Recurrent epidemics: disentangling the disease dynamics of viral biocontrol agents for rabbits

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Abstract

1. European rabbits (*Oryctolagus cuniculus*) have been exposed to rabbit haemorrhagic disease virus (RHDV) and myxoma virus (MYXV) in their native and invasive ranges for decades. Yet, the long-term effects of these viruses on rabbit population dynamics remain poorly understood.

2. In this context, we analysed 17 years of detailed capture-mark-recapture data (2000 – 2016) from Turretfield, South Australia, using a probabilistic state-space hierarchical modelling framework to estimate rabbit survival and epidemiological dynamics.

3. While RHDV infection and disease-induced death were most prominent during annual epidemics in winter and spring, we found evidence for continuous infection of susceptible individuals with RHDV throughout the year. RHDV-susceptible rabbits had, on average, 25% lower monthly survival rates compared to immune individuals, while the average monthly force of infection in winter and spring was ~ 38%. These combined to result in an average infection-induced mortality rate of 69% in winter and spring.

4. Individuals susceptible to MYXV and immune to RHDV had similar survival probabilities to those having survived infections from both viruses, whereas individuals susceptible to both RHDV and MYXV had higher survival probabilities than those susceptible to RHDV and immune to MYXV. This suggests that MYXV may reduce the future survival rates of individuals that endure initial MYXV infection.

5. There was no evidence for long-term changes in disease-induced mortality and infection rates for either RHDV or MYXV.

6. We conclude that continuous, year-round virus perpetuation (and perhaps heterogeneity in modes of transmission and infectious doses during and after epidemics) acts to reduce the efficiency of RHDV and MYXV as biocontrol agents of rabbits in their invasive range.

However, if virulence can be maintained as relatively constant through time, RHDV and MYXV will likely continue realising strong benefits as biocontrol agents.

Key-words: biocontrol, disease transmission, epidemiological dynamics, host-pathogen interactions, invasive species management, myxoma virus, rabbit haemorrhagic disease virus (RHDV), virulence

Introduction

Understanding temporal changes in infection rates and mortality is crucial for predicting the effects of infectious diseases on wildlife populations. This is because the effect of fatal diseases, at the population level, depends on the intricate interplay of disease-induced mortality, host reproductive behaviour, and individual heterogeneity in infection propensity and intensity (Frank 1996; Alizon *et al.* 2009; Wells *et al.* 2017).

The virulence of a pathogen (infection-induced mortality rates of hosts) and infection rate (the propensity of individuals susceptible to a disease to become infected) can depend on the mode of spread and the dose in which pathogens are transmitted, as well as the resistance/immunity of host individuals, all of which can vary temporally. Therefore, it is crucial to quantify temporal as well as spatial variation in apparent virulence and infection rate if host-pathogen (co) eco-evolutionary processes are to be better understood (Woolhouse *et al.* 2002). For example, if pathogens do not constantly persist in host populations but are repeatedly introduced, variation in the resulting virulence of different pathogen strains can cause temporal changes in the impact of the disease on host populations (Manning *et al.* 2008). If the pathogen transmission process involves heterogeneity in the dosage of exposure/inoculum, the epidemiological dynamics can change fundamentally because of dose-dependent variation in mortality rates or variation of within-host replication of the pathogen and subsequent differences of transmission dynamics (Regoes, Ebert & Bonhoeffer 2002).

Understanding the consequences of such epidemiological processes in wild animal populations is of crucial interest for informing strategic actions in disease control and host management. These include eliminating unwanted diseases and improving biocontrol agents (Dwyer, Levin & Buttel 1990; Duffy, Shackelton & Holmes 2008).

The European rabbit, *Oryctolagus cuniculus*, is a well-studied disease-burdened species. Its two major viral diseases in the wild are myxomatosis, caused by the myxoma virus (MYXV), and rabbit haemorrhagic disease (RHD), caused by the rabbit haemorrhagic disease virus (RHDV). These viruses are particularly well studied, partly because they have been used as biocontrol agents in Australia and New Zealand. While rabbits are a keystone species that is traditionally hunted in their native range (Delibes-Mateos, Ferreras & Villafuerte 2009), they cause severe damage to native biodiversity and agricultural resources in their exotic range (Cooke 2012). Extensive population declines of rabbits, following the initial releases of MYXV and RHDV in their exotic range, are well-documented (Dwyer, Levin & Buttel 1990; Mutze, Cooke & Alexander 1998; Kerr 2012); as are declines in their native range (Moreno *et al.* 2007). However, long-term trends in relative pathogen virulence and infection rates have never been quantified in wild rabbits, despite their obvious importance in managing populations.

In Australia, MYXV caused high mortality in infected rabbits upon release in 1950 but disease severity waned with time (Kerr 2012) and, initially, the virulence of the virus declined (Fenner, Day & Woodroofe 1956; Dwyer, Levin & Buttel 1990). Something similar applies to RHDV, where rabbits appear to be increasing in disease resistance with multiple genes associated with immune defence (Schwensow *et al.* 2017a; Schwensow *et al.* 2017b), while laboratory experiments indicate that RHDV strains collected a decade after initial virus release in 1995 appear more virulent than the original strain in resistant wild rabbits (Elsworth *et al.* 2014). RHDV can be transmitted through direct contact with infected individuals which are shedding viral particles in their secretions and excretions, or indirectly by means of fomites-contaminated food, bedding or water (Abrantes *et al.* 2012). Furthermore, RHDV can be transmitted by widely-dispersing insect vectors (e.g. blowflies), which can transmit the virus from rabbit carcases across wide distances to other geographic

regions (Kovaliski 1998; Schwensow *et al.* 2014). By comparison, MYXV strains of moderate virulence rely upon infected rabbits retaining virus-laden skin lesions for sufficient time to enable transmission across shorter distances by mosquitoes or fleas. MYXV is a large DNA virus able to ameliorate the rabbit's immune responses and prolong infection. In contrast, RHDV replicates quickly, often killing the host before an effective anti-viral response can be initiated.

Both RHDV and MYXV cause lifelong immunity for rabbits that survive infection and, in addition, RHDV maternal antibodies passed to kittens (young, immature rabbits) can prevent fatal disease in these individuals during infection. Therefore, these individuals are not at risk of dying from RHD before they have lost their natural resilience and/or maternal antibody protection against RHDV (McPhee *et al.* 2009; Matthaei *et al.* 2014). Consequently, the timing of seasonally driven birth-pulses in rabbits can affect the pool of susceptible host individuals, leading to temporal variation in disease (Mutze *et al.* 2014; Wells *et al.* 2015). The impact of such demographic fluctuations on disease epidemiology is of particular importance in rabbits, because they exhibit high fecundity along with pronounced changes in population size under changing environmental conditions (Rödel *et al.* 2004; Wells *et al.* 2016b).

Recent computational and methodological innovations are improving knowledge of disease dynamics through the development of advanced statistical and mechanistic models (Metcalf & Lessler 2017). This includes the development and application of Bayesian state-space models of capture-mark-recapture data to disease burdened populations (Schofield & Barker 2011; Wells *et al.* 2017), allowing individual heterogeneity in disease status to be directly fitted to data (King 2012). Alternatively, disease impacts on survival parameters can be modelled by the delineation of 'disease states', using a 'multistate' capture-mark-recapture model (Lebreton & Pradel 2002). This is done by discretizing time-varying continuous

individual covariates, such as disease status, into a finite number of states. Doing so, avoids needing to model disease status as a time-varying continuous individual covariate, whose value must be known for all individuals on all occasions (Jones *et al.* 2015).

In this analysis, we examined the impact of RHDV and MYXV on infection and survival rates of European rabbits, *Oryctolagus cuniculus*, using 17 years of detailed capture-mark-recapture (CMR) surveys of rabbit population fluctuations and health status. We incorporated an ontogenetic growth model into a Bayesian state-space CMR model to estimate age-specific demographic processes and rates of infection. To account for uncertainty arising from incomplete details on when individuals became infected or died from the diseases (or other causes) we modelled a latent Markov process of infection dynamics (Schofield & Barker 2011) (**Fig. 1**).

Materials and Methods

2.1 Study area and rabbit monitoring

Rabbits have been live-trapped at Turretfield Agricultural Research Centre (34°33′S, 138°50′E, South Australia) at 8-12 week intervals, continuously since 1996 (Peacock & Sinclair 2009; Fordham *et al.* 2012; Mutze *et al.* 2014). The study area has a Mediterranean climate with cool, moist winters and hot, dry summers. The rabbit population is relatively closed, with neighbouring populations more than 2 km away.

All live captures were uniquely marked with serially numbered ear tags (Leader Products Pty Ltd., Craigieburn, Australia), weighed to the nearest 10 g with Salter spring balances and sexed. Blood was collected from an ear vein for serological tests of RHDV and MYXV antibodies. Additionally, the study area and warrens were regularly surveyed for dead rabbits at intervals ≤ four weeks, increasing to weekly searches during spring (Sept-Nov) when epizootics were most likely to occur, and at 1-7 day intervals following any

evidence of disease-related mortality. Each carcass was spot-sprayed with permanent non-toxic dye to avoid repeated sampling and was returned to its original location to minimize bias on the natural spread of diseases. Ear tags on fresh carcasses gave evidence of the age of some of the dead individuals, and time since death was estimated according to the onset of *rigor mortis*, size of fly maggots or the state of decay.

We analysed CMR and serology data from between January 2000 and August 2016, using a subset of 2,200 individuals with unequivocal serology data for all capture events. Each capture session (n = 83) was assigned to one of the following (southern hemispheric) seasons according to local climate: *(i)* Autumn: Mar – May, *(ii)* Winter: Jun – Aug, *(iii)* Spring: Sept-Nov, *(iv)* Summer: Dec-Feb. The date of the capture session was calculated as the median date of all captures made during a capture session.

2.2 Disease state classification by immunological assays

To detect RHDV antibodies, competition ELISA (cELISA), and ELISA tests for detecting IgA, IgG, IgM isotypes were used (Capucci, Nardin & Lavazza 1997; Cooke *et al.* 2000). We used threshold levels (**Appendix 1**) to classify RHD disease states as *(i)* seronegative ("susceptible") *(ii)* seropositive kittens with maternal antibodies ("protected young") and *(iii)* seropositive due to previous infection ("immune"). Possible serological cross-reaction with benign calicivirus (RCV-A1) was taken into account when interpreting ELISA results from the combination of tests outcomes (Liu *et al.* 2012). Antibodies against MYXV were detected using a specific ELISA (Kerr 1997), classifying rabbits as *(i)* seronegative with no detectable antibodies and therefore susceptible to infection/disease ("susceptible") or *(ii)* seropositive with antibodies against disease. For MYXV, seropositive classifications may involve maternal antibodies in young rabbits or those produced after infection such that only old

individuals can be classified as "immune" (see below for the analytical approach to account for this uncertainty).

2.3 Bayesian multistate capture-mark-recapture model

To estimate the effects of diseases on rabbit survival and epidemiological parameters, we used a hierarchical state-space modelling framework to account for partially observed birth-death and disease state transitions processes (**Fig. 1**). A full model description, code and model graph (**Fig. S1**) can be found in the Supporting Information. In brief, we modelled a (partially known) state variable $z(i,t)$ to establish whether an individual is alive at time step t according to individual encounter histories (i.e. presence-absence data) and the underlying capture probability, which we allowed to vary among capture sessions. The survival probability $\Phi(i,t)$ estimates if individuals were alive conditional on whether they have been alive at a the previous time step. We used a scaling factor to account for unequal time intervals between capture sessions (average length of time intervals = 74 days, 1 SD = 27 days). We used individual measures of body mass $b(i,t)$ for estimating individual age and birth dates using the West-Brown-Enquist ontogenetic model and we projected all data on a continuous time scale (the first day of the study set to one) to express individual age and ontogenetic growth as Euclidean temporal distances (Wells *et al.* 2016a).

We modelled $\Phi(i,t)$ based on *logit*-link functions as

$$logit(\Phi(i,t)) = \mu_{\Phi}\,[age_{cat}(i,t),\, year(t)] + \beta_{sex}[sex(i)] + \beta_{DZ}[\eta_{DZ}(i,t),\, t] \qquad \textbf{(eqn 1)}.$$

Here, μ_{Φ} is the intercept, which we allowed to vary among different age classes and over years. We considered individual age as a categorical variable $age_{cat}(i,t)$ with six unequal levels: 1) 1 – 180 days; 2) 181 – 365 days; 3) 1 – 3 years; 4) 3 – 6 years; 4) > 6 years. The coefficient β_{sex} allows for variation in survival probability due to rabbit gender. The coefficient β_{DZ} captures variation in survival of individuals in different disease states based

on five different categories of the auxiliary parameter η_{DZ}, which summarizes serostatus for both RHDV (state variable η_{RHD} as specified below) and MYXV (η_{MYXV}), respectively. Specifically, the categories for η_{DZ} were 1) all individuals < 90 days old, including η_{RHD} = 'maternal antibodies' *AND/OR* η_{MYXV} = 'antibodies against MYXV, 2) η_{RHD} = 'susceptible' *AND* η_{MYXV} = 'susceptible' (individuals ≥ 90 days old), 3) η_{RHD} = 'susceptible' *AND* η_{MYXV} = 'immune' (individuals ≥ 90 days old), 4) η_{RHD} = 'immune' *AND* η_{MYXV} = 'susceptible' (individuals ≥ 90 days old), and 5) η_{RHD} = 'immune' *AND* η_{MYXV} = 'immune' (individuals ≥ 90 days old). We used these categories to be able to make inference on the relative survival and infection rates for only those disease states for which direct comparison can be made, such as those only susceptible to a single virus and immune to the other versus those immune to both viruses.

Disease status in state-space: We estimated unknown disease states η_{RHD} and η_{MYXV} for time steps where individuals were not captured based on their previous disease state. We assumed that only young rabbits < 90 days old can have effective maternal antibodies to RHDV or MYXV (Robinson *et al.* 2002). The transition probabilities between the different disease states can be summarized into $C \times C$ matrices (C = 3 according to three different disease states; probabilities in these matrices may vary according to individual age) with row sums of one. We accounted for a directional transition (governed by an underlying Markov process) between disease states, i.e. the probability to be in any disease state is conditional on the previous state, meaning that once a rabbit is infected/immune they cannot become seronegative again. We modelled disease states for each individual and time step based on the matrix of transition probabilities using the sum to unity constraint of the multinomial distribution (once conditioned on age and previous disease state, each individual set of

transition probabilities Ψ is a vector of length C, depicting the probabilities of different disease states). In the case of RHD, the equation was:

$$\eta_{RHD}(i,t) \sim Multinomial[\Psi^*_{RHD[\eta RHD(i,t-1),\, age(i,t)\, t]}\ (C)] \qquad \textbf{(eqn 2)}.$$

We used indicator variables to distinguish transition probabilities Ψ_{RHD} when individuals are alive ($z(i,t)=1$) from those prior to individual birth ($z(i,t)=0$, $I_{died}(i,t)=0$) in order to constrain unborn individuals ($I_{born}(i,t)=0$) to the immature state. In this case, the respective transition probabilities Ψ^0_{RHD} comprise a vector of length C with the first value set to 1 and all others to 0. Additionally, the indicator variable $I_{90}(i,t)$ constrained younger individuals < 90 days to transition into any disease state given Ψ^{juv}_{RHD} (thus, Ψ^*_{RHD} corresponds to either Ψ_{RHD}, Ψ^0_{RHD}, or Ψ^{juv}_{RHD} according to individual age and may vary over time steps; see model code in **Appendix 1**). Here, we used the Dirichlet distribution (with equal underlying alpha-values) as conjugate prior of the multinomial distribution. Older individuals could not have maternal antibodies ($I_{90}(i,t) = 1$). The probability for different disease states Ψ_{RHD} was estimated from the transition probability to sero-convert $\lambda(t)$ (e.g. the transition from sero-negative to sero-positive), where the probability to remain sero-negative is 1 - $\lambda(i,t)$.

We modelled the sero-conversion rate $\lambda(t)$ with a *logit*-link function as

$$logit[\lambda(t)] = \mu_\lambda(t) \qquad \textbf{(eqn 3)}.$$

We used a hierarchical hyperprior model for the time-varying intercept $\mu_\lambda(t)$ as detailed below.

The rate at which susceptible individuals acquire RHDV or MYXV at each time t (i.e. force of infection $FoI(t)$) was calculated as the proportion of seronegative individuals at the previous time step $t-1$ that have either sero-converted to seropositive or have died. Since death may have been caused by multiple drivers, we calculated the proportion of dead individuals likely to have died from disease based on the estimated disease effects on survival (β_{DZ}). We chose this approach to calculate FoI, because sero-conversion rates $\lambda(t)$ consider

only sero-conversion of alive individuals, disregarding the individuals that have died in the respective time step.

To test whether temporal fluctuation and correlations in *FoI* for the two viruses were driven by the serology data (i.e. observed individual sequences of sero-conversions) or mortality we run an additional model as described above that excluded the disease state effect β_{DZ} from the model of Φ.

Model fitting and diagnostics: The model was fitted in a Bayesian framework with Markov Chain Monte Carlo (MCMC) sampling, using the Gibbs Sampler in OpenBUGS 3.2.2 (Lunn *et al.* 2009). Chain mixing was inspected both visually and with the Gelman-Rubin diagnostic (most values < 1.2). We expressed all rates/probabilities as monthly (31-day-period) values. All parameter estimates from the state-space model are shown as posterior modes and 95% highest posterior density credible intervals (CI) from 5,000 MCMC samples (including 50% CI in plots). See Supporting Information for details on the model fit and code. Data formatting and visualization were conducted in the R software for statistical and graphical computing Version 3.4 (R Development Core Team 2017).

2.4 Virulence estimation from capture-mark-recapture data

The infection-induced mortality rate γ could not be directly estimated from the given data, as the interplay of virulence (γ) and force of infection rate (*FoI*) determines changes in population-level survival rates of susceptible versus immune rabbits (Hethcote 2000). However, assuming that susceptible rabbits that do *not* become infected have the same survival rate as immune individuals (i.e. no prolonged disease effect), and if *FoI* is the proportion of individuals to become infected, infection-induced mortality rate can be approximated as follows:

$$\gamma = 1 - (\Phi_S - (1 - FoI)) / FoI \qquad \textbf{(eqn 4)}$$

with Φ_S being the average survival rate of the pool of all susceptible rabbits and the survival rate for immune rabbits set to $\Phi_I = 1$. Note that this approach only gives reliable output if $\Phi_S > FoI$, because only then would the proportion of susceptible rabbits not to become infected have equal survival probabilities as immune rabbits.

Results

The disease dynamics induced by rabbit haemorrhagic disease virus (RHDV) and myxoma virus (MYXV) showed different patterns of short and long-term effects of infection on population-level survival rates (**Figs. 2, Fig. S2**).

Rabbits fully susceptible to RHDV (and immune to MYXV) had significantly lower survival rates (estimated at the population level) than immune adults throughout the year, with monthly survival rates being on average 25% less than for susceptible rabbits (odds ratio of hyperprior-level estimate 0.75, CI 0.68 – 0. 82) (**Fig. 2**). There was no evidence for any long-term temporal trend in changes in the survival rates of RHDV susceptible versus immune adults (**Fig. S2**). We did not identify any clear seasonal differences in the relative survival rates of RHDV susceptible versus immune adults (**Fig. S2**) despite a variable force of infection (*FoI*, estimated across all age classes) as detailed below.

In contrast, survival rates of rabbits susceptible to MYXV (and immune to RHDV) were slightly higher than those of immune adults at the population-level (all odds ratios for seasonal hyperprior-level estimates 1.18 – 1.22, CIs ranging between 1.04 – 1.35) (**Fig. 2**). As with RHDV, there was no apparent long-term temporal trend in estimated survival rates of MYXV susceptible and immune adults (**Fig. S2**). The absence of very different survival rates for individuals susceptible to MYXV versus individuals immune to MYXV was *not* caused by the absence of infection since *FoI* estimates were well above zero during the study period

(**Fig. 3**). Therefore, in most capture sessions, subsets of the pool of susceptible individuals were infected. Individuals susceptible to both RHDV and MYXV had significantly higher survival rates than immune rabbits in all seasons (all odd ratio 1.13 – 1.67 with CIs between 1.02 – 1.99) (**Fig. 2, Fig. S3**). Crucially, we found relatively higher survival rates of individuals susceptible to both viruses compared to those susceptible to RHDV and immune to MYXV throughout the year (**Fig. 2)**, indicating that rabbits immune to MYXV have a lower survival rate than susceptible individuals. Young rabbits, including those with maternal antibodies to either virus, had significantly lower survival rates than immune rabbits in spring (**Fig. 2, Fig. S4**), indicating that waning protection by antibodies result in infection and potentially, mortality, later in the same year.

The estimated force of RHDV infection across capture sessions peaked annually in most years in winter and spring (**Fig. 3**). The force of RHDV infection was constantly < 53% in 2003 and 2004, indicating that at least in some years large proportions of susceptible individuals are likely to escape infection (see **Fig. S5** and **Fig. S6** for the proportion of estimated and observed individuals in different disease states, respectively). The *FoI* for RHDV dropped close to zero in only a few capture sessions, providing evidence for potential continuous infection of susceptible rabbits throughout most years (**Fig. 3**). However, there is a possibility that this could be because the use of hyperpriors in our modelling pulled unknown values to the 'average'. Seasonal fluctuations in the *FoI* were less pronounced between 2011 – 2015 than in previous years.

The average monthly infection-induced mortality rate for RHDV was ~ 69% according to an average monthly *FoI* in winter and spring of 38% (average of all winter and spring posterior mode estimates) and 25% lower average survival rates of RHDV-susceptible individuals (see Methods). Due to large uncertainty in all estimates, we were not able to

approximate changes in infection-induced mortality over time with a high level of confidence.

The monthly force of MYXV infection peaked in spring/summer in various years, indicating some evidence of seasonality in infections (**Fig. 3**). The proportions of MYXV-immune adults tended to peak every 2-4 years, which contrasts to the mainly annual oscillations found for RHDV antibody status (**Fig. S5**).

Changes in the force of MYXV infection correlated strongly with the force of RHDV infection (Spearman's r = 0.80, CI 0.70 – 0.88), suggesting some synchrony in infection rates with the two diseases. This observed synchrony is driven largely by the serology data and not only mortality events, as evident from a model without the disease state effect on survival (i.e. excluding β_{DZ} from the model of Φ; see **Fig. S7** and **Fig. S8**).

Overall, monthly survival rates of those rabbits immune to both diseases were 92% (CI 90 – 93%; corresponding to annual survival rates of 28 – 43%). Survival rates did not differ between males and females (odds ratio male/female 0.97, CI: 0.85 – 1.14). Capture rates in most capture sessions were < 40% and varied over time (**Fig. S9**), likely explaining why uncertainty in the estimates of individual disease states and the time-specific disease effect on rabbit survival led to large credible intervals.

Discussion

The threat of diseases to wildlife populations, and the efficiency of pathogens as biocontrol agents, can only be evaluated with an adequate understanding of how different components of demography and epidemiology interact and, ultimately, how such interplay affects survival rates prior to and after contracting diseases (Di Giallonardo & Holmes 2015). Analysing the effects of rabbit haemorrhagic disease virus (RHDV) and myxoma virus (MYXV), using data from the longest running wild rabbit capture-mark-recapture (CMR) program, provided new

insights into the epidemiology of these diseases and their effects on the survival (at the population level) of rabbits. We show that despite a strongly seasonal force of infection (*FoI)* for RHDV and MYXV, it is likely that susceptible rabbits can be infected (at least at low levels) throughout the year, having implications for rabbit conservation and biocontrol. We also show that the negative effect of MYXV on susceptible rabbits is not as immediate as for RHDV, with the pool of rabbits still susceptible to MYXV having similar monthly survival rates to animals that have contracted myxomatosis (and may die sometime after seroconversion). The force of infection for RHDV and MYXV was weak in some years, suggesting that large numbers of susceptible individuals can occasionally escape infection (Wells *et al.* 2015). However, this occurred rarely, and is unlikely to be a major driver of rabbit disease dynamics.

We did not find any evidence of long-term changes in disease induced mortality and infection rates. However, this is despite the viruses having devastating impacts on rabbit survival when the epidemics first occurred, likely because initial disease dynamics are often transient and differ from long-term outcomes (Hastings 2004). Relatively constant rates of RHDV and MYXV induced mortality and infection rates over time are likely to be the result of strongly coupled co-evolutionary changes in host resistance and tolerance and pathogen invasiveness, each working to keep the other at bay. It might be that virulence of both viruses in the study population is being maintained at an optimum (assuming that viruses track changes in host resistance as they are capable of fast selection and genetic changes due to fast replication), which is most efficient for viral spread. If so, this has important implications for the use of these viruses as biocontrol agents for rabbits in their invasive range because these feedback processes carry long-term benefits for invasive species management, by maintaining negligible losses of virus virulence.

4.1 Epidemiological dynamics revealed from the CMR analysis

Our results suggest that heterogeneity in key factors such as mode and dose of virus transmission, and/or the infection process, may reduce the efficiency of RHDV as a biocontrol agent at the population level, independent of the virulence of the virus. This is because we found *(i)* that individuals can potentially become infected after annual epidemics; (*ii*) that the force of RHDV infection oscillates over the year, leading to variation in the chances individuals become infected; and (*iii*) the average infection-induced mortality rate (69%) at Turretfield is lower than rates reported when RHD first spread (up to 95%; Mutze, Cooke and Alexander (1998)). Taken together, our results suggest that prolonged exposure of rabbits to RHDV (extending beyond seasonal outbreaks) and factors that cause variation in infection-induced mortality (such as variation in rabbit resistance to infection and virulence of the virus - the latter resulting potentially from variable modes and doses of infection) are among the likely mechanisms explaining the observed lower than expected mortality rate for RHDV at the study site.

We show that the two diseases have rather different effects on rabbit survival rates. The pool of individuals susceptible to RHDV had lower survival rates compared to those that had survived a previous infection (immune individuals). In contrast, we found that individuals susceptible to both viruses had almost always higher survival rates than individuals susceptible to RHDV but immune to MYXV. This indicates that individuals immune to MYXV have relatively lower survival rates than those susceptible to MYXV. Therefore, myxomatosis has a longer term effect on rabbit survival than RHDV for individuals that ‘run the gauntlet’ of perpetual disease burdens.

Our finding that the pool of rabbits that have survived MYXV infection have lower survival rates than equivalent, unchallenged rabbits, is supported (albeit indirectly) by field research from other sites in Australia. For example, Parer, Conolly and Sobey (1985) found

that MYXV consistently kept rabbit abundance at low levels for several months after an epidemic; and rabbit survival in dry and food-scarce summer months tends to be lower after a MYXV epidemic earlier in the year. A possible explanation is that infection with MYXV in spring depletes the fat reserves of rabbits, leading to morbidity and mortality during summer months when food resources are scarce (Brian Cooke, personal correspondence).

Alternative drivers that could cause lower survival rates for rabbits that have survived MYXV infection (compared to those susceptible to infection) include MYXV directly affecting the ability of rabbits to digest food, following the acute stages of the disease. This is because receptors involved in the immune response have been linked to digestive disorders in domestic rabbits (Rahman & McFadden 2011; Yang *et al.* 2013). Another possible explanation is that exposure to MYXV compromises the health of rabbits in such a way that it reduces the survival of individuals subsequently infected by RHDV. These suggestions are speculative, and not mutually exclusive, but could be a starting point for examining why rabbits challenged with MYXV have survival rates similar to susceptible animals.

When interpreting these results, it is important to consider that the odds ratios of the survival of susceptible and immune rabbits (i.e. those surviving infection) do not provide precise estimates of infection-induced mortality rates. This is because only a fraction of individuals in the pool of susceptible rabbits may get infected at any one time step, due to the underlying force of infection and disease transmission rate (Hethcote 2000). Furthermore, it should be noted that *(i)* our inferences were drawn from data collected over average time intervals of 74 days, whereas viral spread can potentially occur over shorter time periods (Mutze *et al.* 2014); *(ii)* our analysis did not directly explore whether exposure to MYXV compromises the immunity of rabbits in such a way that it reduces survival to subsequent infection from RHDV. If there is a strong interaction between the two diseases, whereby RHDV is more likely to cause the death of MYXV-immune compared to MYXV-susceptible

individuals, then the reported time-delayed effects of MYXV could be being fostered (in full or part) by RHDV infection. Therefore, it is very possible that this new evidence of lower survival rates for rabbits that survived initial infection from MYXV (compared to susceptible rabbits) is the result of an interaction between MYXV and RHD on rabbit survival rates.

In contrast to Mutze *et al.* (2014) we did not find evidence for any long-term temporal trend in changes in the survival rates of susceptible or protected young rabbits versus immune adults to RHDV. This is likely to reflect differences in the two approaches used to analyse the data. Where, in this instance, we were able to model directly the effect of disease status of individual rabbits on survival, using a larger number of individuals, without assuming discrete periods for RHD epizootics.

4.2 Variable transmission modes and the efficiency of RHDV

Our finding that RHDV can persist at low levels across the year is independently supported by relatively short-lived immunoglobulin M (IgM), being detected (at titres ≥ 40) in low numbers of rabbits throughout the year (**Fig. S10**). Since IgM is the first antibody to appear in response to initial exposure to RHDV (Lavazza & Capucci 2008) it confirms a likely annual persistence of RHDV at low levels in the rabbit population at Turretfield. Previously, it was observed that RHDV epidemics were generally initiated by variants of the virus, which were unlikely to have persisted and evolved in the local environment (Schwensow *et al.* 2014). However, this pattern has changed in more recent years. Since 2010, single RHDV isolates collected at times following annual epidemics have shown variants most closely related to those from previous years (NS, unpublished results), suggesting that some RHDV variants perpetuate in the local environment.

If RHDV does indeed infect some susceptible individuals well after or before annual epidemics (i.e. during which time most carcasses with signs of disease-induced death are

found), what are the modes of disease transmission? The different modes of transmission could include *(i)* direct transmission from an infected alive rabbit, *(ii)* contact with a contaminated carcass in a burrow, and *(iii)* flies feeding on contaminated carcass and then defecating on burrow walls, pasture, or feeding around the eyes of rabbits.

There is evidence that high abundances of arthropod vectors, such as flies (Calliphoridae and Muscidae), during epidemics, result in fly-borne virus transmission even over large geographic distances (Asgari *et al.* 1998), facilitating RHD epidemics through repeated virus introductions and enhanced spread. Furthermore, during and after epidemics, carcasses of RHDV-infected rabbits could potentially be a major source for viral spread, since infected carcasses have been found to contain viable viral particles for up to three months (Henning *et al.* 2005). Consequently, we hypothesize that infection from older carcasses could, at least in theory, provide lower doses of infectious particles for a short period of time, which cause lower infection-induced mortality rates outside epidemics. Alternatively, lower abundance of virus-carrying flies may result in lower abundance of virus particles in the environment, which, in turn, may lead to low dose contraction. Infection dose is likely to play an important role for the progression of RHDV. Experimental infections show that mortality rates are dose-dependent, with lower doses tending to result in fewer deaths (Nyström *et al.* 2011).

If reasonably large proportions of susceptible individuals are only exposed to low dose infections, population-level infection-induced mortality will be much less than the mortality rate linked to high dose infections during epidemics. In this context, it would be interesting for future research to explore how temporal changes in the availability and decay rate of RHDV-infected carcasses, immediately following epidemics, impacts the rate and intensity (i.e. infection dosage) that susceptible rabbits become infected. If viruses are less likely to survive in carcasses that dry out more quickly (Henning *et al.* 2005) or decay more

rapidly, one would expect that changing environmental conditions would affect virus dose and the chance that susceptible rabbits become infected. These dynamics, could potentially explain the observed continuous force of infection in concert with lower average infection-induced mortality rates compared to 20 years ago, i.e. when the first RHDV epidemics occurred in Australia.

Therefore, it is likely that factors influencing RHDV transmission rather than virulence limit the number of rabbits killed by the disease. This argument could partly explain recent on-ground observations of increased survival (i.e. less infection-induced mortality) and abundance of South Australian rabbit populations (Mutze *et al.* 2015), and in silico evidence of rabbits escaping infection in some years (Wells *et al.* 2015).

4.3 Future research into transmission pathways

We believe that future research avenues should include investigating disease transmission dynamics at finer temporal scales to test the importance of heterogeneity in modes of RHDV transmissions and doses of infection on the mortality rates of rabbits susceptible to RHDV. Our analysis was restricted by practical limits to relatively long time intervals (ca. ten weeks) between capture sessions. This potentially affected our ability to capture important aspects of more rapid disease dynamics (e.g. short epidemics that last only a few days) in our CMR analysis. Furthermore, recapture rates of rabbits were low-to-moderate throughout the study period (mostly < 40%). Consequently, the accurate timing of sero-conversion of a large number of individuals in the Turretfield population remains unknown, perhaps affecting our population-level estimates of the force of infection or hazard ratios.

Further work is still needed to understand whether the time-delayed effect of MYXV reported in our study can be linked to interactions between the two co-circulating viruses. Therefore, in addition to more targeted analysis of CMR data, experiments should be used to

determine the strength and structure of possible interactions. Aspects to be studied include *(i)* whether infection by MYXV results in significant lower survival during subsequent RHDV infection and vice versa; and *(ii)* whether the timing of infection by one virus depletes the pool of susceptible rabbits for the other virus. These sorts of interactions could strongly affect the epidemiological dynamics of rabbits at the population level.

The analytical framework and results from this study lead to new questions regarding the importance of year-round epidemiological dynamics, modes of disease transmission and possible dose-response relationships in the wild. While these can only be solved with future empirical research, our study highlights that different factors may set limits on the efficacy of using RHDV and MYXV as biocontrol agents for invasive rabbits. If rabbits experience low dose exposure after epidemics, resulting in fewer fatalities, the population level effect of RHDV would be moderate, regardless of infection-induced mortality. This would have important ramifications for rabbit management, because modes of viral transmission needed to ensure high dose exposure would have to be given as much priority as engineering and releasing more virulent strains of RHDV for improved rabbit pest management. Nevertheless, if virulence remains relatively constant for RHDV and MYXV as we found, both viruses will continue to produce strong benefits as biocontrol agents, even if virulence is not as high as was observed shortly after the initial disease outbreaks.

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548

549 Authors' contribution

550 KW developed the statistical framework with input from RBO'H, DAF, and BWB and wrote
551 the first draft. NS contributed to data collection and study concept. All authors contributed to
552 writing the manuscript and gave final approval for publication.

553

554 Data accessibility

555 If the manuscript is accepted for publication, the data supporting the results will be archived
556 in Dryad and the data DOI will be included at the end of the article.

557

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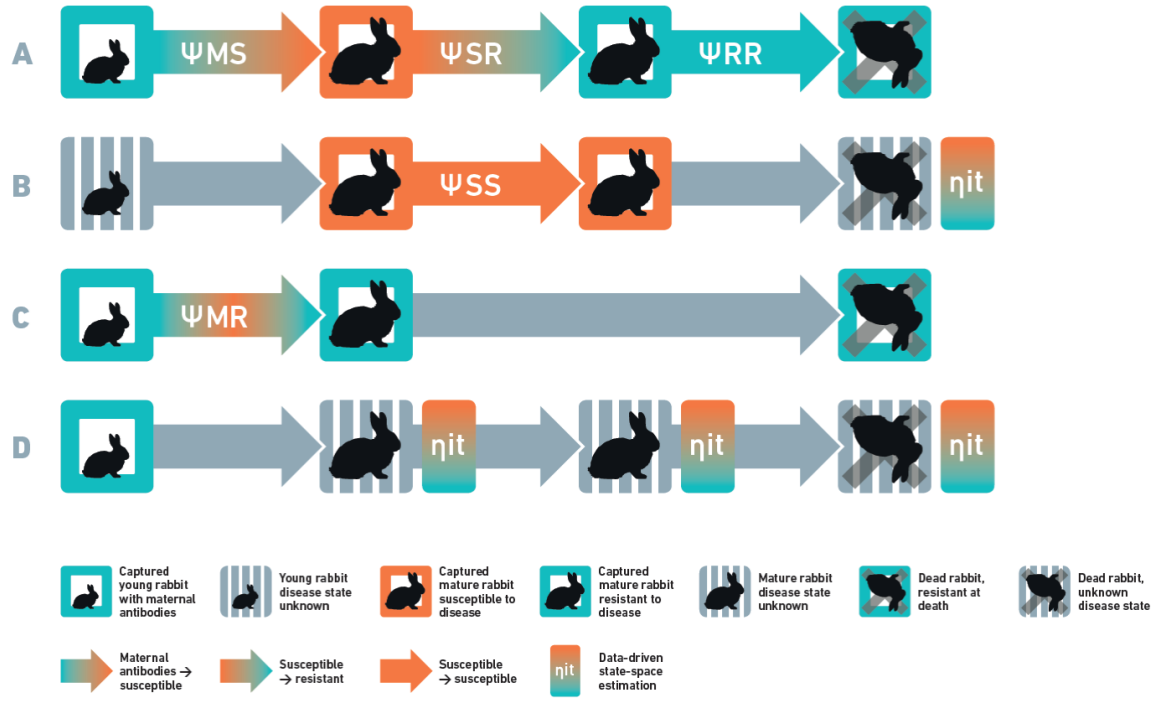

713

714 **Figure 1**. Illustration of the state-space inference pathway for estimating the effects of
715 disease on the survival of rabbits using capture-mark-recapture data (A – D). Disease state
716 and size/age of individuals are known or inferred for each capture event: small/young rabbit
717 with maternal antibodies, large/mature rabbit susceptible to disease, large/mature rabbit
718 resistant to disease after surviving an infection. Unknown classes (grey vertical bars) of
719 rabbits occur when rabbits are not captured: rabbits likely to be alive but disease state
720 unknown, rabbit with unknown disease state likely to have died. Transition probabilities
721 between different disease states arise from sequences of observed disease states (i.e. maternal
722 antibodies → susceptible Ψ_{MS}, susceptible → susceptible Ψ_{SS}, susceptible → resistant Ψ_{SR}),
723 allowing data-driven state-space estimation of unknown disease states for all individuals any
724 time during their lifespans (η_{it}). This allows relative survival rates of rabbits in different
725 disease states and age classes to be estimated.

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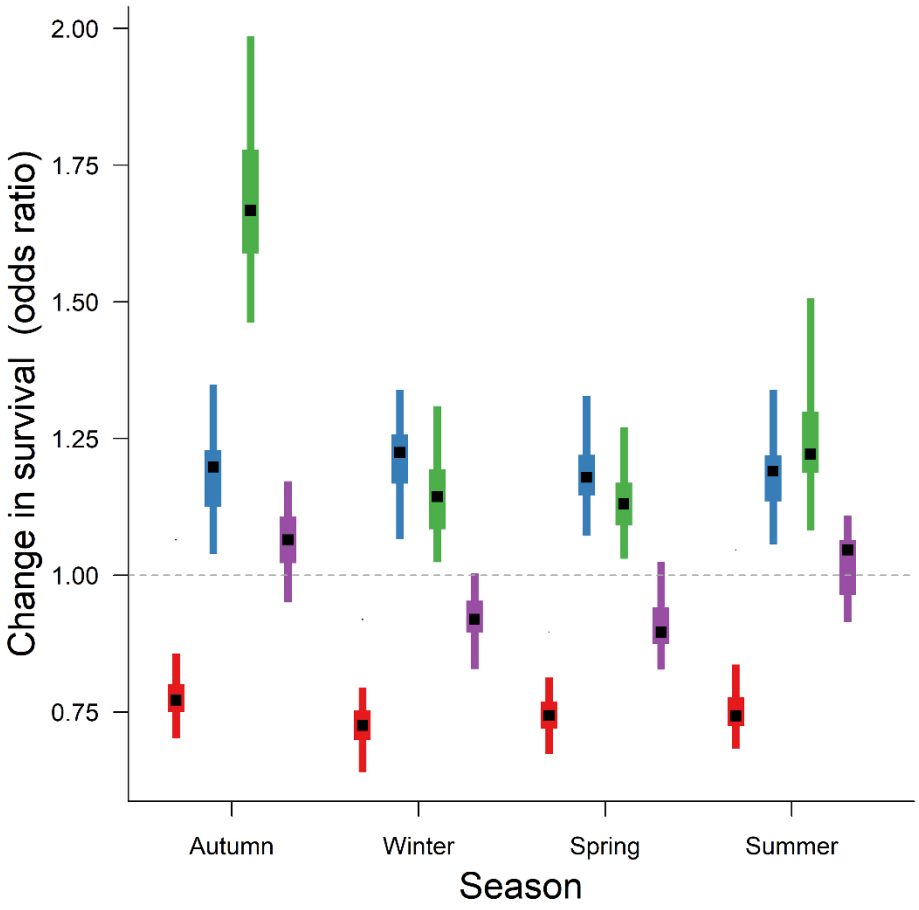

728

729 **Figure 2**. Estimated average changes in monthly survival rates of rabbits in different disease
730 states, namely 1) susceptible to rabbit haemorrhagic disease virus (RHDV) and immune to
731 myxoma virus (MYXV) (red bars), 2) susceptible to MYXV and immune to RHDV (blue
732 bars), 3) susceptible to both RHDV and MYXV (green bars), and 4) young rabbits < 90 days
733 old of various disease states, including individuals with maternal antibodies against RHDV
734 and/or MYXV (purple bars). Values represent odds ratios that compare survival rates to
735 rabbits that survived previous infection of RHDV and MYXV (as indicated by seropositive
736 antibody status for the respective virus for individuals > 90 days old). Black squares are
737 posterior modes; vertical thick and thin bars are 50% and 95% credible intervals. Estimates
738 are based on hyperpriors that 'average' the effects over the entire study period (2000-2016).

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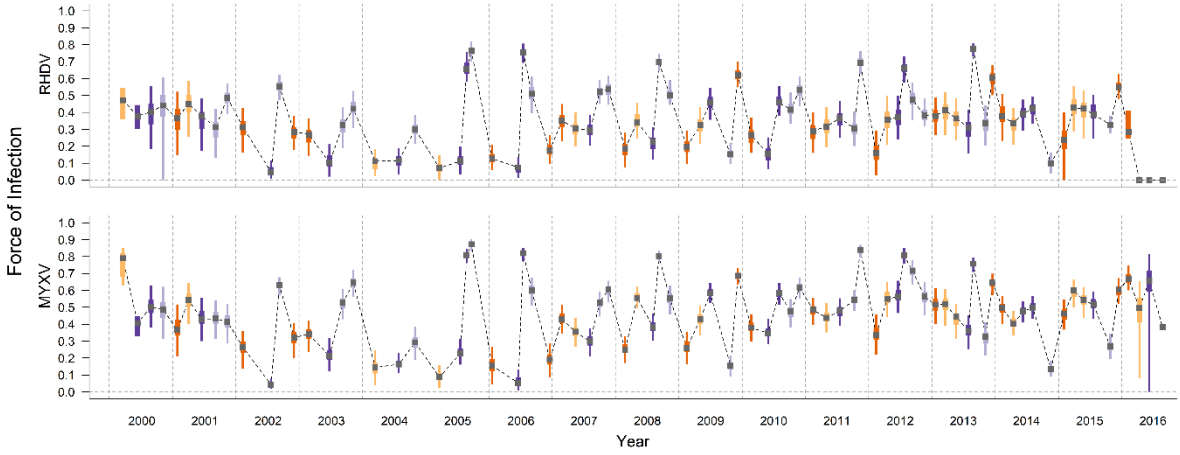

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741 **Figure 3**. Estimated monthly rate at which susceptible rabbits (> 90 days old) become
742 infected (force of infection) with rabbit haemorrhagic disease virus (RHDV) and myxoma
743 virus (MYXV), respectively. Colours represent different seasons (light orange: autumn, dark
744 violet: winter, light violet: spring, dark orange: summer). Black squares are posterior modes;
745 vertical thick and thin bars are 50% and 95% credible intervals. Estimates are plotted on a
746 continuous time scale, vertical broken lines indicate the 1st day of each year.