Recommendations for a long-term rabbit biocontrol research and innovation plan

Tanja Strive, CSIRO (Compiler)

2016

An Invasive Animals CRC Project

Invasive Animals CRC

Australian Government
Department of Industry, Innovation and Science

Business Cooperative Research Centres Programme

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Report to the Invasive Plants and Animals Committee
Endorsed by the Invasive Animals CRC Rabbit Biocontrol Scientific Committee

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Executive Summary

Biocontrol of rabbits in Australia using Myxoma virus and Rabbit Haemorrhagic Disease Virus (RHDV) has resulted in $70 billion of increased agricultural productivity over 60 years, reduced impacts on a number of the 304 nationally listed threatened species, and improved landscape condition.

However, rabbits and viral biocontrol agents are in an 'arms race' as rabbits gradually develop genetic resistance, which reduces their effectiveness over time. As a result, to efficiently manage rabbit impacts a pipeline of biocontrol agents needs to be developed that ideally can be released every 8-10 years.

Governments and industry have co-invested in the first five year plan of a 20 year rabbit biocontrol strategy through the Invasive Animals CRC. This plan was based on a three pronged approach to: 1. evaluate and select a naturally occurring overseas RHDV strain demonstrated to complement the one existing RHDV strain already in Australia; 2. develop a platform technology to naturally produce new RHDV strains under laboratory conditions; 3. prospect, assess and identify any potential new biocontrol agents that warrant further research.

This report provides a framework for the next phase of the 20 year rabbit biocontrol strategy, to 1. complement and/or improve existing viral biocontrol tools, 2. investigate the suitability of novel biocontrols, and 3. outline long-term strategic approaches for pest animal population control. The plan comprises 17 recommended areas of research activity.

Viral biocontrol of rabbits

Concurrent with the proposed national release of the RHDV1 K5 strain, RHDV2 - a new exotic rabbit calcicivirus that arrived in Australia in 2015 – needs to be evaluated for its utility as a complementary viral biocontrol agent. The ability of RHDV2 to kill rabbit kittens and the significant capacity to partially overcome immunity to RHDV1 suggest that it may have potential as an important additional biocontrol tool. ***"RHD Accelerator – stage 3"*** builds on an existing platform technology that aims to accelerate and direct evolution of virus strains able to overcome immunity to other circulating strains.

The recommended projects are:

<u>RHDV2 evaluation – adding RHDV2 to the rabbit biocontrol toolkit</u>

1. Experimental assessment of RHDV-2 for its utility as an additional rabbit biocontrol agent

2. Mitigate effects of RHDV2 on pet and domestic rabbits by supporting the development of a specific vaccine

<u>RHDV Accelerator – Stage 3</u>

3. Adapt the Accelerator platform to counter effects of genetic resistance and maintain a program to select for superior RHDV strains for subsequent releases

<u>Mitigate risk of genetic resistance</u>

4. Establish a captive wild rabbit breeding colony

5. Understand genetic resistance mechanisms to rabbit diseases, and develop tests for broad-scale screening of genetic resistance to RHDV in Australian rabbits

Increased predictive capability and the potential for active intervention by continuing Australia-wide rabbit disease impact assessment

6. Increased coordinated, nation-wide rabbit monitoring and sampling effort to understand the impacts and interactions of the various viruses circulating in wild Australian rabbit populations, in particular the effects of the exotic RHDV2 strain and its potential impacts on the pending K5 release.

7. Develop state of the art diagnostic facilities for rabbit disease epidemiology

8. Increase scientific capability to improve the understanding of virus ecology and epidemiology.

9. Develop new efficient diagnostic tools to measure landscape-scale RHDV infection dynamics based on fly transmission and sampling.

10. Determine seasonality of non-pathogenic caliciviruses impeding effective RHDV biocontrol, and identify windows of opportunity for RHDV releases for rabbit management.

11. On-going nation-wide baseline monitoring of rabbit diseases

Other biocontrol options

Prospecting for new potential rabbit biocontrol agents will provide information on new emerging pathogens of rabbits and other pest species. Virulent forms of the protozoan parasite ***Eimeria*** are potential biocides. Studies investigating its natural ***distribution*** in Australia ***and efficacy*** will determine the suitability of this parasite as a rabbit control tool.

The recommended projects are:

Continued prospecting for new rabbit biocontrol agents

12. Maintain existing watching brief, and extend to include pathogens of other vertebrate pest species, such as foxes, feral cats and cane toads

13. Preparation (and regular updating) of a draft response plan

Eimeria intestinalis and E. flavescens as additional rabbit biocontrol agents and a possible biocide

14. Determine the baseline-prevalence of protozoan parasites in wild rabbits across Australia

15. Undertake efficacy testing of selected Eimeria species in domestic and wild Australian rabbits

Long term strategic approaches

Revolutionary molecular techniques that combine precision genetic engineering with so called ***'Gene drive technology'*** open up novel, humane, genetic control options to control feral pest populations. Feasibility studies will investigate the potential of these approaches in the Australian context.

The recommended activities are:

16. Investigate RNA-guided gene drive strategies for pest animal control

17. Examine the ethical, social and regulatory licences for novel genetic control options for feral animals

18. Consider experimental feasibility studies investigating the potential use of genetic tools for the humane suppression of invasive animals

1. Introduction: A long-term rabbit biocontrol pipeline strategy

Biological control of any vertebrate pest species is never a silver bullet (Morin, Sheppard et al. 2014). The vertebrate host evolves together with the pathogen: development of genetic resistance to the pathogen and/or reduced virulence of the pathogen leads to a lessening of the effect of biocontrol over time. Long term strategies need to be in place providing a pipeline of tools that will allow for ongoing control of rabbit numbers and impacts and provide a series of options for targeted rabbit control in different situations.

The current Invasive animals CRC recognises the environmental, economic and social benefits of long term sustainable rabbit control and has developed a multi-pronged, long term strategic approach to harness the unique opportunities and potential high returns on investment that successful biocontrol initiatives can provide (Cox, Strive et al. 2013). The current biocontrol research portfolio of the Invasive Animals CRC has therefore developed a series of projects that are either aiming at improving and complementing existing biocontrol tools, or identifying new agents, for future applications to keep rabbit numbers below the rabbit impact threshold (Figure 1).

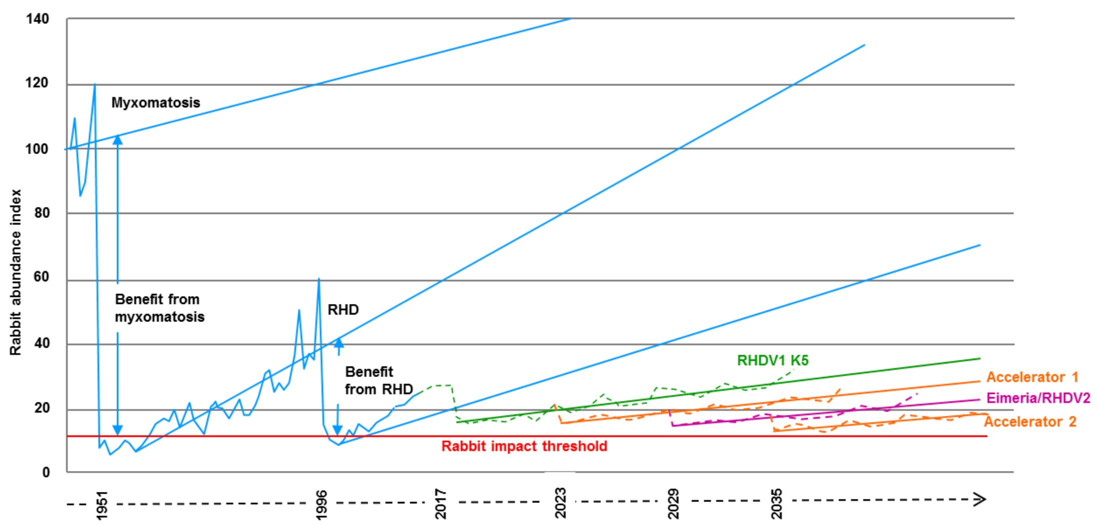

Figure 1: Impacts of previous biocontrol initiatives on rabbit abundance and possible benefits of future biocontrol initiatives developed through a rabbit biocontrol pipeline strategy. Adapted from Cox et al., 2013

Phase one of this strategy has been completed: the 'RHDV Boost' project compared different overseas strains of RHDV to assess their suitability to complement the current RHDV strain in Australia. A Korean strain of RHDV (K5) was selected as a product for release, on the basis of its increased ability to overcome partial cross protective immunity provided by non-pathogenic endemic caliciviruses as well as its increased ability to infect genetically resistant wild rabbits. K5 has been approved by the Australian Pesticides and Veterinary Medicines Authority (APVMA) and the nation-wide release is planned for March/April 2017.

The subsequent ‘RHD-Boost Rollout’ initiative (currently underway) is providing a comprehensive, pre- and post- release epidemiological monitoring and surveillance network for the accurate performance evaluation and impact measurement of RHDV K5.

A second, longer term strategy currently pursued by the Invasive Animals CRC is the ‘RHD Accelerator’ project. This approach is aiming to develop a platform technology that allows for the accelerated natural selection of viral variants that are able to overcome immunity to existing strains or otherwise outperform them. If successful, this platform can be used to produce a continuous supply of superior virus strains for successive releases.

In addition, a ‘Bioprospecting’ project is actively looking for new pathogens of rabbits, to complement the current biocontrol agents Myxoma virus and RHDV.

The sudden appearance of exotic strains of rabbit caliciviruses in Australia, in particular RHDV2 (Hall et al., 2015) will likely have the greatest impact on current biocontrol initiatives. The uncontrolled spread of exotic strains of caliciviruses could mask or potentially reduce the impact of the RHDV K5 strain. Conversely, the appearance of these exotic viruses may also represent an opportunity, if we can harness these new arrivals, and direct and maximise additional impacts they may have on Australian rabbit populations. In any case, specific diagnostic tools are essential to determine exactly how the different viruses are affecting rabbit populations.

Finally, new ground breaking biotechnological approaches such as gene drives are being developed, and research activities are increasing globally to assess these new technologies for their potential to deliver humane, species specific and safe pest animal control.

The long-term strategy proposed here is an update and extension of the recommendations made in the Cox et al. (2013) report and takes into account recent developments relevant to the existing control strategy. It outlines recommendations for a broad, three pronged approach consisting of

1. **Viral biocontrol of rabbits**
2. **Other biocontrol options**
3. **Long-term strategic research approaches**

The long-term objectives of the strategy are to ensure new rabbit biocontrol agents can be released on a regular basis, to counteract reduced effectiveness of existing agents due to increasing immunity and genetic resistance, and to accurately measure impacts on rabbit biocontrol, as part of a larger integrated pest management approach.

Due to the somewhat unpredictable impacts that the incursion of the exotic caliciviruses will have on rabbit biocontrol, and due to the rapid developments in the area of precision genome engineering and other biotechnological approaches for pest population control, this strategy will require regular updating and should therefore be reviewed every 3-5 years.

2. Viral biocontrol of rabbits

2.1. RHDV2 evaluation – adding RHDV2 to the rabbit biocontrol toolkit

In May 2015 a new calicivirus termed RHDV2 was discovered in Australian wild rabbits (Hall, Mahar et al. 2015) and is very closely related to an RHDV2 strain recently described in southern Europe. This virus, a recombinant consisting of 'prototype' genes of 'classical' RHDV with a RHDV-2 capsid protein gene (Lopes, Dalton et al. 2015), spread through Europe in domestic and wild rabbits. It appears to be replacing previous strains of RHDV on the Iberian Peninsula and accelerating the decline in wild rabbit numbers (Delibes-Mateos, Ferreira et al. 2014, Lopes, Correia et al. 2015). RHDV2 has been reported to cause higher mortality in young rabbits than RHDV1 (Dalton, Nicieza et al. 2012) and overcome immunity to other RHDV strains (Le Gall-Recule, Lavazza et al. 2013), but to what extent is not clear. RHDV2 is the only rabbit calicivirus known to infect species other than European rabbits (*Oryctolagus cuniculus*), namely two species of hare, the Sardinian cape hare *(Lepus capensis mediterraneus)* and the Italian hare *(Lepus corsicanus*) (Puggioni, Cavadini et al. 2013, Camarda A. and M. Legretto 2014). Very recently, RHDV2 infections of the European brown hare *(Lepus europaeus*) have also been reported in Australia (Hall et al., in print). RHDV2 is potentially a suitable candidate to add to the rabbit biocontrol toolkit due to its increased impact on young rabbits and the significant capacity to overcome immunity to other strains.

<u>Recommendations:</u>

1. **Experimental evaluation of RHDV-2 for its utility as an additional rabbit biocontrol agent**

Of particular importance is detailed, quantitative analyses of the virus' virulence and ability to overcome natural immunity to other caliciviruses, such as RHDV1 K5, the non-pathogencic calicivirus RCV-A1, naturally circulating Australian RHDV1 field strains, and any other calicivirus that may be present in Australian wild rabbits (and vice versa). This includes quantitative analyses of the ability of RHDV2 to infect and kill rabbit kittens at various ages, the ability to overcome passive immunity (maternal antibodies) to field strains, and a quantification of the ability to overcome vaccination. This data is a prerequisite for the development of predictive models that will aid in the targeted application and impact maximisation of this additional biocontrol tool. A thorough assessment and potential development of RHDV2 into an additional registered product for rabbit biocontrol has been included into the '*Business case to advance the selection of new rabbit biocontrol agents*' (submitted as a separate document).

2. **Mitigate effects of RHDV2 on pet and domestic rabbits by supporting the development of a specific vaccine**

RHDV2-specific vaccines are now commercially available in Europe (Barcena, Guerra et al. 2015) (K. Dalton, personal comm.) and should be made available in Australia to ensure effective protection of pet and domestic rabbits. As government and industry are the main financial beneficiaries of any new rabbit biocontrol agent, a shared responsibility approach towards mitigating the risks would therefore be desirable should RHDV2 be considered as an additional biocontrol tool, to help protect commercial rabbit breeders and pet owners from the effects of RHDV2.

Benefits/Outcomes: 'horses for courses' – a comprehensive RHDV toolkit

RHDV2 could be the missing link providing ongoing effective rabbit biocontrol, bridging imminent short term solutions (e.g. Boost Rollout with K5) and more long term strategies such as Accelerator, which is still at the R&D stage, or Bioprospecting, which relies on the assessment of detected (eg. Eimeria) or the emergence of new, potential highly pathogenic and species specific rabbit pathogens. The advantage lies in the ability of RHDV2 to partially overcome cross protective immunity to other rabbit caliciviruses, which should work both ways. For example, if a rabbit population predominantly has antibodies to Australian field strains, RHDV2 could be used as a control tool in these populations. In populations where there is predominantly immunity to RHDV2, the K5 strain (or an RHD-Accelerator strain) can be released to achieve maximum impact. Individual rabbits surviving exposure to multiple strains will have immunity against all of them, however the average lifespan of wild rabbits is relatively short (<12 months, T. Cox personal comm.), such that patterns of population immunity can be expected to change over time. As RHDV2 also kills a higher proportion of young rabbits than RHDV1, RHDV2 might be more effectively applied during the breeding season than RHDV1 strains (e.g. the Czech strain currently in use and K5). This recommendation is closely linked to Recommendation 7B (development of specific serological tools) and 10 (ongoing epidemiological monitoring and surveillance).

2.2. RHDV Accelerator - Stage 3

The current RHD Accelerator – Stage 1 project aims to develop a platform for the acceleration of natural selection to produce virus variants that are able to overcome immunity to existing RHDV strains for subsequent virus releases. The current pilot project is now in its second year and has delivered proof-of-concept that virus variants with altered properties can be selected, and remain highly virulent in rabbits. Funding until June 2018 has been secured for RHD Accelerator – Stage 2 to further develop the platform and explore options to speed up the selection process. What is not included (and subject to availability of a wild rabbit colony) is the adaptation for the platform to address genetic resistance. In addition, if the approach proves feasible, work should continue to keep producing superior virus strains for subsequent releases. For any RHD Accelerator virus strain proposed as an additional control tool, an effective vaccine will need to be produced and made available prior to any virus release.

Recommendations:

3. **Adapt the RHDV Accelerator platform to counter effects of genetic resistance and maintain a program to select for superior RHDV strains for subsequent releases.**

This needs to take into account the nature of current virus strains circulating in wild rabbit populations in Australia. Originally designed to develop a strain that can overcome immunity to circulating Australian field strains, it is now possible that within a few years any or all of the exotic caliciviruses may be the dominant field strains. An effective vaccine needs to be available for each proposed candidate release virus produced by the Accelerator program.

Benefits/Outcomes:

This aspect will complete the Accelerator platform technology and will allow us to overcome two of the main problems facing effective ongoing rabbit biocontrol – pre-existing immunity and genetic resistance. It complements the RHDV2 evaluation and K5 rollout initiatives, and would also mitigate the risk of not finding any new suitable emerging diseases of rabbits using the Bioprospecting approach.

2.3. Mitigate risk of genetic resistance

There is now evidence for mounting genetic resistance to RHDV infection in wild Australian rabbits (Elsworth, Kovaliski et al. 2012). Although some mechanisms relating to viral attachment factors have been suggested (Nystroem, Le Gall-Recule et al. 2011) we are a long way from understanding the molecular and/or immunological mechanisms that account for genetic resistance to RHDV infection in rabbits. There is some encouraging evidence that RHDV may be co-evolving with its host to maintain very high levels of virulence (Elsworth, Cooke et al., 2014), however rabbit numbers are increasing (Mutze et al., 2014), and more work is needed to understand the underlying genetic mechanisms that are driving host-pathgogen co-evolution and virulence, in order to develop tailored strategies to maximise the impact of rabbit biocontrol.

Recommendations:

4. **Establish a captive wild rabbit breeding colony.**

A small ongoing base investment is needed to ensure continuous animal husbandry and veterinary support for the breeding program and maintenance of several different genetic lines of rabbits. The output of this breeding colony can then be increased and funded on a project basis in the short to medium term as the need arises.

5. Understand developing genetic resistance mechanisms to rabbit diseases, and develop tests for broad-scale screening of genetic resistance to RHDV in Australian rabbits. As genetic resistance to RHDV appears to be geographically localised (Elsworth, Kovaliski et al. 2012) it is essential to understand its distribution to develop tailored biocontrol strategies. Work should also continue to identify the nature of genetic resistance and if it is generic against all rabbit caliciviruses or virus specific.

Benefits/Outcomes:

Recent work describing how RHDV appears to adapt to the pressure of genetically resistant rabbits by evolving to maintain high levels of virulence (Elsworth, Cooke et al. 2014) highlights the importance of testing RHDV on field caught or captive bred wild rabbits. Domestic rabbits are not suitable for these types of studies. The lack of any facility in Australia that is able to successfully breed wild rabbits is currently hampering progress in this field. It is difficult to capture large numbers of rabbits from field populations with low levels of immunity to other viruses or other confounding factors. The use of seronegative field caught or captive bred wild rabbits is essential to support research on genetic resistance in rabbits, virus virulence and to inform the strategic release of RHDV.

2.4. Increased predictive capability and potential for active intervention through Australia-wide rabbit disease monitoring and impact assessment

The release of the new RHDV1 K5 strain that was identified during the 'RHD-Boost' project is imminent. In addition, two exotic rabbit caliciviruses have now been confirmed in Australian wild rabbits: a Chinese strain of the variant type RHDVa that was first reported in domestic rabbits in January 2014 has been confirmed in wild rabbits in Canberra in March 2015. More recently, RHDV2 was identified in wild rabbits in the ACT and has since spread to all Australian states except Queensland. This means that following the K5 release, there will be four different pathogenic calicivirus strains in the Australian environment. In addition, non-pathogenic caliciviruses known to interfere with effective RHDV mediated biocontrol are widespread in south-east Australia. Myxoma virus still regularly affects most rabbit populations, and more recently, the potential role of endemic protozoan parasites to rabbit population control has also been increasingly discussed. There is a clear need to understand the spread and potential interactions between all these rabbit pathogens, to identify possible antagonistic or synergistic effects that could be exploited to assist planned strategic approaches for more efficient rabbit control.

Recommendations:

6. Increased coordinated and nation-wide rabbit monitoring and sampling effort to understand the impacts and interactions of the various viruses circulating in wild Australian rabbit populations, in particular the effects of the exotic RHDV2 and its potential impacts on the pending K5 release.

It is essential to evaluate the effectiveness of RHDV2 and the new RHDV1 K5 strain and understand their epidemiology, ecology and interaction with other circulating strains to guide future biocontrol releases. Understanding spread and impact of naturally circulating RHDV2 in the field is a prerequisite of recommendation 1 (adding RHDV2 to the biocontrol tool kit). The combined outcomes of recommendations 1 and 6 will allow the identification of any synergistic effects of different virus strains that can be exploited for active intervention, thereby maximising biocontrol benefits.

7. Develop state of the art diagnostic facilities for rabbit disease epidemiology.

The increased number of pathogens in Australian rabbits highlights the urgent need for the development of reliable and discriminatory diagnostic tools to monitor the effect each of the viruses is having on rabbit populations and how they interact. This includes:

7A. Develop high throughput molecular diagnostic and genotyoping tools using next generation sequencing technology as a priority

Such tools will allow the detection and identification of the different caliciviruses, identification of possible recombination events and tracking the evolution of the viruses simultaneously.

7B. Develop specific differential serological tests for different rabbit caliciviruses.

This is challenging due to the increased number of virus strains in the environment and the high level of antigenic similarity between certain virus strains. It should nevertheless be attempted, as serological tools allow reliable large scale epidemiological studies of RHDV. This aspect is also funded until June 2018, and there will also be a need to maintain capability for ongoing diagnostic support.

8. Increase scientific capability to understand virus ecology and epidemiology. There is a growing needed for this type of capability to ensure the timely and ongoing analysis of the large amount of data that will be produced during the increased monitoring and surveillance efforts associated with the K5 release.

9. Develop a new efficient diagnostic tool to measure landscape-scale RHDV infection dynamics based on fly transmission and sampling

Fly transmission of RHDV in the field has been well documented (Asgari, Hardy et al. 1998, McColl, Merchant et al. 2002, Schwensow, Cooke et al. 2014). What is not clear at this stage is if flies can be used to track the movements of RHDV in the landscape, and to help determine the cause of any given outbreak. Retrieving dead rabbits following a virus outbreak before scavengers remove them and when most die underground can be difficult, particularly in remote areas. If flies prove to be a sufficiently sensitive indicators of the various caliciviruses at a site this could potentially transform landscape-scale monitoring. Such research should be closely aligned with the intensive monitoring efforts associated with the RHDV K5 release, as this will allow comparative evaluation of the various diagnostic tools (see also Recommendations 6 and 7).

10. Determine seasonality of non-pathogenic caliciviruses impeding effective RHDV biocontrol, and identify windows of opportunity for targeted rabbit management.

Non-pathogenic caliciviruses are widespread in south-east Australia (Liu et al., 2014) and are known to provide partial cross protective immunity to lethal RHDV1 infection (Strive et al., 2010). It has been hypothesized that the spread of these benign viruses may be greatest during the breeding season (T. Strive, personal comm.); its occurrence and greatest impact on rabbit biocontrol may therefore be seasonal. Notably, the protection conveyed by non-pathogenic caliciviruses was found to be transient (Strive et al., 2013). Therefore, understanding any patterns of seasonality will enable windows of opportunity to be identified where impacts of RHDV biocontrol initiatives are likely to be greatest.

11. **Ongoing and nation-wide base-line monitoring of rabbit diseases.**

Beyond the intense pre- and post- K5 release monitoring, structures need to be put in place to allow for reduced but ongoing and nation-wide monitoring of rabbit disease epidemiology. This could in part be achieved by providing on-ground vertebrate pest managers with training and basic resources, to ensure a minimum of recording disease outbreaks and collecting samples of dead wild rabbits (to be analysed for known viral diseases or other causes). Collection and analysis of fly samples to study RHDV2 epidemiology may also provide a means of increasing the surveillance area at comparatively low cost. Within this initiative there is clear scope for investment and innovation into integrating and utilising citizen science and community-engagement.

Benefits/Outcomes:

Ongoing monitoring is a prerequisite for the evaluation and impact assessment of any new biocontrol agent used for the control of wild Australian rabbit populations. It will allow a more accurate estimate of return on previous investments (e.g. RHD-Boost/K5), as well as an estimate of any collateral benefits and/or damage from co-circulating exotic rabbit caliciviruses.

Determining if and how different strains of RHDV can co-exist, and which genetic and epidemiological factors define effective field strains, and how the rabbit pathogens interact with each other on a temporal and spatial scale will be needed to guide future control efforts.

3. Other biocontrol options

3.1. Continued prospecting for new rabbit biocontrol agents

The benefits of biocontrol agents for controlling pests in a country as large as Australia are well recognised. The current IA CRC rabbit biocontrol agent prospecting project utilises Wildlife Health Australia to tap into the international wildlife health network to efficiently scan for potential new rabbit biocontrol agents. Monthly reports on diseases affecting wildlife world wide are collated and circulated by Wildlife Health Australia. This ensures rapid flow of information at very low cost, facilitating responses to new or emerging diseases of rabbits or other vertebrate pest species. Overseas disease outbreaks may be short-lived, requiring quick intervention to obtain data or material for analysis.

Recommendations:

12. **Maintain existing watching brief, and extend to include pathogens of other vertebrate pest species, such as foxes, cats, and cane toads.**

13. **Preparation (and regular updating) of a draft response plan.**

    This should include likely regulatory requirements (quarantine, animal welfare) and timeline estimates for importation and assessment of new agents.

Benefits/Outcomes:

The extended watching brief and draft response plan will allow rapid responses to promising new pathogens of rabbits or other pest species.

3.2. *Eimeria intestinalis* and *E. Flavescens* as additional rabbit biocontrol agents and a possible biocide

Protozoan parasites such as Eimeria are recognised as causing high mortality primarily in young rabbits (Coudert et al 1993, Duszynski and Couch 2013), and interacting with myxomatosis (Boag et al 2013). They may therefore be useful as an additional biocontrol tool for Australian wild rabbits. Two species of virulent coccidia, *Eimeria intestinalis* and *E. flavescens,* have been reported in Western Australia (Hobbs and Twigg 1998), but have not been described on the eastern seaboard. Recent studies on the prevalence and distribution of protozoan parasites in Australian rabbits are scarce. A detailed business case investigating the possible translocation of *Eimeria* species within Australia to add to the existing rabbit biocontrol tool kit has been prepared and is submitted as a separate document (*Business case to advance the selection of new rabbit biocontrol agents*).

Recommendations:

14. **Determine the baseline prevalence of protozoan parasite profiles across Australia.**

    A PCR-based pilot survey of protozoan parasites in rabbit populations across Australia is necessary to confirm presence and determine current distribution of these organisms. This study could capitalise on existing sampling efforts carried out for the RHDV Boost Rollout project. As certain intestinal coccidia species can be highly immunogenic (Pakandl et al., 2008), the possibility of a serological pre-screen should be investigated to identify populations suitable for PCR testing.

15. **Undertake efficacy testing of selected *Eimeria* species. in domestic and wild Australian rabbits**

    Any putative new biocontrol agent will need to undergo efficacy testing prior to approval for release by the relevant authorities (APVMA). This will likely include detailed assessment of the mortality and morbidity in wild and domestic rabbits (young and adult), as well as considerations of how a protozoan parasite could be delivered as a commercial control agent if deemed suitable.

Benefits/Outcomes:

A potential alternative strategy to control certain rabbit populations, if efficacy can be demonstrated.

4. Long-term strategic approaches

4.1 Novel humane genetic control technologies for the suppression of vertebrate pest populations

Emerging gene drive technology has potential for use in feral animal control programs by genetically altering entire populations. RNA-guided gene drives can be designed to edit any gene with extremely high precision (using the CRISPR/Cas9 method) in order to alter a trait of an individual. Following fertilisation of gametes during reproduction, the gene drive has the ability to copy itself onto the second parental chromosome alongside the altered trait; the resulting organism will be homozygous for the modified trait, as will its offspring. This way any trait can be spread through entire populations quickly through sexual reproduction (Esvelt, Smidler et al. 2014). Gene drive strategies can be utilised to achieve population suppression, for example by altering the sex bias of populations that will eventually lead to a population crash, or sensitizing specific species to a particular toxin (Esvelt, Smidler et al. 2014).

Any such approaches for vertebrate pest species including rabbits can capitalise on a large body of research that was previously carried out through the Vertebrate Pest CRC and the Pest Control CRC. An approach termed virally vectored immunocontraception (VVIC) investigated the development of genetically modified virus vectors that could be used to sterilise vertebrate pests. While not producing any VVIC product, a vast body of knowledge was generated during this time (Tyndale-Biscoe and Hinds, 2007), increasing our understanding of induced sterility, reproductive targets, reproductive biology, pest animal ecology, and analysis of risks associated with the release of genetically modified organisms into the environment.

Rabbits were one of the key species researched during this program, which is now presenting **a golden opportunity to combine proven sterilisation strategies with novel delivery mechanisms such as gene drives.**

Recommendations:

16. Investigate RNA-guided gene drive strategies for pest animal control.

Pest animal researchers should keep abreast of this rapidly developing research field, with a particular focus on technical solutions that increase the safety and controllability of gene drive approaches.

17. Examine the ethical, social and regulatory environments for novel genetic control options for feral animals.

For any potentially contentious technologies involving the release of genetically modified organisms into the environment, transparency and social licence to operate are paramount. Therefore, ongoing dialogue between researchers, stakeholders and key regulatory bodies (e.g. APVMA and the Office of the Gene Technology Regulator), risk analysis specialists, ethicists, and the general public will be essential when scoping any potential applications for this powerful new technology in the area of pest animal management.

18. Consider experimental feasibility studies investigating the potential use of genetic tools for the humane suppression of invasive animals.

For example, gene drive technology could be used to target well understood key reproductive proteins that have been proven to induce sterility in female mammals (including rabits, mice and others) when removed or impaired. Because of the revolutionary and extremely powerful novel molecular tools for precision genome editing, proof of principle of any such approaches can now be achieved within a few years.

Benefits/Outcomes/Opportunities:

Gene drives are not ‘contagious’ like classical biocontrol agents but are spread through sexual reproduction. It is therefore a very attractive option for controlling invasive species such as rabbits, which are relatively short-lived, have high reproductive rates, have no close relatives amongst native species and rarely interact with domestic species. In addition, and unlike rodents or cane toads, live rabbits are not likely to accidentally get to other countries as stow-aways. As a non-lethal control option that aims to control populations, gene drive technology also addresses important animal welfare and community concerns surrounding lethal pest animal control, although any approach involving the release of GMO animals will be controversial in its own right. These caveats notwithstanding, gene drive technology has the potential to transform the way we manage invasive species, although probably not within the next decade (Strive and Sheppard 2015).

Gene drive technology is still in its infancy for application in vertebrate pest management and may require substantial research and development time. Consideration also needs to be given to the risk of movement (legal or illegal) of gene-drive modified populations internationally to countries where Australia’s feral animals are native or desirable species. However, this exciting new technology offers many advantages, and it is important to be involved early in this rapidly expanding new research field to ensure pest animals are a focus. Gene drive research in rodents is already underway, driven by pioneers in the field. In contrast to rodents, pest species such as the rabbit are a problem specific to Australia (and New Zealand), presenting a unique opportunity to Australian research providers to be part of an exciting new research field from the beginning and to deliver innovative tailored solutions to protect Australia’s agriculture and unique biodiversity.

Conclusions

Due to the extensive ground work of the current Invasive Animals CRC, we have never been better placed to advance a strong biocontrol program for vertebrate pest management, to integrate with conventional approaches for the long-term sustained control of rabbits and mitigation of their impacts. Past and current research confirms that although biocontrol is the most cost-effective and feasible means for landscape scale management of pests, it is never a silver bullet. Host-pathogen interactions and co-evolution inevitably reduce effectiveness and, following release of a new control agent, work needs to start immediately on a follow-up solution. A proactive approach is essential to protect previous investments in the area. A small strategic but ongoing investment can provide basic infrastructure. It is essential to maintain key capability and networks required for broad scale sampling and monitoring, ensure ongoing diagnostic services (capability and supply of reagents) and maintain a wild rabbit breeding colony. This will support all aspects of future rabbit biocontrol research, and allow quick response to expand effort when the opportunity arises.

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