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

by

PETER GEOFFREY ELSWORTH

B.Sc. (Honours) – University of Queensland

Institute for Applied Ecology

University of Canberra

Australia

A thesis submitted in fulfilment of the requirements of the Degree of Doctor of Philosophy at the University of Canberra

December 2011

ACKNOWLEDGEMENTS

This thesis represents the culmination of a journey that began with the simple statement “Hey can I do a PhD out of that?” It has been a great journey with many highs and a few lows and some fantastic experiences both in the research activities and the people I have had the opportunity to meet and work with. The piece of work that this thesis has grown into has been the result of input and contributions from a lot of people.

First and foremost a great amount of thanks goes to my supervisors Stephen Sarre and Brian Cooke. They have guided the development of research activities and kept me progressing forward and striving for more polished writing. I thank you both for the countless hours you have put into reading through drafts of manuscripts and finally this thesis. Your advice and direction throughout has greatly helped in producing some excellent research and results for RHDV understanding. Thanks also to Brett Lidbury for early supervision and advice on matters of virology.

A great deal of thanks goes to the staff at the Robert Wicks Pest Animal Research Centre. The care of animals when I was not on-site was first rate and I never had any worries that they were being well cared for. The facilities were also well maintained and anything I needed was always ready on time and to specification. Thanks to Maria, Glen, Jack, Andrew and David for the help. The staff in the Toowoomba office have also been very supportive, taking on some of my duties when the PhD pulled me away and reading and commenting on drafts. Thanks to Dave, Matt, Michael, Speedy, Lee and Jennifer. The support of Qld DEEDI for me to undertake this work as part of

my primary duties has been incredibly generous and logistically I could not have done it without that support. The funding support helped make these projects run extremely smoothly.

The Invasive Animals Cooperative Research Centre took me on as a student and their financial support and education program has been fantastic. The student camps have been a great opportunity to interact with other people in the same situation, going through the same problems and forged some great friendships. The education program has allowed me to develop further skills and meet leaders in the pest animal world in a very interactive environment. Thanks to Steve Sarre for overseeing these programs, Nina Jenkins, Tom Heinsohn and Tony Buckmaster for running the camps and all the presenters and mentors who made them so successful. The students of the IACRC have made these camps a pleasant break from the hardships of a PhD. From the adventures at Wee Jasper to the "Shed" at Kangaroo Island, the pub at Eaglehawk and the campfire at Kioloa where the wine has flowed freely, the tales have been tall and the camaraderie has been great.

Thanks to the Institute for Applied Ecology for providing me home on my infrequent trips to the Uni. The staff and students were always accepting of me and made me feel a part of the group. Thanks for not asking too many tough questions at the initial seminar and always being willing to help out with the logistics of the University. Thanks also to Arthur Georges and the wonderful paper writing courses that were run at Kioloa. They helped focussed some of my manuscripts and provided excellent direction for the writing process.

Logistically, the projects undertaken for this thesis were a nightmare. Rabbits had to be collected from all over the country and transported to Queensland and serum and inoculums samples needed testing on pretty tight timelines. Thanks to Ron Sinclair, Greg Mutze and John Kovaliski at APCC in South Australia for collecting and transporting rabbits and running all the sera tests for me. Thanks to Steve McPhee for rabbit collection and transport from Victoria and for always providing great conversation whenever we met up. Thanks to Maija Marsh and Brian Cooke for help with collecting rabbits from New South Wales. Thanks to Bill Lowe and Tom Newsome for setting up collection sites around Alice Springs. And thanks to John Augusteyn (QPWS) and Clint Heyworth (Centrogen) for help with collecting rabbits from Diamantina Lakes and Townsville. Thanks to all the property managers whose land rabbits were taken from. Thanks to Tanja Strive, John Wright and June Liu for running PCRs on the inoculum samples.

Finally and by no means least, a great deal of thanks goes to my family. Thanks to my parents who have always supported me in all of my endeavours and provided me with a willingness and want to learn and explore and a work ethic to see things through. Thanks to my sister-in-law Margaret for all you're pushing to get things done and for always being interested in where I was up to and what I was doing. Thanks to my mother-in-law Anne for your supportive words and babysitting to allow me time to get on with the work. Most importantly of all, to my wife Katherine who has provided so much support over the time of this thesis, you have my most sincerest thanks. I doubt you fully understood what it would entail when we started out but you have

been so incredibly supportive. Thanks also to my little boy Lachlan whose arrival partway through this project threw things into disarray for a while, but is a constant source of joy and grounding when things seemed so difficult.

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.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

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.