CENTRE FOR
INVASIVE SPECIES SOLUTIONS

AUSTRALIA'S RABBIT BIOCONTROL PIPELINE STRATEGY

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The strategy was reviewed by the CISS rabbit biocontrol steering committee (now ended). Its members comprised of key investors and end-user stakeholders (DAFF/DCCEEW, MLA, AWI, land managers, a National Farmers Federation representative, and Rabbit Free Australia).

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COVER IMAGES
Sheep grazing. Tablelands near Oberon. New South Wales. Australia. Source: Shutterstock

Rabbit warren Oaky Ck on 29 Aug 2007 Source: Brian Lukins

European rabbit (Oryctolagus cuniculus) Toledo, Spain. Source: Shutterstock

White cypress pine (Callitris glaucophylla), Ikara-Flinders Ranges National Park, SA. Source: Brian Cooke & David Peacock

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EXECUTIVE SUMMARY

Biological control of rabbits has been successfully used in Australia since the initial release of the myxoma virus in 1950 followed by the introduction of two flea species in the 1970s and 1990s to boost myxoma virus transmission. The release of the initial Czech-351 strain of Rabbit Haemorrhagic Disease Virus (RHDV1) took place in 1995–96. A further Korean-sourced strain, RHDV1-K5, was released in 2017 and a novel virulent RHDV (RHDV2) was discovered in Australia in 2015.

The managed release of these biocontrol agents and the serendipitous arrival and spread of RHDV2 had a dramatic impact on rabbit abundance across its extensive range. The resulting continent-wide, sustained reduction in rabbit numbers since 1950 has delivered significant economic, environmental and social benefits. Many studies show that biocontrols facilitated the recovery of native species and ecological communities heavily impacted by rabbits.

Agriculture has also benefited from reduced rabbit impacts since the 1950s. The cumulative benefit of myxoma virus and RHDV1 to Australia's livestock and farming industries have been estimated at approximately $70 billion from 1950 to 2011 and more recent economic assessments demonstrated that the positive estimated economic benefits of rabbit biocontrol in Australia after 2014 underpins the value of ongoing investment in rabbit biocontrol RD&E pipelines.

However, biological control to date has not provided a silver bullet for rabbit control. Pathogen-host coevolution selects for the most efficiently transmitted virus with a likely ongoing dynamic between host resistance and pathogen virulence. This often leads to a lessening of the effect of biocontrol over time. In addition, once a biocontrol agent has become established in a population, a proportion of animals will have acquired immunity to the biocontrol agent, reducing the overall susceptibility of the population to recurrent outbreaks.

This document was developed with input from scientists experienced in the development and application of viral biological controls. Based on the information available at the time of writing, its main focus is biological control, with the aim to provide a broader framework to scientifically guide current and future research directions in this area and build a pipeline of biocontrol options for sustainable landscape scale rabbit control. While this document highlights the importance of better integration of biological control with conventional controls as well as an increased focus in extension and adoption, it does not expand on any non-biological rabbit control tools and strategies, and as such does not intend to provide a comprehensive list of recommended integrated rabbit control activities going forward through government or industry- funded related research activities.

The long-term rabbit biocontrol pipeline strategy

The Centre for Invasive Species Solutions (CISS), together with its members and partners, and prior to that the Invasive Animals Cooperative Research Centre (IA CRC), recognise the environmental, economic and social benefits of long-term sustainable rabbit control. Consequently, these organisations have championed a multi-pronged, long term strategic approach to harness the unique opportunities and potential high returns on investment that successful biocontrol initiatives can provide.

Given that rabbits and viral biocontrol agents are in an 'arms race' as rabbits gradually develop genetic resistance that reduces their effectiveness over time, to efficiently manage rabbit impacts a pipeline of biocontrol agents needs to be developed, ideally to enable a new agent to be released every 10 to 15 years or so.

The pipeline strategy is the key mechanism to enable nationally coordinated and collaborative rabbit biocontrol research and innovation to implement key priority actions under the EPBC Act *Threat Abatement Plan for Competition and Land Degradation by Rabbits* and relevant industry plans.

Foundational activities of the rabbit biocontrol pipeline strategy were undertaken between 2007 and 2012 by the IA CRC. Following this work, over the past decade, governments and industry have co-invested in two five-year R&D plans, driving forward the long-term rabbit biocontrol pipeline strategy. Phase 2 was implemented by the IA CRC (2012–2017) and phase 3 was completed through CISS (2017–2022).

In addition, Meat & Livestock Australia and CSIRO are co-funding a series of projects informed by and closely aligned with the rabbit biocontrol pipeline strategy. Major project components are the development of organoid tissue culture systems for rabbits and assessment of their suitability for the cultivation of rabbit caliciviruses *ex vivo*; bioprospecting of lagomorph (rabbit and hare) pathogens within Australia and abroad; and modelling using population genomics data to investigate whether emerging genetic control technologies may feasibly assist in the control of rabbits in Australia.

Phase 1, IA CRC foundational activities 2007–12

The primary focus of these activities was on the RHDV strains already present in Australia and understanding geographic variation in their efficacy.

Research by the IA CRC was the first to uncover the presence of an endemic, non-pathogenic form of calicivirus, Australian rabbit calicivirus (RCV-A1), and showed that it was able to provide transient and partial immunity to lethal RHDV1 infection. Experimental infection studies showed that while some rabbit populations were developing genetic resistance to infection with RHDV1, the virus was partially compensating for this genetic resistance amongst rabbits by evolving towards relatively increased virulence.

Phase 2, 2012 to 2017

The 'RHDV Boost' project investigated foreign strains of RHDV to assess their suitability for introduction to Australia. This led to the selection of the Korean K5 strain (RHDV1-K5) as the virus for release based on an increased ability to overcome partial cross protective immunity provided by the non-pathogenic RCV-A1 and its increased ability to infect genetically resistant wild rabbits. Following approval of the Australian Pesticides and Veterinary Medicines Authority (APVMA), RHDV1-K5 was released nationwide in March 2017.

The increased surveillance efforts put in place by the RHDV Boost project identified the exotic RHDV2 in Australia in 2015, and later demonstrated it had arrived earlier in 2014. RHDV2 rapidly spread within the Australian rabbit population. Within 18 months it had mostly replaced the existing RHDV1 strain and become the dominant calicivirus in the Australian landscape. The impact of RHDV2 on rabbit populations varied but achieved an average reduction in rabbit numbers of 60%.

An 'RHD Accelerator' approach was adopted to facilitate the accelerated natural selection of RHDV variants able to overcome immunity to naturally circulating strains or outperform them. This delivered proof-of-concept that virus variants with altered immunological properties can be selected for and remain highly virulent in rabbits. However, no variants were produced in the course of the project, largely due to the need to carry out selection and growth in experimentally infected animals. The decision was made to delay this approach until substantial progress has been made in the development of culture systems that allow the cultivation of RHDV *in vitro.*

A systematic review of known rabbit pathogens was established as a method for Bioprospecting, with assessments through expert consultations and stakeholders for their potential as suitable biocontrol agents/biocides.

Phase 3, 2017 to 2022

Phase 3 continued to monitor the spread, evolution and interactions between the various virus strains in Australian wild rabbit populations and focussed on the case for RHDV2 to be registered as an additional tool for the rabbit biocontrol pipeline. This involved undertaking a thorough characterisation of RHDV2 to assess its suitability as an additional biocide and generating the data needed for an application to the APVMA for its registration.

One CISS project explored the potential use of genetic biocontrol technologies as an alternative long-term, non-lethal means of managing invasive animals (including rabbits) in Australia through a series of stakeholder consultations. These approaches often involve genetic modifications that will skew the sex bias of the offspring towards all-male, which would eventually result in the collapse of the population.

Phase 4, 2022 to 2030

Based on consideration of the first three phases of the implementation of the long-term biocontrol R&D pipeline, this report provides a framework and biocontrol R&D recommendations to move forward into phase 4. A strong proactive approach is recommended that considers the available and potential short-, medium- and long-term options, and balances the risks and likelihood of success with the potential benefits. The approach must maintain critical capability and the ability to quickly react to new opportunities (pathogens or technologies) as they arise.

Phase 4 highlights the importance of maintaining underpinning science capability and infrastructure, as well as the need to continue to improve integration of biocontrol applications with conventional control methods for maximum impact.

Ten recommendations are made for the 2022 to 2030 phase under six strategic options. These are listed below and discussed in section 5 of the report.

RECOMMENDATIONS

A. Optimise the use of existing biocontrol tools available to maximise impact.

Recommendation 1: Develop more efficient ways of deploying available biocides and improving their integration with existing and new conventional tools to maximise their impacts, in particular in high value agricultural and environmental settings.

Recommendation 2: Continue coordinated national rabbit monitoring and sampling to better understand the impacts of, and interactions between, the various viruses (RHDV1, RHDV2, RCV-A1, RHDV1-K5 and myxoma virus) circulating in Australian rabbits. This supports Recommendation 1 by identifying possible windows of opportunity to better deploy available tools. Monitoring intensity should increase from current levels to mimic or exceed coverage achieved through CISS Phase 2 and include optimisation of tools for more effective sample and population data collection.

Recommendation 3: Develop improved and streamlined serological (and molecular) diagnostic methods and consider the development of reliable hand-held serological diagnostic tools to facilitate strain selection and estimate the likely success of biocide applications. This includes establishing a local source of critical reagents (monoclonal antibodies and antigens).

Recommendation 4: Facilitate underlying science to increase understanding of the detailed genetic resistance mechanisms in wild Australian rabbits to the various circulating biocontrol agents using whole genome sequencing approaches, confirmatory functional studies, and the development of high throughput screening tools for resistance in Australian rabbit populations.

B. Select for better versions of existing pathogens, for example by accelerating or directing evolution towards more suitable strains.

Recommendation 5: Re-start the RHD Accelerator platform technology development using novel organoid technologies for the accelerated selection of improved RHDV variants, based on the recent success in growing RHDV in rabbit and hare liver organoids (MLA-CSIRO aligned research).

C. Identify new pathogens (onshore or offshore) suitable for self-disseminating or augmentative biocontrol (review in 2023/24).

Recommendation 6: Continue bioprospecting for new rabbit biocontrol agents and maintain the capability required to react to emerging opportunities.

D. Develop and assess novel technologies for non-lethal biocontrol (for example genetic biocontrol).

Recommendation 7: Undertake proof-of-concept studies on the technical feasibility of genetic biocontrols for rabbits modelled on successful strategies applied in model vertebrate organisms. This includes all aspects of the transfer of these molecular technologies to the rabbit system, in parallel with genetic and ecological modelling.

E. Increase underpinning & enabling science and capability.

Recommendation 8: Increase rabbit biocontrol-focussed, multi-disciplinary, scientific capability in virology, epidemiology, genetics/genomics and ecology with a particular emphasis on training and succession planning.

Recommendation 9: Maintain or increase infrastructure required for rabbit biocontrol-related work, including experimental animal facilities and breeding colonies for domestic and wild rabbits.

F. Improve adoption and integration with conventional control.

Recommendation 10: Implement the integration of rabbit biocontrol R&D with stakeholder and community engagement strategies to enable effective and coordinated rabbit management using a best practice combination of conventional management techniques and biocontrol.

1. INTRODUCTION: A LONG-TERM RABBIT BIOCONTROL PIPELINE STRATEGY

This report reviews Australia's initiatives in biocontrol of the European rabbit (*Oryctolagus cuniculus*) which quickly became the nation's worst terrestrial vertebrate pest after its successful release on the mainland in 1859. The report discusses the successful impact of the biocontrol agents in reducing rabbit abundance beginning with myxoma virus in 1950 followed by the introduction of two flea species in the 1970s and 1990s to boost myxoma virus transmission. Subsequent releases were the initial Czech-351 strain of Rabbit Haemorrhagic Disease Virus (RHDV1) in 1995–96 and a Korean-sourced strain, RHDV1-K5 in 2017. A novel virulent RHDV (RHDV2) was discovered in Australia in 2014 and this added to the impact of Rabbit Haemorrhagic Disease on the wild rabbit population.

The focus of the report is the implementation of a long-term rabbit biocontrol pipeline that commenced with phase 1 foundational activities of the IA CRC from 2007 to 2012, progressed to a second phase R&D Plan for 2012 to 2017 under the IA CRC, advanced to the third phase for 2017 to 2022 under the Centre for Invasive Species Solutions (CISS), and recommendations for progressing to a fourth stage from 2022 to 2030, and beyond.

The underlying rationale of a pipeline strategy has been the experience of the lessening impact of introduced biocontrol agents over time. This is a function of pathogen-host coevolution which selects for the most efficiently transmitted virus with a likely ongoing dynamic between host resistance and pathogen virulence. In addition, once a biocontrol agent has become established in a population, a proportion of animals will have acquired immunity to the biocontrol agent, reducing the overall susceptibility of the population to recurrent outbreaks.

The ecological benefits and economic benefits and return on investment of a successful, self-disseminating rabbit biocontrol agent are unparalleled, as outlined in a recently updated report outlining the benefits of rabbit biocontrol (CISS 2021). It provides important background on the proven benefits and imperatives for the development and implementation of long-term strategic approaches outlined in this document and should be read as a companion publication.

Research and experience have shown long term and varied strategies need to be implemented to provide a pipeline of new tools and strategies that can be applied in 10-15 year intervals, which will allow for the ongoing control of rabbit numbers and impacts on the Australian continent.

In this context, it is critical to sustain low levels of rabbit populations and associated impacts long-term, to protect the gains made by previous successful biocontrol initiatives.

The main focus of this document is on research and development of biological control tools and strategies. It was developed with input from scientists with expertise in the development and application of viral biological controls, based on the information available at the time of writing. The aim is to provide a broader framework to scientifically guide current and future research directions in this area with a view to creating a pipeline of options for ongoing landscape scale rabbit control, therefore aligning with and complementing the current Threat abatement plan for competition and land degradation by rabbits (Rabbit TAP) (Dept. Environment and Energy 2016). The Rabbit TAP clearly outlines the need to "*improve the effectiveness of rabbit control programs*" and acknowledges that *"as improving the effectiveness of control programs and control methods (particularly biocontrol agents) or developing of new tools can take many years, it is imperative that [sic] is begun prior to any significant increase in rabbit numbers".*

While this Rabbit Biocontrol Pipeline Strategy report highlights the importance of better integration of biological control with conventional controls as well as an increased focus in extension and adoption, it does not expand on comprehensive recommendations on the broader (non-biological control) research, management and other actions needed to ensure ongoing long-term mitigation of rabbit impacts which are more comprehensively covered in the existing Rabbit TAP.

2. PHASE 1 FOUNDATIONAL ACTIVITIES 2007–12

During the period 2007–2012, the primary focus of the long-term biocontrol R&D strategy was on the RHDV strains already present in Australia and understanding geographic variation in their efficacy.

It had long been suspected that Australian rabbits harboured an endemic, non-pathogenic form of calicivirus that could provide some immunity to virulent RHDV (Robinson, Kirkland et al. 2002). This suspicion stemmed from serological cross reactivity to caliciviruses observed in Australian rabbits prior to the release of the first RHDV in the 1990s (a Czech strain RHDV1 v351) in areas where it had a lesser impact on rabbit populations.

Work funded by the IA CRC was the first to uncover the presence of this endemic, non-pathogenic form of calicivirus, Australian rabbit calicivirus (RCV-A1) (Strive, Wright et al. 2009), and showed that it was indeed able to provide transient and partial immunity to lethal RHDV1 infection (Strive *et al.* 2010 Strive, Elsworth *et al*. 2013). Furthermore, researchers demonstrated that RCV-A1 was most prevalent in the temperate zones of south-east Australia where the initial RHDV1 impacts were low and continued to be reduced to this day (Liu *et al*. 2014) (Figure 1).

Experimental infection studies showed that some rabbit populations were developing genetic resistance to infection with RHDV1 (Elsworth *et al.* 2012). However, RHDV1 appeared to be partially compensating for this genetic resistance in rabbits by evolving towards relatively increased virulence (Elsworth *et al.* 2014), thereby maintaining a highly virulent phenotype. Later studies showed that the genetic resistance developing amongst rabbits was likely through RHDV driven selection (Schwensow *et al.* 2017a, Schwensow *et al.* 2020), although the molecular and immunological mechanisms by which this resistance develops in rabbits are not well understood.

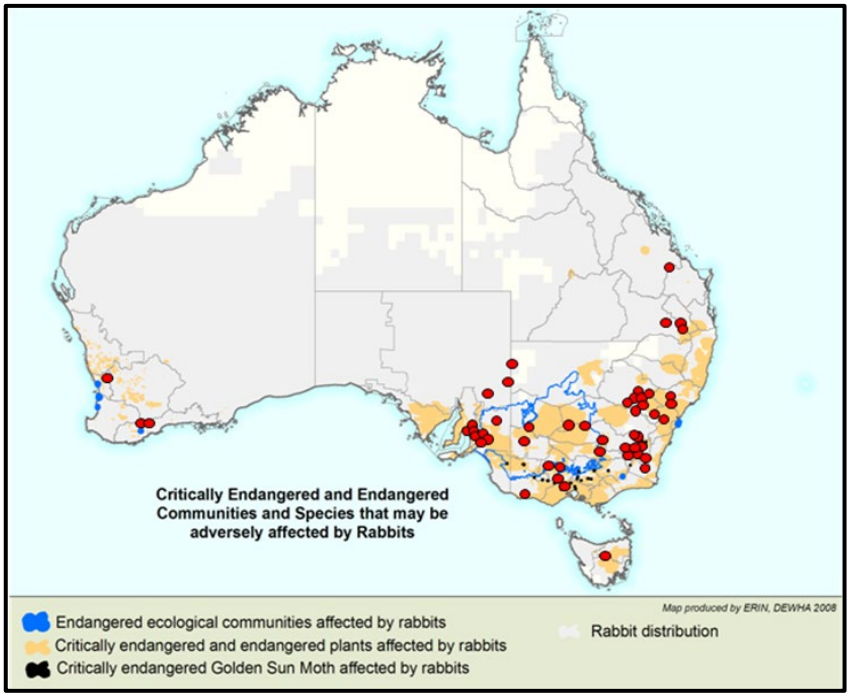

Figure 1. Distribution of the endemic non-pathogenic calicivirus RCV-A1 in Australia.
Red dots indicate current and historical sites that have tested positive for RCV-A1. (Cox *et al*. 2013).

3. PHASE 2, 2012–17

RHDV Boost

The 'RHDV Boost' project investigated 38 foreign strains of RHDV and RHDV-like viruses to assess their suitability for introduction to Australia to complement the existing strain RHDV1-v351. A Korean strain of RHDV (K5) was selected as the virus for release, based on its increased ability to overcome partial cross protective immunity provided by the non-pathogenic RCV-A1, as well as its increased ability to infect genetically resistant wild rabbits. RHDV1-K5 was approved by the Australian Pesticides and Veterinary Medicines Authority (APVMA) and released nationwide in March 2017 (Strive and Cox 2019).

While preparations for the release were underway, two exotic strains of RHDV were identified in Australia through the increased surveillance efforts put in place by the RHDV Boost program. These newly identified strains were a previously unknown RHDV, a variant most closely related to an isolate described in China (Mahar *et al*. 2018b), and a new rabbit calicivirus (Rabbit Haemorrhagic Disease Virus 2, RHDV2) that emerged in Europe in 2010 (Le Gall-Récule *et al*. 2013, Hall *et al*. 2015, Mahar *et al.* 2018b).

RHDV2 rapidly spread within Australian rabbit populations and within 18 months had mostly replaced the existing RHDV strains and become the dominant calicivirus in the Australian landscape (Mahar *et al*. 2018a). The impact of RHDV2 on rabbit populations varied with an average reduction in rabbit numbers of 60% and greater reductions in Western Australia and arid South Australia (Mutze *et al*. 2018, Ramsey *et al*. 2020).

The arrival and spread of RHDV2, prior to the release of RHDV1-K5, impacted the effectiveness of the K5 release, which was reported to achieve local population knockdowns of approximately 34% (Cox *et al*. 2019) but did not appear to have become widely established in wild rabbit populations. It is likely that RHDV1-K5 was outcompeted by RHDV2 (Ramsey *et al*. 2020).

RHD Accelerator phase 1 and 2

The RHD Accelerator approach aimed to develop a re-usable platform technology that facilitates the accelerated natural selection of RHDV variants able to overcome immunity to naturally circulating strains, or otherwise outperform them. The intention was that, if successful, this platform could be used to repeatedly produce new and superior RHDV strains for successive releases.

The project delivered proof-of-concept that virus variants with altered immunological properties can be selected for, and remain highly virulent, in rabbits. However, the project did not succeed in producing a variant that was able to completely overcome immune protection conveyed by recovering from infection with other strains of RHDV (Hall *et al*. 2017). The main impediment to the success of this project was the need to carry out selection and growth in experimentally infected animals.

At the end of the project the recommendation was made to delay the project until substantial progress has been made in the development of culture systems that allow the cultivation of RHDV *in vitro.* Should this approach be revisited in the future, for any RHD Accelerator virus strain proposed as an additional control tool, an effective vaccine would also need to be produced and made available prior to any virus release.

Bioprospecting

Modelled on previous approaches (Henzell *et al*. 2008), this project carried out a systematic review for known rabbit pathogens and assessed them through expert consultations and a stakeholder review. While the review, published as 'Business case to advance the selection of new rabbit biocontrol agents' considered most pathogens unsuitable, two were selected for further assessment; RHDV2 and two species of the intestinal protozoan parasite *Eimeria* (Peacock 2015). These *Eimeria* parasites were known to be present in Western Australia but were assumed to be absent from rabbit populations in the eastern states, making them potentially suitable as biocontrol agents/biocides in these regions (Peacock 2015).

Two further pathogens were earmarked as potentially interesting, but less likely to be suitable biocontrol agents/biocides based on the available information at the time and it was recommended to keep a 'watching brief' on these agents. These candidates were virulent strains of myxoma virus and a rabbit herpesvirus.

Based on the available information in the existing literature, the rabbit herpesvirus was deemed not suitable due to not being considered sufficiently virulent, while at the same time having very high welfare impacts (Sunohara-Neilson *et al*. 2013). Since the report by Peacock (2015), no additional information has become available that would encourage substantial additional investment to further investigate this pathogen.

In relation to virulent strains of myxoma virus, field trials dating back to 1954 have shown that attempts to spread new strains of myxoma virus tend to be unsuccessful (Fenner *et al.* 1957; Merchant *et al.* 2003; Berman *et al.* 2006). Despite the widespread releases of the Lausanne strain of myxoma virus from the 1970s through to the 1990s, no field strains descended from this virus have been identified.

Since 2015 a substantial amount of additional evidence has been added to the literature for myxoma virus (Di Giallonardo and Holmes 2015, Kerr *et al*. 2017, Kerr *et al*. 2022). It is now better understood that for optimal transmission, myxoma virus needs to exhibit optimal levels of virulence, which is in turn influenced by the level of genetic resistance in the respective rabbit populations (Di Giallonardo and Holmes 2015). This means that any myxoma virus isolate, either highly virulent or attenuated, would likely always be outcompeted by locally adapted field strains of the virus. There is, however, potential scope in utilising possible synergies between the already circulating myxoma virus strains and other available control tools, which are outlined later in this document. The dynamics of myxoma virus appears to have changed following the introduction of RHDV. Previous exposure to myxoma virus increases the mortality from subsequent RHDV outbreaks, possibly due to immune suppressions of rabbits surviving myxomatosis (Barnett *et al.* 2018).

Field studies over the last 5 years, and published reports confirm that myxoma virus still regularly infects rabbit populations and causes substantial mortality (Wells *et al*. 2018). Recently assessed myxoma virus field isolates from Australia are far more virulent than the initially released strains in laboratory rabbits, indicating that virus and the rabbit host are continuing their biological 'arms race') (Kerr *et al* 2022). Even with apparent virus attenuation in resistant rabbits, the selection against typical lesions of myxomamatosis, leading to an amyxomatous phenotype of disease (without normal myxoma induced benign tumour), may be leading to enhanced virus replication and transmission (Kerr et al. 2022). Kerr et al. (2022) proposed that selection against inflammation at cutaneous sites prolongs virus replication and enhances transmission, leading to the amyxomatous phenotype. In some virus backgrounds this creates an immunosuppressive state that predisposes to high virulence and acute death. The alterations in disease pathogenesis, particularly the overwhelming bacterial invasions that characterize the modern viruses, suggest that their virulence grades are not directly comparable with earlier studies.

4. PHASE 3 2017–2022

4.1 CISS Rabbit Biocontrol Program

RHDV2 as a registered biocontrol agent and national optimisation of rabbit biocontrol

RHDV2 has unique abilities that could potentially make it a candidate for registration as an additional tool for the rabbit biocontrol pipeline (Figure 2) even though it is already circulating naturally in Australia. These abilities include, for example, the ability of RHDV2 to overcome immunity to other strains and to infect and kill rabbits at any age. It therefore was hypothesized that RHDV2 has potential to be a superior biocide that could be released at any time of the year, possibly even during the breeding season. Furthermore, an RHDV2 biocide may have the potential to be released in tandem with RHDV1-K5 or other conventional rabbit control methods to maximise population reductions.

Projects within the CISS biocontrol program undertook a thorough characterisation of RHDV2 to assess its suitability as an additional biocide and generated the data needed for a potential application to the APVMA for its registration as a product. To achieve this, detailed information on critical aspects of the biology and epidemiology of RHDV2 were investigated:

- Quantitative analyses of the virus' virulence and ability to overcome natural immunity to other caliciviruses (for example, RHDV1-K5, the non-pathogenic calicivirus RCV-A1, RHDV1); (Hall *et al*. 2021a; Patel *et al*. 2022; O'Connor *et al*. 2022)
- The ability of, and extent to which, RHDV2 can infect and kill rabbit kittens; (Hall *et al*. 2021a)
- The ability of RHDV2 to overcome passive immunity (maternal antibodies) (Hall *et al*. 2021b); and
- An updated welfare assessment for RHDV2 (https://pestsmart.org.au/toolkit-resource/rabbit-control-methods-humaneness-matrix/).

Key findings from this project have been summarised in a final report (Taggart and Strive, 2022). The combined results suggested there would be little benefit in registering RHDV2 as an additional biocide for local control at this point in time. Major factors underpinning this evidence-based recommendation included the high seroprevalence of RHDV2 in wild rabbit populations, its frequent natural-transmission events (Taggart *et al*. 2022), and the attenuating effect from RHDV2 maternal immunity. The recommendation was additionally supported by an independent economic assessment that found a negative return on investment ratio for an RHDV2 registered product (Hardaker *et al.* 2022).

Notably, outputs from this project indicate that with RHDV2 being the dominant virus in the landscape, K5 may be a more effective biocide today compared to when it was released in 2017, when the majority of wild rabbits had natural immunity to RHDV1 viruses that were completely protective against K5 (Patel *et al*. 2022).

Simultaneously, the project continued to monitor the spread, evolution and interactions between the various virus strains in Australian wild rabbit populations. This includes the analysis of rabbit carcasses submitted by members of the public, assessing the merit in strategically sampling carrion-feeding blow flies as an additional tool to improve landscape scale monitoring of rabbit diseases, and ongoing monitoring of long-term rabbit biocontrol monitoring sites nationally.

The outputs demonstrated the value of ongoing national disease monitoring and reported

- A Sustained reduction in wild rabbit populations of ~ 60% following the arrival of RHDV2, between 2028 and 2022 (Ramsey *et al*. 2023)
- Evidence of changes in transmission patterns of RHDV2 (Taggart *et al*. 2022),
- The emergence of several recombination events of RHDV2 (mixing and matching of genes of different RHDVs and related viruses to increase their fitness) (Mahar *et al*. 2021)
- The establishment of RHDV-K5 in some western Australian rabbit populations (Peng *et al*. 2023).

Virus sequence data obtained through this work was also used to infer epidemiological parameters such as reproductive number and viral population sizes (Pacioni *et al*. 2022a; Pacioni *et al*. 2022b).

In addition, the CISS rabbit program supported research and development towards a multivalent vaccine to protect non-target pet and farmed animals from all RHDV strains known to circulate in Australia, including RHDV2 (O'Connor *et al*. 2022) however this process has been ceased due to the importation of the European Filavac vaccine.

Eimeria intestinalis and *E. flavescens* distribution in Australia

Certain species of the rabbit protozoan parasite *Eimeria* can cause high mortality in young rabbits (Pakandl 2013). Furthermore, it has been suggested that these parasites may enhance the lethal effects of myxomatosis (Boag *et al*. 2013). *Eimeria* parasites have, therefore, been suggested as potential candidates for an additional rabbit biocide in Australia.

In particular, two highly virulent species of *Eimeria*, *E. intestinalis* and *E. flavescens,* have been reported in Western Australia (Hobbs and Twigg 1998) but have not been described in rabbit populations on the eastern seaboard. If absent from the eastern states, it was hypothesized that relocation and introduction of these parasites into wild rabbit populations in the east could provide additional pathogen pressure and contribute to population reduction. In 2019 a survey of 26 Australian rabbit populations revealed that both virulent *Eimeria sp*. appear to be widespread in wild Australian rabbits, including in the eastern states (Peacock *et al*. 2021). This suggests that there would be little benefit from the relocation of these parasites.

Genetic biocontrol technology for vertebrate pest decision framework

One CISS project explored the potential use of genetic biocontrol technologies as an alternative long-term and non-lethal means of managing invasive animals (including rabbits) in Australia (Ruscoe *et al*. 2021). These genetic biocontrol concepts including, but not limited to RNA-guided gene drives (Esvelt *et al*. 2014), involve molecular tools that genetically modify the genome of the target animal. This is to introduce a desired trait which, when the animal reproduces, will ideally be passed on to 100% of the offspring rather than the usual 50% of offspring that would inherit a particular trait from one parent.

In the context of invasive species control, these approaches often involve genetic modifications that will skew the sex bias of the offspring towards all-male, which would eventually result in the collapse of the population. While such approaches have been demonstrated to work in model organisms such as insects and nematodes, real-world applications in vertebrates are still under development (Grunwald *et al*. 2019, Pfitzner *et al*. 2020, Gierus *et al*, 2022). As any of these genetic technologies would involve the release of a genetically modified organism (GMO) into the environment, risk management, regulatory implications and social licence to operate are critical factors that need to be explored prior to such management approaches being implemented in wild populations (Hayes *et al*. 2018, Moro *et al*. 2018).

Through a series of key stakeholder consultations, the CISS Genetic biocontrol technology for vertebrate pest decision framework project explored the demand, knowledge gaps and key issues associated with genetic control technologies. the resulting prioritisation framework provides a useful tool to help inform investment decisions towards the development of these technologies for the management of pest animals (Carter *et al*. 2021; Ruscoe *et al*. 2021), as part of a long-term biocontrol pipeline approach for a range of species including rabbits.

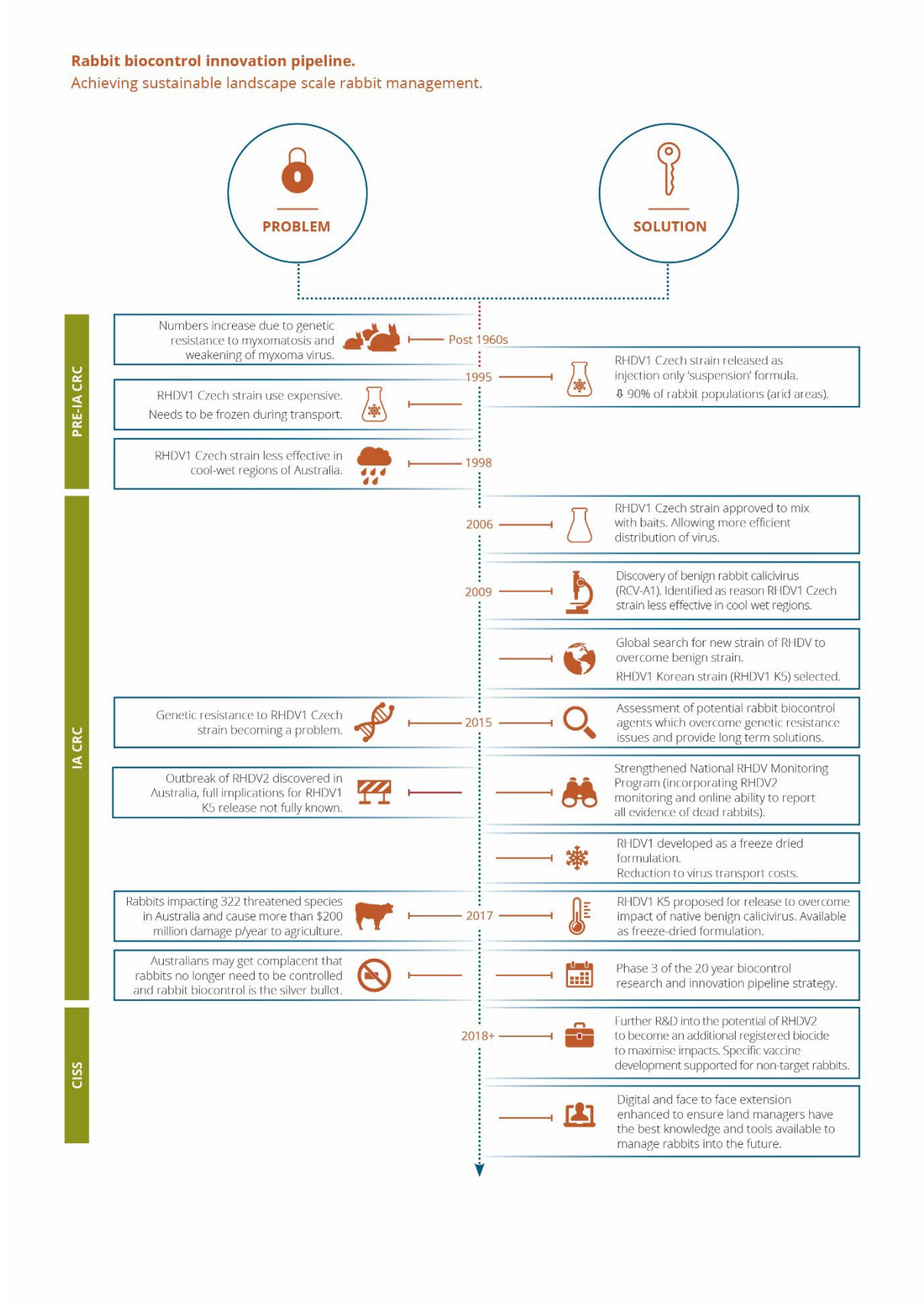

Figure 2. Current Rabbit Biocontrol Innovation Pipeline (CISS 2021).

4.2 Current MLA/CSIRO-funded initiatives strengthening the CISS rabbit biocontrol pipeline (2020 – 2024)

Under the previous rabbit biocontrol pipeline strategy, the Meat & Livestock Australia (MLA) and CSIRO co-funded a project that will address the gaps in the current CISS rabbit research portfolio. In particular, this project aims to address the medium-term and medium risk research approaches that could deliver biocontrol products and strategies within a 10–15 year timeframe, thereby complementing and strengthening the rabbit biocontrol pipeline. The major project components planned are:

- The development of organoid tissue culture systems for rabbits and assessment of its suitability for the cultivation of rabbit caliciviruses *ex vivo*. If successful, this will turbo-charge the RHD Accelerator Platform technology approach.
- Conducting bioprospecting of lagomorph (rabbit and hare) pathogens within Australia and abroad, to gain a better understanding of lethal pathogens and possible emerging diseases of rabbits that may be suitable for rabbit biocontrol.
- Genetic modelling using population genomics data to investigate whether emerging genetic control technologies may feasibly assist in the control of rabbits in Australia.

Organoid culture systems for the successful cultivation of rabbit caliciviruses

Organoids are miniaturised and simplified versions of organs cultured *ex vivo* from stem cells that retain the different cell types, micro anatomy, host genetics and some of the functions of organs. Organoid cultures allowed the robust and repeatable cultivation of human norovirus (a calicivirus specific to humans) for the first time which could not be grown in cultured cells (Ettayebi *et al*. 2016). Therefore, it was hypothesized that an organoid culture system may also support the replication of RHDV, which does not grow in more commonly used cell culture systems.

Recent results report the world-first first reproducible replication of rabbit caliciviruses (RHDV1 and RHVD2) in liver organoid cultures (Kardia *et al*. 2023). The project was also able to demonstrate that organoid cultures can be used to study species specificity of the various caliciviruses. The system, which is currently undergoing successful optimisation, represents a critical stop and go point for the Accelerator work to proceed and revisit the selection of superior, highly virulent strains for subsequent release by accelerating natural selection processes.

The successful culture of RHDV within an organoid system also has a host of potential other applications, including the development of reverse genetics systems for RHDV, functional studies into virulence mechanisms, functional studies into genetic resistance development in rabbits, and the mechanisms underlying host-pathogen co-evolution processes.

Off-shore and on-shore bioprospecting

This approach differs from previous bioprospecting initiatives pursued in Phase 2. Whereas previous projects used available information and literature searches to identify possible additional pathogens of rabbits, this project focusses on the identification of the cause of death in rabbits that are negative for RHDV.

Since 2015, as part of the IA CRC and CISS funded rabbit projects, samples from both wild and domestic dead rabbits have been submitted by the public for genetic analysis to confirm if rabbit biocontrol viruses were the likely cause of death. This service, offered by CSIRO, has revealed that for approximately 30% of submitted samples the cause of death was not due to any of the known strains of virulent RHDVs or myxoma virus. This raised the question, what killed these animals and are there

potentially other unknown rabbit pathogens that may be harnessed as an additional biocontrols for rabbit management? This project uses diagnostic deep sequencing analyses to establish if infectious agents may explain a proportion of these unexplained rabbit mortalities. Any identified infectious agents are investigated further for their potential as an additional biocide.

To date, apart from confirming the a series of known rabbit pathogens as the likely cause of death (Jenckel *et al*. 2021a), the project has identified the presence of Hepatitis E virus in Australia, a potentially zoonotic pathogen that was not known to be present in Australian rabbits (Jenckel *et al*. 2021a, Jenckel *et al*. 2021b), a new species of hepacivirus in North American Lagomorphs (Jenckel *et al.* unpublished data – manuscript in preparation). Based on the characteristics of these virus families, none of the pathogens identified to date are likely suitable candidates for additional biocontrols or biocides. However, the project outputs demonstrated repeatedly that the methods used are highly suitable for the detection of new and known pathogens and to make inferences if these are likely causes of death.

Throughout this project, Office International des Epizooties (now World Organisation for Animal Health -OIE) reports will be closely monitored for any reports of mass lagomorph mortalities. The project will also proactively seek out international collaborations with a view to identifying lagomorph pathogen profiles abroad. In addition to developing and strengthening existing networks in Europe and North America, a new international focus is Patagonia. Patagonia is home to the same species of introduced lagomorphs as Australia (i.e. European rabbits and European brown hares) and, like Australia, has no native lagomorphs. In addition to potentially identifying new opportunities for rabbit biocontrol in Australia, this project will strengthen international lagomorph disease surveillance networks.

Suitability of Australian rabbit populations for genetic biocontrol approaches — Gene drive modelling informed by population genomics

This project component aims to ascertain if Australian wild rabbit populations may be amenable to future genetic biocontrol approaches. The project will utilise the 'Gene drive Utility and Risk Determination Pipeline (GUARD)' (Rane *et al*, in preparation), a user-friendly bioinformatics platform for the design and risk assessment of genetic biocontrol approaches that is currently being developed for insects.

To provide the input data, the project has sequenced >300 individual entire genomes of representative Australian rabbit populations. Subsequent GUARD analyses will then take into account the genetic variability of the population to help identify suitable genetic targets for any genetic control options. For example, for a 'gene drive-type' control tool to spread efficiently, every rabbit of the target population needs to have the target genetic sequence, otherwise they will be resistant.

Furthermore, for a gene drive to be safe, the putative target sequence for the genetic manipulation must not be conserved or occur at high frequencies in non-target populations, such as rabbits in their native range in Europe. In addition, the tool can use the genetic information to model the Australian rabbit population structure and in turn to model the likely spread of a putative genetic control tool within Australian rabbit populations.

The 'nuts and bolts' of genetic biocontrol — for example, the development of animals that will produce all male offspring — are currently being developed in 'easier-to-work-with' vertebrate model species such as mice (Gierus *et al*. 2022) and zebra fish. However, once this work has identified a suitable way forward, the outputs from this modelling work will lay the foundations for the future transfer of suitable genetic biocontrol strategies to rabbits. In addition, the Australian wild rabbit

genome data will also provide an invaluable resource to study other aspects relevant to biocontrol — for example, the ongoing development of genetic resistance to pathogens, including RHDV2.

5. BIOCONTROL PIPELINE STRATEGY WAY FORWARD 2022–2030 AND BEYOND

Myxoma virus and especially RHDV1 and RHDV2 have set a high bar for rabbit biocontrol with regards to safety, efficacy and relative humaneness. A proven track record in safety and species specificity is essential for any biocontrol agent. Since its emergence in the mid-1980s, RHDV1 has been shown to be highly species specific to European rabbits, and while RHDV2 is less species specific, reports of effective transmission since its emergence in 2010 has been restricted to lagomorphs (rabbits, hares and cottontails).

All three viruses (myxoma virus, RHDV1 and RHDV2) are mechanically transmitted by insect vectors over large distances and between populations that are not in direct contact (Schwensow *et al*. 2014; Hall *et al*. 2019). This enables these biocontrols to effectively self-disseminate across the entire range of the rabbit and cause repeated outbreaks in populations without the need for repeated applications. This is particularly beneficial in remote areas.

Myxoma virus initially caused very high mortality, but rabbits rapidly developed genetic resistance to the original virus strain, resulting in the emergence of strains with moderate mortality rates and long disease periods in genetically resistant wild rabbits. In turn with developing genetic resistance in wild rabbits the virus is continuing to evolve higher levels of virulence, resulting in an ongoing arms-race between the host and the virus, resulting in a phenotype causing the prolonged disease periods that ensure optimal virus transmission by biting insects (Di Giallonardo and Holmes, 2015). Since the 1990s, field isolates of Myxoma virus have now become so virulent that they now cause a completely different disease in domestic rabbits which, unlike wild rabbits, have not co-evolved for decades to be highly genetically resistant against myxoma virus (Kerr *et al*. 2017, Kerr *et al*. 2022). This suggests that domestic rabbits are no longer a suitable animal model for studying myxoma virus virulence. In addition, recently assessed myxoma viruses from the field are increasingly virulent, indicating that virus and the rabbit host remain in a biological 'arms race'. There are now significant gaps in the myxoma virus-host co-evolution knowledge which warrant further investigation in wild rabbits. Further research is required to better understand the humaneness and virulence grades of currently circulating variants compared to historic versions of the virus. This would be progressed through experimental studies systematically comparing virulence grades in unselected laboratory and wild rabbits.

RHDV remains highly virulent and continues to kill wild rabbits quickly, i.e. with no prolonged periods of clinical disease (Elsworth *et al*. 2014). Virulent caliciviruses have emerged at least three times independently. On the first two occasions RHDV and European Brown hare syndrome virus (a disease similar to RHD in hares) emerged independently in the 1980s, and in 2010 RHDV2 emerged as a new virulent disease of rabbits and hares (Frölich and Lavazza 2008, Abrantes *et al*. 2012, Le Gall-Recule *et al*. 2013). The emergence of additional RHDVs in the future (e.g. RHDV3) is feasible and these may represent possible candidates to investigate as additional biocontrol agents. However, relying on such serendipitous events is clearly risky as a long-term rabbit management strategy.

It is currently impossible to predict if, when and where new RHDVs or other suitable rabbit pathogens will emerge. Therefore, a proactive approach is needed that considers the available and potential short, medium and long-term options, and balances the risks and likelihood of success with the

potential benefits (Figure 3). At the same time, such a proactive approach must maintain critical capability and the ability to quickly react to new opportunities (pathogens or technologies) as they arise.

Broad categories of these potential options are outlined below:

A. Optimising the application of biocontrol tools that are already available.
B. Selecting for better versions of existing pathogens.
C. Identifying new pathogens that may be suitable as biocontrol agents.
D. Developing alternate non-lethal biocontrol technologies.
E. Increase underpinning and enabling science.
F. Improve adoption and integration with conventional control.

Such an approach also highlights the importance of maintaining underpinning science capability and infrastructure, as well as the need to continue to improve integration of biocontrol applications with conventional control methods for maximum impact.

5.1 Recommendations

Outlined below — and illustrated in Figure 3— are a series of recommendations that take into account current rabbit biocontrol research initiatives. The various options outlined cover both short- and long-term strategies, with various levels of associated risks as well as potential benefits. In addition, they aim to find the optimal balance between pursuing the most promising management approaches, while at the same time continuing to look forward and incorporate long term future strategies.

The way forward 2022–2030 should be guided by the outcomes of the CISS (2017-2022) rabbit control program as well as the additional MLA/CSIRO funded work (2020–2024) investigating some of the medium-term options identified in the previous rabbit biocontrol strategy. For example, the establishment of a robust culture system for RHDV may warrant revisiting the RHD Accelerator natural selection platform technology. Any suitable pathogens identified through the ongoing watching brief and/or bioprospecting projects, or any additional information on new or existing pathogens suggesting a possible additional biocontrol/biocide, could also trigger recommendations for further investigation.

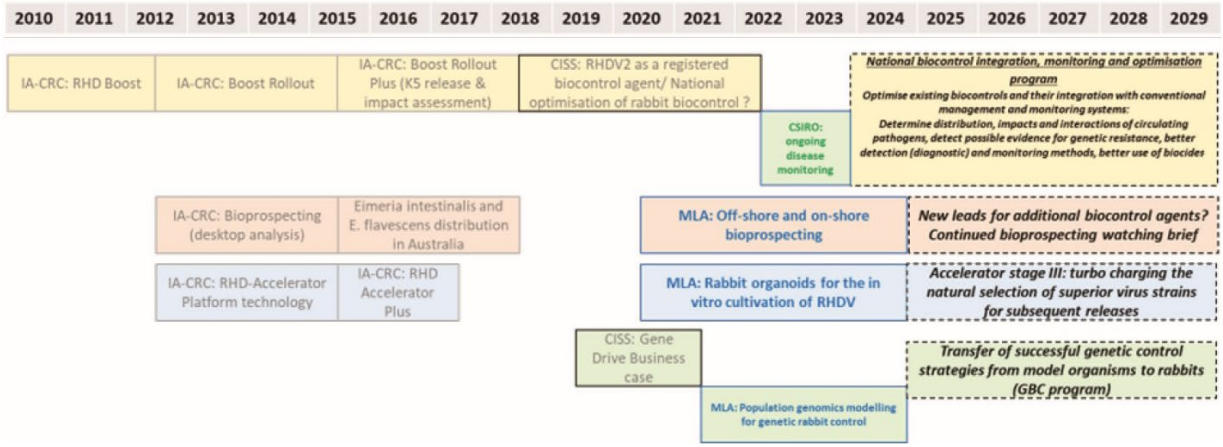

Figure 3. Rabbit biocontrol pipeline with suggested future research pathways for CISS Phase 4 (2022–2030). Black font indicates current CISS Rabbit Program activities (2018–2022). Blue font indicates current MLA/CSIRO co-funded Rabbit Biocontrol activities.

A. Optimise the use of existing biocontrol tools available to maximise impact.

Further optimisation may be possible if we simultaneously increase efforts to better understand the impacts of, and interactions between, the various circulating biocontrol agents in Australia, namely RHDV1, RHDV2, RHDV1-K5, and myxoma virus, as well as the benign RCV-A1. This should include further studies into the genetic resistance mechanisms and if these differ between RHDV1, RHDV2 and myxoma virus. A more detailed understanding of these processes driving or inhibiting the activity of these viruses across the landscape may open further opportunities to find more beneficial combinations of existing biocontrol agents which, in turn, may further increase impact and/or extend the useful life of registered biocontrol tools. Determining if and how different strains of RHDV can co-exist, and which genetic and epidemiological factors define effectiveness is essential knowledge to guide future control efforts.

Recommendation 1: Develop more efficient ways of deploying available biocides and improving their integration with existing and new conventional tools to maximise their impacts, in particular in high value agricultural and environmental settings.

CISS Phase 3 2018–2022 assessed the suitability of RHDV2 as a candidate for an additional, registered biocide and the likelihood of its application leading to Australia wide, regional, and/or local benefits in the immediate or longer-term future. It was determined that there was insufficient benefit above that already gained through the currently circulating RHDV2 strain to warrant the additional cost to have the virus registered as a biocontrol. It has already become apparent that the epidemiological patterns of RHDV circulation appear to be changing and a fresh view on how, when and where to apply existing biocontrol tools in conjunction with existing and new conventional controls seems clearly warranted to achieve the best possible long term rabbit impact reduction. An ongoing and improved national monitoring program is a key enabler, by continuing monitoring of disease activity through molecular testing of dead rabbits, monitoring population numbers and pathogen impacts through ongoing serological testing, possibly including the use of flies as ‘drones’ to support broad scale monitoring of disease spread and activity, as well as other factors that may facilitate or inhibit pathogen spread.

In the context of an ongoing rabbit monitoring program, there is a strong case for further development of improved and streamlined serological diagnostic tools. Currently, seven different assays are performed on every single rabbit serum which is very resource intensive, in many cases samples require testing several times to obtain the final antibody titre. More recent available technologies should be utilised to allow for higher throughput analysis and potentially multiplexing of these assays (for example Luminex technology or similar). In this context it is also paramount to generate an Australia-based source for critical serological reagents such as strain specific monoclonal antibodies. Obtaining these critical reagents from international collaborators has repeatedly caused bottlenecks in the past and led to substantial delays on project delivery. Furthermore, point-of-use handheld antibody tests available to land managers could facilitate fast decision making by determining which virus strain would be best used in any population and what the expected knock down would be under ideal conditions. More detailed immunological cross protection studies may be needed to maximise the benefits of this approach.

Recommendation 2: Continue coordinated national rabbit monitoring and sampling to better understand the impacts of, and interactions between, the various viruses (RHDV1, RHDV2, RCV-A1, RHDV1-K5 and myxoma virus) circulating in Australian rabbits. This supports Recommendation 1 by identifying possible windows of opportunity to better deploy available tools. Monitoring intensity should ideally increase from current levels to mimic or exceed coverage achieved through CISS Phase 2, and include optimisation of tools for more effective sample and population data collection.

Recommendation 3: Develop improved and streamlined serological (and molecular) diagnostic methods, generate Australian-owned tools for serological diagnostic and consider the development of hand-held reliable serological diagnostic tools to facilitate strain selection and estimate the likely success of biocide applications.

The outputs from the first three phases of the rabbit biocontrol pipeline program have shown that it is important to understand the variety and interactions between the growing number of rabbit pathogens in Australia.

Three different types of virulent lagoviruses are known to circulate in Australian rabbits; RHDV1, RHDV1-K5 and RHDV2. Non-pathogenic caliciviruses still interfere with effective RHDV-mediated biocontrol, although their prevalence appears to have declined since the emergence of RHDV2. However, multiple recombination events between RHDV2 and RHDV1/RCV-A1 have been described and they appear to become locally dominant, indicating a potential selective advantage (Mahar *et al*. 2021). The mechanism underlying this selective advantage has not yet been determined but is clearly of importance for the dynamics of these viruses and, if identified, could be used to guide any RHD-Accelerator pipelines if revisited in the future.

Myxoma virus still regularly affects most rabbit populations and can cause substantial mortality (Wells *et al*. 2018). Furthermore, previous exposure to myxoma virus can increase the mortality from subsequent RHDV outbreaks, possibly due to immune suppression of rabbits surviving myxomatosis (Barnett *et al*. 2018).

There remains a clear need to understand the spread and potential interactions between all these rabbit pathogens by pulling together long-term data on virus spread, genetic variability, and virus evolution. This also includes serological analyses and data on rabbit population fluctuations to estimate impacts on rabbit populations. Such analyses will allow identification of possible synergistic effects that could be exploited, or possible antagonistic effects that should be avoided. In this context, there is clear opportunity to harness new technologies to further streamline high throughput molecular diagnostic and genotyping tools, or to develop serological multiplexing methods to increase efficiency of analyses.

Recommendation 4: Facilitate underlying science to increase understanding of the detailed genetic resistance mechanisms in wild Australian rabbits to the various circulating biocontrol agents using whole genome sequencing approaches, confirmatory functional studies, and the development of high throughput screening tools for resistance in Australian rabbit populations.

Development of genetic (heritable) resistance to RHDV infection in wild Australian rabbits has been reported in several studies (Nyström *et al*. 2011, Elsworth *et al*. 2014, Schwensow *et al*. 2017a, Schwensow *et al*. 2017b, , Schwensow *et al*. 2020). However, we are a long way from understanding the molecular and/or immunological mechanisms that account for genetic resistance to RHDV infection in rabbits.

More recently, in-depth sequencing approaches and functional studies have been used to unravel the development of genetic resistance to myxoma virus, resulting in a better understanding of the underlying mechanisms (Alves *et al*. 2019). With the decreasing costs of full-length genome re-sequencing, similar approaches should be applied to revisit the issue of genetic resistance to the different RHDVs and identify the underlying genetic/molecular/immunological mechanisms.

Following candidate genes being identified, functional studies need to be carried out. This could be achieved either through *ex vivo* organoid work or by infection of captive-bred wild rabbits. Once identified, diagnostic tests can be designed to study prevalence and distribution of disease resistance

markers and investigate possible strategies to counteract their effects. If genetic resistance mechanisms to one biocontrol agent were different to those for another (for example RHDV1-K5 versus RHDV2) then populations could be screened to help identify the most suitable control tool or combination of tools to increase impact and extend the period in which they can be used effectively. The current MLA-funded project '*Suitability of Australian rabbit populations for genetic biocontrol approaches*' will provide a wealth of genetic information that can serve as a starting point to further build upon to answer these questions.

The expected outcomes of recommendations 1–4 discussed above are:

- Maximisation of the long-term impact of existing biocontrol/biocide tools by developing and implementing tailored applications and increase community awareness and literacy in rabbit control.
- Increased predictive capability and potential for active intervention through Australia-wide rabbit disease monitoring and impact assessment. This would enable more accurate assessments of the returns on previous and ongoing investments. This would also enable detection of any major epidemiological changes that could potentially warrant re-visiting the option of a registered RHDV2 biocide.

B. Select for better versions of existing pathogens, for example by accelerating/directing evolution towards more suitable strains (review in 2022/2023)

A major limitation of the RHD Accelerator pipeline (CISS Phase 2) was the need for virus selection to be conducted in live rabbits. This was laborious, time consuming and had significant welfare impacts. In addition, the need to maintain a calicivirus-free rabbit breeding colony to supply animals for these experiments makes this a costly undertaking in terms of infrastructure and personnel. However, the previous RHD Accelerator project demonstrated proof-of-concept that RHDV strains with altered properties could be selected.

The current MLA-funded rabbit organoid project has demonstrated that RHDV1 and RHDV2 can successfully be amplified *ex vivo* in organoids, paving the way for renewed attempts for selection and characterisation of novel virus variants, at much faster speed and lower cost. Prerequisite for the accelerated evolution of antigenic variants is the availability of a panel of monoclonal antibodies to RHDVs, the production of which would also support recommendation 3.

In addition, other non-GM approaches can be explored in this system. This includes facilitating natural recombination between two caliciviruses or attempts to select for antigenically different enteric caliciviruses (such as RCV-A1) with a liver tropism, thus re-creating one of the suggested mechanisms for the emergence of virulent calicivirus strains.

Recommendation 5: Re-start the Accelerator Platform technology using novel organoid technologies for the accelerated selection of improved RHDV variants.

The expected outcomes of recommendation 5 are:

- A platform for the ongoing production of suitable rabbit biocontrol agents for long-term sustainable rabbit management.
- A platform enabling functional studies of fundamental aspects of RHDV replication and genetic resistance mechanisms.

C. Identify new pathogens (onshore or offshore) suitable for self-disseminating or augmentative biocontrol (review in 2023)

To identify new pathogens that may be suitable as additional rabbit biocontrols, it will be important to maintain a watch of OIE reports and continue to scan the scientific literature for emerging new pathogens of lagomorphs.

In addition, it is suggested to carefully follow the genetic evolution of RHDVs in Europe. This has been a successful strategy previously with RHDV1-K5 and would have been with RHDV2 if it had not arrived in Australia unintentionally. RHDV1 and RHDV2 may further evolve into new serotypes, either by genetic drift or recombination. It is also possible that another form of RHDV will emerge.

The outcomes of the mid-term review of the current MLA project should be considered when looking at possible future biocontrols. To date the project has not identified a suitable candidate, but has validated the methods used for the bioprospecting approach.

Recommendation 6: Continue bioprospecting for new rabbit biocontrol agents and maintain the capability required to react to emerging opportunities.

The expected outcome of recommendation 6 is the strengthening of the pipeline of additional biocontrol agents and maintaining the capability to act quickly if something suitable emerges.

D. Develop and assess novel technologies for non-lethal biocontrol (for example genetic biocontrol)

The development of non-lethal, genetic biocontrol technologies is subject to a proof-of-concept prototype in a model vertebrate/mammalian species. This is currently a very active area of research with world leading research laboratories at several Australian universities pursuing cutting edge research in rodent and fish model organisms respectively.

Depending on the success of the current research streams, future projects can be envisaged that investigate the transfer of the most successful genetic biocontrol approaches from model organisms into rabbits, which would include proof of concept rabbit work as well as genetic and ecological modelling.

In this context, the ongoing MLA/CSIRO co-funded rabbit project, which will generate an Australian wild rabbit population genome database, provides essential baseline data to inform the genetic design of such approaches. It will also enable modelling of the likely spread, effectiveness and target population specificity. In addition to experimental work to transfer successful genetic technologies to the rabbit system, any such projects going forward will need to be accompanied by continued communication with stakeholders. Constructive, transparent, pro-active, and regular engagement with stakeholders and the public will be required to effectively manage risk and social licence issues.

While genetic technologies are high risk (both in terms of technical feasibility and public acceptance) and long-term, the potential benefits of such approaches for the sustainable control or eradication of vertebrate pests are substantial. Therefore, it is important to include consideration of genetic technologies as part of a long term and balanced research portfolio, provided they are demonstrated to be safe, effective, and socially accepted.

Recommendation 7: Undertake proof-of-concept studies on the technical feasibility of genetic biocontrols for rabbits modelled on successful strategies applied in model vertebrate organisms. This includes all aspects of the transfer of these molecular technologies to the rabbit system, in parallel with genetic and ecological modelling.

The expected outcome of recommendation 7 is a long-term option for sustainable and humane landscape-scale control (and possibly even eradication) of rabbits.

E. Increase underpinning & enabling science and capability

Maintaining world class cross-disciplinary capability in understanding rabbit biology, disease epidemiology and impact, as well as host-pathogen co-evolution is essential. Developing relevant research capability by attracting young scientists through collaboration or developing them through the CISS PhD program. This and working closely with partner universities is key to the long-term success of this essential enabling component of a long-term biocontrol pipeline strategy.

Recommendation 8: Increase rabbit biocontrol-focussed, multi-disciplinary, scientific capability in virology, epidemiology, genetics/genomics and ecology with a particular emphasis on training and succession planning.

Recommendation 9: Maintain or increase infrastructure required for rabbit biocontrol-related work, including experimental animal facilities and breeding colonies for domestic and wild rabbits.

The expected outcome of these recommendations is that relevant and necessary knowledge and research capacity is maintained and grown for long term sustainable rabbit control during the 20-year pipeline strategy and beyond.

F. Improve adoption and integration with conventional control

Biocontrols should never be stand-alone tools. For maximal sustained rabbit knockdown, it is essential to combine biocontrols with conventional rabbit management for the greatest long-term impact. Conversely, biocontrol is essential for landscape scale management of wild rabbits.

While conventional rabbit management techniques can be effectively used in localised geographic areas, self-disseminating control methods are required in order to suppress rabbit populations as much as possible across Australia, to minimise reintroductions into control areas and to mitigate impacts on public land. Extensive knowledge exists regarding the use of biocontrols in combination with conventional rabbit management techniques, but detailed recommendations — for example, regarding the timing of conventional management in combination with biocontrol — may need to be adjusted based on changes in disease epidemiology of the various pathogens that are now circulating in Australia, as well as their interactions with other pathogens and ecological factors.

Recommendation 10: Implement stakeholder and community engagement strategies to enable effective and coordinated rabbit management using a best practice combination of conventional management techniques and biocontrol.

The expected outcome of recommendation 10 is the better uptake and integration of rabbit control strategies leading to better long-term outcomes for rabbit management and impact reduction.

6. CONCLUSION

Past and current research confirms that although biocontrol is the most cost-effective and feasible means for landscape-scale management of rabbits, it may never a silver bullet. Host-pathogen interactions and co-evolution inevitably reduce effectiveness of biocontrol agents.

When a new biocontrol agent is released into the landscape, work needs to start immediately on a follow-up solution. A proactive approach is essential to protect previous investments and achievements and gains in rabbit management. Coordinated investment into the implementation of a long-term rabbit biocontrol pipeline research strategy has built a strong, multi-pronged and ongoing biocontrol research program that has, and will continue to, contribute to the continental scale control of rabbits and their economic and environmental impacts.

Australia has never been better placed to strategically plan ahead. Staggered long-term investments in rabbit biocontrol will continue to ensure that the impacts of rabbits are kept in check into the future.

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