

**DEVELOPMENT OF GENETIC RESISTANCE TO
RABBIT HAEMORRHAGIC DISEASE IN WILD
RABBITS *Oryctolagus cuniculus***

by

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Animal Ethics Approvals

The research undertaken in this thesis was covered by Animal Ethics Approval from the relevant state agencies. Rabbits were collected under approvals: Queensland Pest Animal Ethics Committee approval PAEC060601, South Australia Wildlife Ethics Committee approval WEC45/2007, PIRSA Animal Ethics Committee approval AEC09/03 and Victorian Animal Ethics Committee approval 062793. Experimental trials were conducted under approvals: Queensland Pest Animal Ethics Committee approvals PAEC060601 and PAEC060801 and Queensland Community Access Animal Ethics Committee approvals CA2007/10/220 and CA2009/06/356.

Publications associated with this thesis

This thesis includes publications for which I am the senior but not the sole author. I took the lead in this research in that I designed the research, undertook the experimental work, analysed the data and wrote the manuscripts. I was, however, assisted by my co-authors.

The publications associated with this thesis are as follows:

Chapter Three

Elsworth, P.G., Kovaliski, J. and Cooke, B.D. in press. Rabbit haemorrhagic disease: Are Australian rabbits (*Oryctolagus cuniculus*) evolving resistance to infection with Czech CAPM 351 RHDV? *Epidemiology and Infection*.

Chapter Four and Five

To be submitted for publication.

Chapter Six

In final preparation for submission to PLoS Pathology:

Elsworth, P.G., Strive, T., Kovaliski, J., Sinclair, R., Kerr, P., Capucci, L., and Cooke, B.D. In prep. Evidence of increased virulence in Rabbit Haemorrhagic Disease Virus associated with genetic resistance in rabbits.

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ABSTRACT

Rabbit haemorrhagic disease virus (RHDV) was introduced into Australia in the mid 1990's to aid the control of rabbits. The impact was immediate and highly successful generally causing major declines in rabbit numbers. Some ten to fifteen years after the introduction of RHDV, increases in rabbit populations were seen, in some cases reaching pre-RHDV levels. This raised questions of the continuing efficacy of RHDV and whether rabbits had developed resistance. Resistance is a natural consequence of co-evolutionary processes and can reduce the impact of viral infection. This was the case as rabbits developed innate resistance against myxoma virus. The impact of myxoma virus was greatly reduced and rabbit populations recovered although not to the levels seen pre-myxomatosis.

To test for resistance in rabbits to RHDV, direct challenge experiments were used to compare different wild populations against each other and against unselected domestic controls. Susceptible rabbits from twelve populations throughout central and eastern Australia were used to represent a range of environmental habitats. In the absence of temporal information of resistance levels, a spatial experiment can be used to infer resistance as different populations are expected to evolve at different rates. The infection and mortality rates varied between the populations (from 0% infection at Ingliston to 73% infection at Bulloo Downs) tested demonstrating that resistance has developed against RHDV.

The establishment that resistance is developing in Australian rabbits raised further questions about the mechanism of resistance given the observed differences between populations. Of key importance is whether there is a genetic basis to the resistance

and whether the virus is evolving to keep pace with the resistance. Further challenge testing provided answers for these questions as did comparative information from molecular research from France.

Resistance levels appeared to be related to average annual rainfall from the sites with lower levels of resistance seen at arid sites and areas with high rainfall. It is likely that this pattern is caused by poor recruitment in arid areas and the presence of a partially protective non-pathogenic lagovirus in cooler-wetter sites. The mechanism of resistance appears to be one which prevents infection. A view that is supported by molecular research from France that suggests that the phenotype of ABH binding antigens influence survival against exposure to low doses of RHDV. Similarly, the challenge experiments presented in this thesis showed that wild rabbits were better able to survive challenge with low doses of RHDV compared to unselected domestic rabbits, but that this difference disappeared at high doses. Taken together, these findings suggest that resistance to a low dose simply delays infection until a large dose is encountered as most adult rabbits have antibodies to RHDV. As the selective advantage for rabbits in doing this is unclear, a dual mechanism of infection is postulated with resistance preventing a virus facilitated uptake which may allow rabbits to slow infection and perhaps better control it.

The phenotype for resistance may readily be achieved. Challenge tests in a laboratory situation were used to expose resistant individuals from a wild population with low resistance. Breeding from only these resistant individuals produced a fully resistant generation. This shows that resistance is heritable.

Finally, preliminary examination of field strains of RHDV indicates that virulence is being maintained at a high level given that recently collected field strains infected a higher proportion of test rabbits and caused greater mortality than the Czech strain virus originally released. The evidence that the virus is maintaining its virulence relative to rabbit resistance is encouraging but given that rabbit numbers are slowly increasing it may be possible that resistance is slowly out-stripping virulence.

Evolution of RHDV and rabbits is a continuing process and the effectiveness of the virus in controlling rabbits may be a population specific prospect and other control tools such as warren ripping, poisoning and fumigation will be required at sites with high levels of resistance.