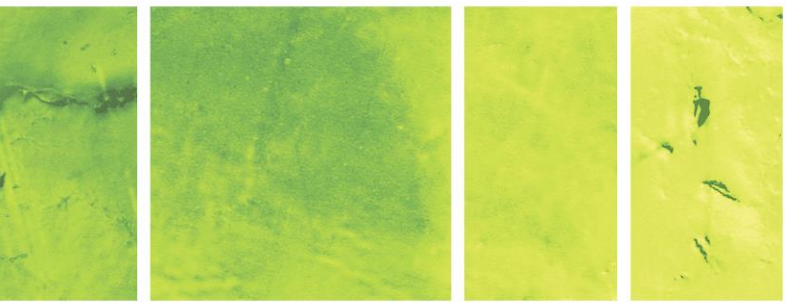
Calculations of the base investment criteria used the best estimates of each input variable, notwithstanding a high level of uncertainty for many of the estimates. All analyses ran from the first year of the investment plus 30 years (2017/18 to 2046/47). The tables following show the results for the total investment in each prospective biocontrol agent.

Table 6: Investment Criteria for Proposed *Eimeria* Investment (Discount rate 5%)

Investment criteria	Years from First Year of Investment 2017/18						
	0	5	10	15	20	25	30
Present value of costs ($m)	0.22	0.56	0.56	0.56	0.56	0.56	0.56
Present value of benefits ($m)	0.00	-1.95	-0.99	15.22	25.59	30.88	35.03
Net present value ($m)	-0.22	-2.51	-1.55	14.66	25.03	30.32	34.46
Benefit-cost ratio	0.00	-3.47	-1.76	27.09	45.55	54.97	62.34
Return on Investment (ROI)	-1.00	-4.47	-2.76	26.09	44.55	53.97	61.34
Internal rate of return (IRR)	negative	negative	3%	27%	30%	31%	31%

Table 7: Investment Criteria for Proposed RHDV-2 Investment (Discount rate 5%)

Investment criteria	Years from First Year of Investment 2017/18						
	0	5	10	15	20	25	30
Present value of costs ($m)	0.38	1.22	1.22	1.22	1.22	1.22	1.22
Present value of benefits ($m)	0.00	-0.22	96.78	192.56	225.92	239.64	250.39
Net present value ($m)	-0.38	-1.44	95.56	191.34	224.70	238.42	249.17
Benefit-cost ratio	0.00	-0.18	79.34	157.85	185.20	196.45	205.26



Return on Investment (ROI)	-1.00	-1.18	78.34	156.85	184.20	195.45	204.26
Internal rate of return (IRR)	negative	negative	111%	112%	112%	112%	112%

Some care should be taken in interpreting the different investment criteria. Apart from the initial R&D investment, any ongoing investment assumed to be required in any year is subtracted from the estimated benefits from the corresponding year. The benefit-cost ratios (BCRs) for both agents appear quite large (62 to 1 for *Eimeria* and 205 to 1 for RHDV-2). One of the main reasons for this is the small magnitude of the size of the initial committed investments represented by the present values of costs (PVC). The same issue also must be taken into account when considering the return on investment (ROI) figures for both proposed biocontrol investments. Calculated by the ratio of the NPV (PVB - PVC) to the PVC, at 61 to 1 for *Eimeria* and 204 to 1 for RHDV-2, the ROI results are similar to those for the BCRs and are high as a result of the small PVCs for each agent.

Perhaps the most meaningful criteria in interpreting the results as the time period increases is the net present value (NPV) as it captures the flow of values of both expected benefits and costs including the original project investment costs. At 15 years the *Eimeria* NPV is $14.7 million and that for RHDV-2 is $191.3 million. By year 30 the RHDV-2 NPV has grown to $249.2 m (1.3 times) but *Eimeria* has increased by 2.3 times its 15 year NPV, but still only to $34.5 m. This demonstrates the earlier impact of RHDV-2 and the assumed greater pathogenicity and more widespread impact of RHDV-2.

The investment criteria for RHDV-2 are all significantly higher than those for *Eimeria*. The principal reason for the higher expected investment criteria for RHDV-2 over 30 years is the higher level of impact of RHDV-2 on rabbit costs (25% compared to *Eimeria* at 10%) and its potential application to all zones compared with *Eimeria* impacts being restricted to the High Rainfall Zone (HRZ).

There are two primary sources of benefits valued in the analysis. The first is the reduction in rabbit damage and control costs resulting from the general use/release of a new biocontrol agent. The second is the additional benefit of rabbit cost reductions through the tactical use (akin to a biocide) of the new biocontrol agent in specific areas and specifically applicable seasons. The following tables show the relative estimates of the contribution from each source.

Table 8: Sources of Benefits for *Eimeria* Investment
(Discount rate 5%, 30 years)

Benefit Source	Present Value of Benefit ($m)[(a)]	Proportion of Total Benefits
General biocontrol use resulting in an overall reduction of rabbit costs.	31.40	89.7%
Ongoing tactical use of biocontrol in	3.62	10.3%

applicable areas and seasons.		
Total	35.03	100.0%

(a) Assumes that a positive synergy exists between *Eimeria* and existing biocontrol agents already active in the Australian wild rabbit population (i.e. myxomatosis and various prevailing RHDV strains).

Table 9: Sources of Benefits for RHDV-2 Investment
(Discount rate 5%, 30 years)

Benefit Source	Present Value of Benefit ($m)[(a)]	Proportion of Total Benefits
General biocontrol use resulting in an overall reduction of rabbit costs.	244.35	97.6%
Ongoing tactical use of biocontrol in applicable areas and seasons.	6.04	2.4%
Total	250.39	100.0%

(a) Assumes that a positive synergy exists between RHDV-2 and existing biocontrol agents already active in the Australian wild rabbit population (i.e. myxomatosis and various prevailing RHDV strains).

The undiscounted annual cash flows for both the proposed *Eimeria* investment and the RHDV-2 investment for the 30 year period are shown in Figure 4.

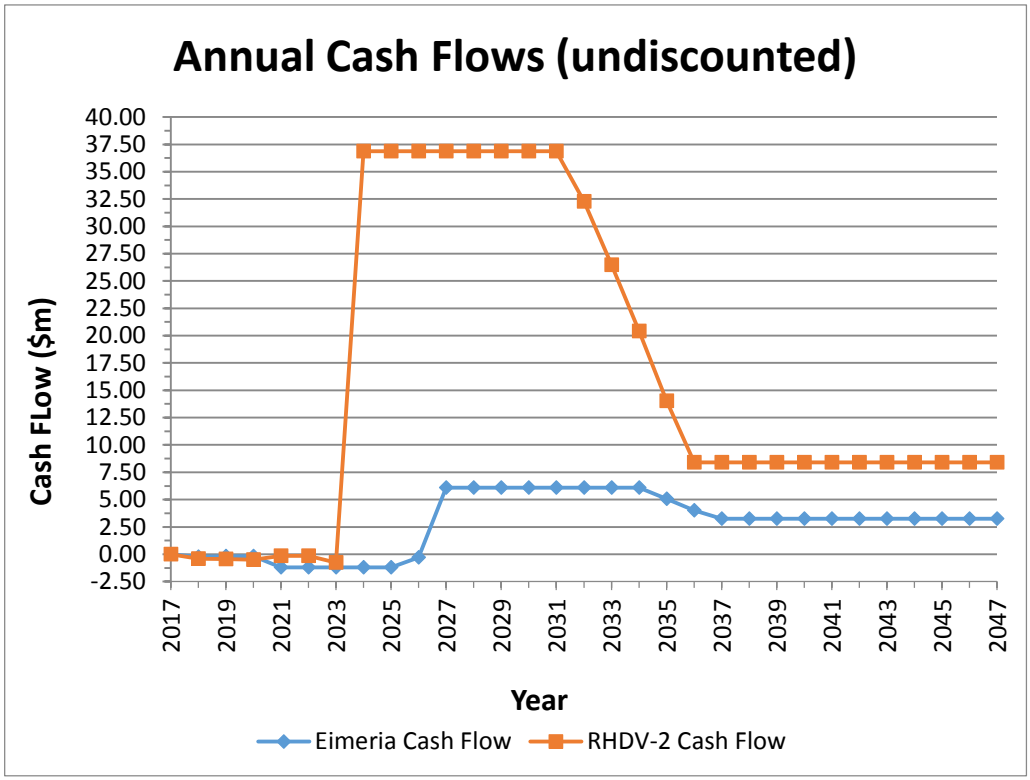


Figure 4: Annual Undiscounted Cash Flows for Each of the Proposed Biocontrol Investments (*Eimeria* and RHDV-2)

Figure 4 demonstrates graphically the differences in magnitude and timing of the expected annual benefits for each proposed biocontrol investment over the 30 year period.

Sensitivity analyses

Sensitivity analyses were carried out on several variables and results are reported in Tables 10 to 18. The sensitivity analyses were performed on the investment results using a 5% discount rate with benefits taken from the first year of investment plus 30 years. All other parameters were held at their base values.

EIMERIA

Table 10: Sensitivity of Investment Criteria to Discount Rate (*Eimeria*, 30 years)

Investment Criteria	Discount Rate		
	0%	5% (base)	10%

Present value of costs ($m)	0.62	0.56	0.51
Present value of benefits ($m)	87.16	35.03	15.22
Net present value ($m)	86.55	34.46	14.71
Benefit-cost ratio	141.34	62.34	29.57

Table 11: Sensitivity of Investment Criteria to Levels of Additional Investment Required (*Eimeria*, Discount rate 5%, 30 years)

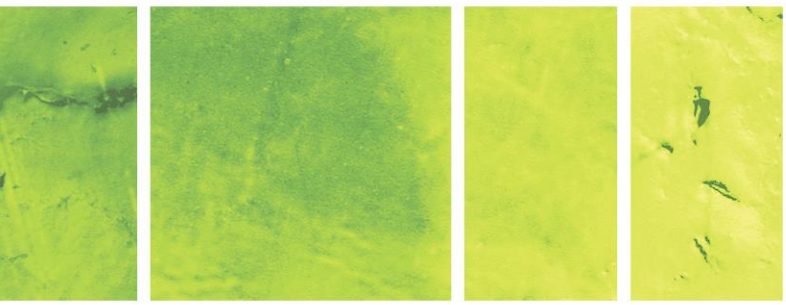
Investment Criteria	Additional Investment		
	0.75 x Base	Base	2 x Base
Present value of costs ($m)	0.56	0.56	0.56
Present value of benefits ($m)	36.35	35.03	29.71
Net present value ($m)	35.79	34.46	29.15
Benefit-cost ratio	64.70	62.34	52.89

Table 12: Sensitivity of Investment Criteria to Probabilities of Success (*Eimeria*, Discount rate 5%, 30 years)

Investment Criteria	Probability of Success Scenario		
	All half of Base	Base	All 100%
Present value of costs ($m)	0.56	0.56	0.56
Present value of benefits ($m)	1.87	35.03	74.06
Net present value ($m)	1.31	34.46	73.50
Benefit-cost ratio	3.33	62.34	131.81

Table 13: Sensitivity of Investment Criteria to Assumed Positive Synergy with Existing Biocontrol Agents (*Eimeria*, Discount rate 5%, 30 years)

Investment Criteria	Synergy Scenario	
	Neutral (impacts return to 100% pre-release levels)	Positive (Base: impacts return to 95% pre-release levels)
Present value of costs ($m)	0.56	0.56
Present value of benefits ($m)	27.26	35.03



Net present value ($m)	26.69	34.46
Benefit-cost ratio	48.51	62.34

RHDV-2

Table 14: Sensitivity of Investment Criteria to Discount Rate (RHDV-2, 30 years)

Investment Criteria	Discount Rate		
	0%	5% (base)	10%
Present value of costs ($m)	1.35	1.22	1.11
Present value of benefits ($m)	488.00	250.39	140.78
Net present value ($m)	486.65	249.17	139.67
Benefit-cost ratio	361.73	205.26	126.85

Table 15: Sensitivity of Investment Criteria to Levels of Additional Investment (RHDV-2, Discount rate 5%, 30 years)

Investment Criteria	Additional Investment		
	0.75 x Base	Base	2 x Base
Present value of costs ($m)	1.22	1.22	1.22
Present value of benefits ($m)	250.98	250.39	248.02
Net present value ($m)	249.76	249.17	246.80
Benefit-cost ratio	205.75	205.26	203.32

Table 16: Sensitivity of Investment Criteria to Probabilities of Success (RHDV-2, Discount rate 5%, 30 years)

Investment Criteria	Probability of Success Scenario		
	All half of Base	Base	All 100%

Present value of costs ($m)	1.22	1.22	1.22
Present value of benefits ($m)	15.51	250.39	515.66
Net present value ($m)	14.29	249.17	514.44
Benefit-cost ratio	12.71	205.26	422.72

Table 17: Sensitivity of Investment Criteria to Assumed Positive Synergy with Existing Biocontrol Agents (RHDV-2, Discount rate 5%, 30 years)

Investment Criteria	Synergy Scenario	
	Neutral (impacts return to 100% pre-release levels)	Positive (Base: impacts return to 95% pre-release levels)
Present value of costs ($m)	1.22	1.22
Present value of benefits ($m)	225.84	250.39
Net present value ($m)	224.62	249.17
Benefit-cost ratio	185.14	205.26

Table 18: Sensitivity of Investment Criteria to Primary Benefit (Benefit 1 – General Biocontrol) Rabbit Damage and Control Cost Reduction Assumption (RHDV-2, Discount rate 5%, 30 years)

Investment Criteria	Rabbit Cost Reduction		
	10%	25% (Base)	50%
Present value of costs ($m)	1.22	1.22	1.22
Present value of benefits ($m)	116.31	250.39	581.27
Net present value ($m)	115.09	249.17	580.05
Benefit-cost ratio	95.35	205.26	476.51

For both *Eimeria* and RHDV-2, the sensitivity of the results to the discount rate (Tables 10 and 14) reflects the protracted nature of R&D related to biocontrol. The benefits to the investment occur predominantly over a future period that is subject to significant discounting. Table 18 also indicates that the investment criteria for RHDV-2 are particularly sensitive to the uncertainty of the assumption for the expected damage and control cost reduction.

The results for both potential biocontrol agents also are sensitive to the probability of success assumed in each stage of the framework. This sensitivity emphasises the uncertain nature of

the impacts and the importance of the steps required to achieve them.

When all risk factors are removed (probabilities are all 100%) the net present value for RHDV-2 increased proportionally the same (approximately) as in the case for *Eimeria* (increase of x 2.06 for RHDV-2 compared to x 2.13 for *Eimeria*).

10. Discussion

The two biocontrol agents that appear in this investment analysis have been recommended as priority for further investment by industry and scientists (Henzell *et al*. 2008; Peacock 2015a). The proposed investment in each is relatively small compared to most biocontrol investments. However, the proposed investments are exploratory and will require further follow-on investment if early stages of research are successful.

One of the most significant and uncertain stages for additional investment for both *Eimeria* and RHDV-2 is the need for APVMA registration and the depth of R&D required to enable spread of these agents. Some costs and extended timelines in terms of registration and spread are built into the analysis. Some of these additional investment decisions are represented in the analysis framework used.

As noted in Table 2, an unspecified strain of RHDV-2 has been detected in the ACT. Should this strain prove virulent and become widespread throughout Australia there may be consequences to the development of the recombinant strain of RHDV-2 as described in this report. This analysis does not report on the impacts and benefits of investing in the recombinant RHDV-2 should the existing virus spread leading to some effect on the Australian rabbit population. As more information about the distribution and virulence of existing strains of RHDV-2 in Australia becomes available, in the future it would be possible to update the model in order to accommodate other potential scenarios and/or the registration of the recombinant strain of RHDV-2 for the more limited purpose of tactical biocontrol only.

While reasonable and conservative assumptions have been made in the analyses, the associated investment criteria have been subjected to various sensitivity analyses to test and report alternative variations to the base assumptions.

The baseline of rabbit damage and control costs assumed for the counterfactual is based on estimated costs provided in the Gong Report but allows for any growing positive impacts of rabbits post 2009 as well as subtracting the expected impacts of IA CRC investments as described earlier and assumptions for which are contained in a current report to IACRC (Agtrans Research, 2015). Time did not allow any sensitivity testing to the counterfactual assumptions, but the major impact on rabbit damage costs before the new biocontrol agents could be released is that for RHD-Boost. The counterfactual would be sensitive to the assumption regarding the impact of RHD-Boost after it is released in 2016.

11. Conclusions

The investment criteria estimated from the base set of assumptions for the initial investment in *Eimeria* are all positive from a period of 15 years after the first year of investment, and are all positive from 10 years for the investment in RHDV-2. The positive investment criteria suggest that the initial investments would be worthwhile given the estimates made of future likely pathways, the additional investment and associated timelines required, the risks involved, and the expected sequencing and level of rabbit impacts.

The proposed investment can be staged conditionally so that as the investment proceeds along a particular pathway, directions can be changed according to any past success and any new information so avoiding or minimising losses and maximising the chances of significant impacts being delivered.

Acknowledgments

Brian Cooke, University of Canberra

Andreas Glanznig, Invasive Animals CRC

David Peacock, Biosecurity Research Officer, Pest Animals, Biosecurity SA

Tanja Strive, CSIRO

John Tracy, Invasive Animals CRC

References

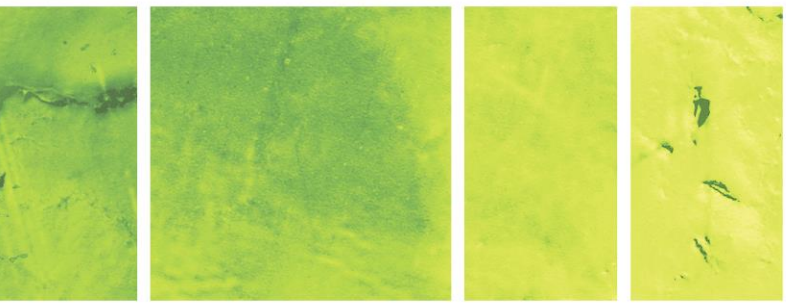
Agtrans Research (2011). Prospective Economic Assessment of Investment in Activities to Manage the Impact of Rabbits up to and Beyond 2020. Canberra: Invasive Animals Cooperative Research Centre.

Agtrans Research (2015) IA CRC Impact Assessment, Draft Report to IA CRC.

Coudert P, Licois D and Drouet-Viard F (1995). *Eimeria* species and strains of rabbit. Office for Official Publications of the European Communities. Luxembourg.

Cox TE, Strive T, Mutze G, West P and Saunders G (2013). Benefits of Rabbit Biocontrol in Australia. PestSmart Toolkit publication, Invasive Animals Cooperative Research Centre, Canberra, Australia.

Ewing M and Flugge F (2005). The benefits and challenges of crop-livestock integration in Australian agriculture. 4th International Crop Science Congress. www.cropscience.org.au/icsc2004/symposia/6/3/2005_ewingma.htm#TopOfPage



Le Gall-Reculé G, Lavazza A, Marchandeau S, Bertagnoli S, Zwingelstein F, Cavadini P, Martinelli N, Lombardi G, Guérin JL, Lemaitre E, Decors A, Boucher S, Le Normand B and Capucci L (2013). Emergence of a new lagovirus related to rabbit haemorrhagic disease virus, *Veterinary Research* 2013, 44:81. www.veterinaryresearch.org/content/44/1/81

Gong W, Sinden J, Braysher M and Jones R (2009). The economic impacts of vertebrate pests in Australia. Canberra: Invasive Animals Cooperative Research Centre.

Henzell RP, Cooke BD and Mutze GJ (2008). The future biological control of pest populations of European rabbits, *Oryctolagus cuniculus*. *Wildlife Research* **35**, 633-650.

Invasive Animals Cooperative Research Centre. (2013). Rabbit biocontrol saves agriculture $70 billion: new study. Media Release: 8 March 2013. Canberra.

Norton CC, Catchpole J and Joyner LP (1979). Redescriptions of *Eimeria irresidua* Kessel & Jankiewicz, 1931 and *E. flavescens* Marotel & Guilhon, 1941 from the domestic rabbit. *Parasitology* 79, 231-48.

Osipovskiy AI (1955). Inheritance of resistance to coccidiosis by rabbits [in Russian]. *Zhurnal Obshchey Biologii* **16**, 64-68.

Peacock D (2015a). Seeking a new biological control agent for the European rabbit (*Oryctolagus cuniculus*) in Australia: a review of potential agents, Discussion Paper (v2), Biosecurity SA.

Peacock D (2015b). Business Case to Advance the Selection of New Rabbit Biocontrols (v4), Biosecurity SA.

Strive T and Read A (2015). RHDV Boost Reloaded – New European RHDV-2 Strains for Rabbit Biocontrol. Invasive Species R,D&E Project Application. Canberra: Invasive Animals Cooperative Research Centre.

Additional Bibliography

Australian Pesticides and Veterinary Medicines Authority (2015). Guideline for the regulation of biological agricultural product. apvma.gov.au/node/11196. Accessed: 16 /08/2015.

Stodart E (1968). Coccidiosis in wild rabbits, *Oryctolagus cuniculus* (L.), at four sites in different climatic regions in eastern Australia I. Relationship with the age of the rabbit. *Australian Journal of Zoology* 16, 69-85.

Stodart E. (1971). Coccidiosis in wild rabbits, *Oryctolagus cuniculus* (L.) at a site on the coastal plain in eastern Australia. *Australian Journal of Zoology* 19, 287-292.

Stodart E and Parer I (1988). Colonisation of Australia by the rabbit, *Oryctolagus cuniculus* (L). CSIRO Division of Wildlife and Ecology.

ISBN (print): 978-0-9943800-1-2
ISBN (online): 978-0-9943800-2-9

PESTSMART

www.pestsmart.org.au