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

3
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24

25 **Abstract**

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

30 **2.** In this context, we analysed 17 years of detailed capture-mark-recapture data (2000 –
31 2016) from Turretfield, South Australia, using a probabilistic state-space hierarchical
32 modelling framework to estimate rabbit survival and epidemiological dynamics.

33 **3.** While RHDV infection and disease-induced death were most prominent during annual
34 epidemics in winter and spring, we found evidence for continuous infection of susceptible
35 individuals with RHDV throughout the year. RHDV-susceptible rabbits had, on average,
36 25% lower monthly survival rates compared to immune individuals, while the average
37 monthly force of infection in winter and spring was ~ 38%. These combined to result in an
38 average infection-induced mortality rate of 69% in winter and spring.

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

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

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

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

51

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

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74 **Introduction**

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

80 The virulence of a pathogen (infection-induced mortality rates of hosts) and infection
81 rate (the propensity of individuals susceptible to a disease to become infected) can depend on
82 the mode of spread and the dose in which pathogens are transmitted, as well as the
83 resistance/immunity of host individuals, all of which can vary temporally. Therefore, it is
84 crucial to quantify temporal as well as spatial variation in apparent virulence and infection
85 rate if host-pathogen (co) eco-evolutionary processes are to be better understood (Woolhouse
86 *et al.* 2002). For example, if pathogens do not constantly persist in host populations but are
87 repeatedly introduced, variation in the resulting virulence of different pathogen strains can
88 cause temporal changes in the impact of the disease on host populations (Manning *et al.*
89 2008). If the pathogen transmission process involves heterogeneity in the dosage of
90 exposure/inoculum, the epidemiological dynamics can change fundamentally because of
91 dose-dependent variation in mortality rates or variation of within-host replication of the
92 pathogen and subsequent differences of transmission dynamics (Regoes, Ebert & Bonhoeffer
93 2002).

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

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

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

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

129 Both RHDV and MYXV cause lifelong immunity for rabbits that survive infection
130 and, in addition, RHDV maternal antibodies passed to kittens (young, immature rabbits) can
131 prevent fatal disease in these individuals during infection. Therefore, these individuals are not
132 at risk of dying from RHD before they have lost their natural resilience and/or maternal
133 antibody protection against RHDV (McPhee *et al.* 2009; Matthaei *et al.* 2014). Consequently,
134 the timing of seasonally driven birth-pulses in rabbits can affect the pool of susceptible host
135 individuals, leading to temporal variation in disease (Mutze *et al.* 2014; Wells *et al.* 2015).
136 The impact of such demographic fluctuations on disease epidemiology is of particular
137 importance in rabbits, because they exhibit high fecundity along with pronounced changes in
138 population size under changing environmental conditions (Rödel *et al.* 2004; Wells *et al.*
139 2016b).

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

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

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

159

160 **Materials and Methods**

161 2.1 Study area and rabbit monitoring

162 Rabbits have been live-trapped at Turretfield Agricultural Research Centre (34°33'S,
163 138°50'E, South Australia) at 8-12 week intervals, continuously since 1996 (Peacock &
164 Sinclair 2009; Fordham *et al.* 2012; Mutze *et al.* 2014). The study area has a Mediterranean
165 climate with cool, moist winters and hot, dry summers. The rabbit population is relatively
166 closed, with neighbouring populations more than 2 km away.

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

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

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

184

185 2.2 Disease state classification by immunological assays

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

197 individuals can be classified as “immune” (see below for the analytical approach to account
 198 for this uncertainty).

199

200 2.3 Bayesian multistate capture-mark-recapture model

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

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

$$216 \quad \text{logit}(\Phi(i, t)) = \mu_{\Phi} [\text{age}_{\text{cat}}(i, t), \text{year}(t)] + \beta_{\text{sex}}[\text{sex}(i)] + \beta_{\text{DZ}}[\eta_{\text{DZ}}(i, t), t] \quad (\text{eqn 1}).$$

217 Here, μ_{Φ} is the intercept, which we allowed to vary among different age classes and over

218 years. We considered individual age as a categorical variable $\text{age}_{\text{cat}}(i, t)$ with six unequal

219 levels: 1) 1 – 180 days; 2) 181 – 365 days; 3) 1 – 3 years; 4) 3 – 6 years; 4) > 6 years. The

220 coefficient β_{sex} allows for variation in survival probability due to rabbit gender. The

221 coefficient β_{DZ} captures variation in survival of individuals in different disease states based

222 on five different categories of the auxiliary parameter η_{DZ} , which summarizes serostatus for
 223 both RHDV (state variable η_{RHD} as specified below) and MYXV (η_{MYXV}), respectively.
 224 Specifically, the categories for η_{DZ} were 1) all individuals < 90 days old, including $\eta_{RHD} =$
 225 ‘maternal antibodies’ AND/OR $\eta_{MYXV} =$ ‘antibodies against MYXV, 2) $\eta_{RHD} =$ ‘susceptible’
 226 AND $\eta_{MYXV} =$ ‘susceptible’ (individuals ≥ 90 days old), 3) $\eta_{RHD} =$ ‘susceptible’ AND $\eta_{MYXV} =$
 227 ‘immune’ (individuals ≥ 90 days old), 4) $\eta_{RHD} =$ ‘immune’ AND $\eta_{MYXV} =$ ‘susceptible’
 228 (individuals ≥ 90 days old), and 5) $\eta_{RHD} =$ ‘immune’ AND $\eta_{MYXV} =$ ‘immune’ (individuals \geq
 229 90 days old). We used these categories to be able to make inference on the relative survival
 230 and infection rates for only those disease states for which direct comparison can be made,
 231 such as those only susceptible to a single virus and immune to the other versus those immune
 232 to both viruses.

233

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

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

$$248 \quad \eta_{RHD}(i,t) \sim \text{Multinomial}[\Psi^*_{RHD}[\eta_{RHD}(i,t-1), \text{age}(i,t) t] (C)] \quad (\text{eqn 2}).$$

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

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

$$262 \quad \text{logit}[\lambda(t)] = \mu_\lambda(t) \quad (\text{eqn 3}).$$

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

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

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

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

277

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

287

288 2.4 Virulence estimation from capture-mark-recapture data

289 The infection-induced mortality rate γ could not be directly estimated from the given data, as
290 the interplay of virulence (γ) and force of infection rate (FoI) determines changes in
291 population-level survival rates of susceptible versus immune rabbits (Hethcote 2000).

292 However, assuming that susceptible rabbits that do *not* become infected have the same
293 survival rate as immune individuals (i.e. no prolonged disease effect), and if FoI is the
294 proportion of individuals to become infected, infection-induced mortality rate can be
295 approximated as follows:

$$296 \quad \gamma = 1 - (\Phi_S - (1 - FoI)) / FoI \quad (eqn 4)$$

297 with Φ_S being the average survival rate of the pool of all susceptible rabbits and the survival
 298 rate for immune rabbits set to $\Phi_I = 1$. Note that this approach only gives reliable output if Φ_S
 299 $> FoI$, because only then would the proportion of susceptible rabbits not to become infected
 300 have equal survival probabilities as immune rabbits.

301

302 **Results**

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

306 Rabbits fully susceptible to RHDV (and immune to MYXV) had significantly lower
 307 survival rates (estimated at the population level) than immune adults throughout the year,
 308 with monthly survival rates being on average 25% less than for susceptible rabbits (odds ratio
 309 of hyperprior-level estimate 0.75, CI 0.68 – 0.82) (**Fig. 2**). There was no evidence for any
 310 long-term temporal trend in changes in the survival rates of RHDV susceptible versus
 311 immune adults (**Fig. S2**). We did not identify any clear seasonal differences in the relative
 312 survival rates of RHDV susceptible versus immune adults (**Fig. S2**) despite a variable force
 313 of infection (FoI , estimated across all age classes) as detailed below.

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

321 (Fig. 3). Therefore, in most capture sessions, subsets of the pool of susceptible individuals
322 were infected. Individuals susceptible to both RHDV and MYXV had significantly higher
323 survival rates than immune rabbits in all seasons (all odd ratio 1.13 – 1.67 with CIs between
324 1.02 – 1.99) (Fig. 2, Fig. S3). Crucially, we found relatively higher survival rates of
325 individuals susceptible to both viruses compared to those susceptible to RHDV and immune
326 to MYXV throughout the year (Fig. 2), indicating that rabbits immune to MYXV have a
327 lower survival rate than susceptible individuals. Young rabbits, including those with maternal
328 antibodies to either virus, had significantly lower survival rates than immune rabbits in spring
329 (Fig. 2, Fig. S4), indicating that waning protection by antibodies result in infection and
330 potentially, mortality, later in the same year.

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

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

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

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

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

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

362

363 **Discussion**

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

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

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

394

395 4.1 Epidemiological dynamics revealed from the CMR analysis

396 Our results suggest that heterogeneity in key factors such as mode and dose of virus
397 transmission, and/or the infection process, may reduce the efficiency of RHDV as a
398 biocontrol agent at the population level, independent of the virulence of the virus. This is
399 because we found (i) that individuals can potentially become infected after annual epidemics;
400 (ii) that the force of RHDV infection oscillates over the year, leading to variation in the
401 chances individuals become infected; and (iii) the average infection-induced mortality rate
402 (69%) at Turretfield is lower than rates reported when RHD first spread (up to 95%; Mutze,
403 Cooke and Alexander (1998)). Taken together, our results suggest that prolonged exposure of
404 rabbits to RHDV (extending beyond seasonal outbreaks) and factors that cause variation in
405 infection-induced mortality (such as variation in rabbit resistance to infection and virulence
406 of the virus - the latter resulting potentially from variable modes and doses of infection) are
407 among the likely mechanisms explaining the observed lower than expected mortality rate for
408 RHDV at the study site.

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

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

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

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

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

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

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

455

456 4.2 Variable transmission modes and the efficiency of RHDV

457 Our finding that RHDV can persist at low levels across the year is independently supported
458 by relatively short-lived immunoglobulin M (IgM), being detected (at titres ≥ 40) in low
459 numbers of rabbits throughout the year (**Fig. S10**). Since IgM is the first antibody to appear
460 in response to initial exposure to RHDV (Lavazza & Capucci 2008) it confirms a likely
461 annual persistence of RHDV at low levels in the rabbit population at Turretfield. Previously,
462 it was observed that RHDV epidemics were generally initiated by variants of the virus, which
463 were unlikely to have persisted and evolved in the local environment (Schwensow *et al.*
464 2014). However, this pattern has changed in more recent years. Since 2010, single RHDV
465 isolates collected at times following annual epidemics have shown variants most closely
466 related to those from previous years (NS, unpublished results), suggesting that some RHDV
467 variants perpetuate in the local environment.

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

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

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

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

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

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

505

506 4.3 Future research into transmission pathways

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

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

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

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

538

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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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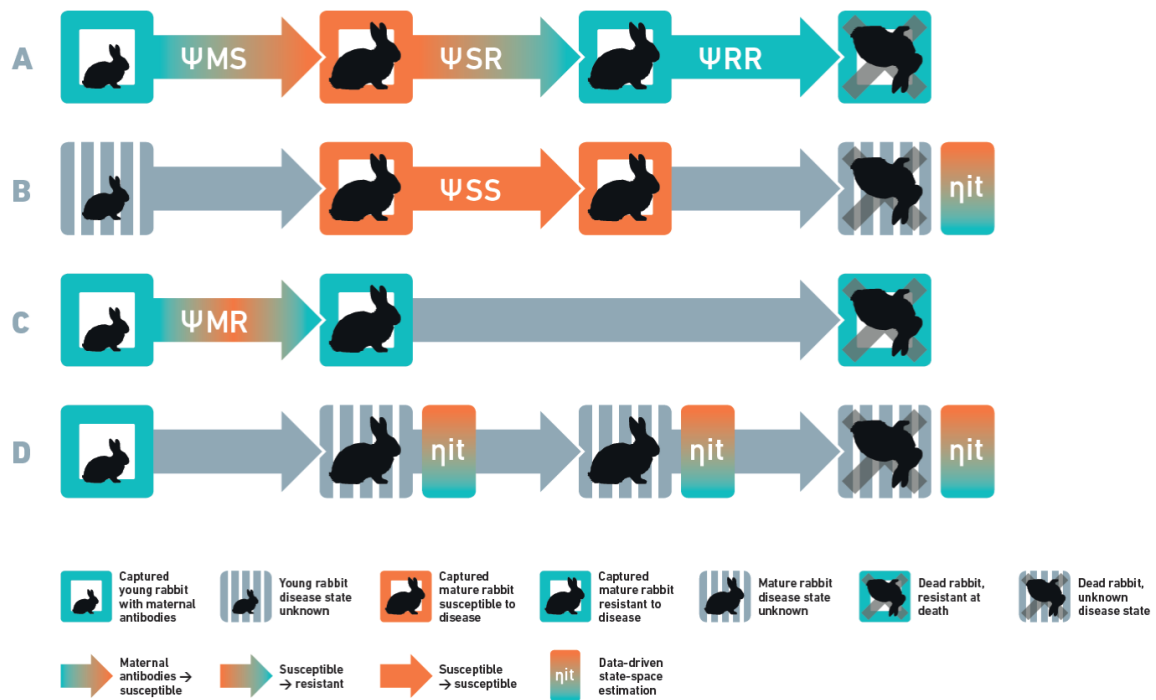
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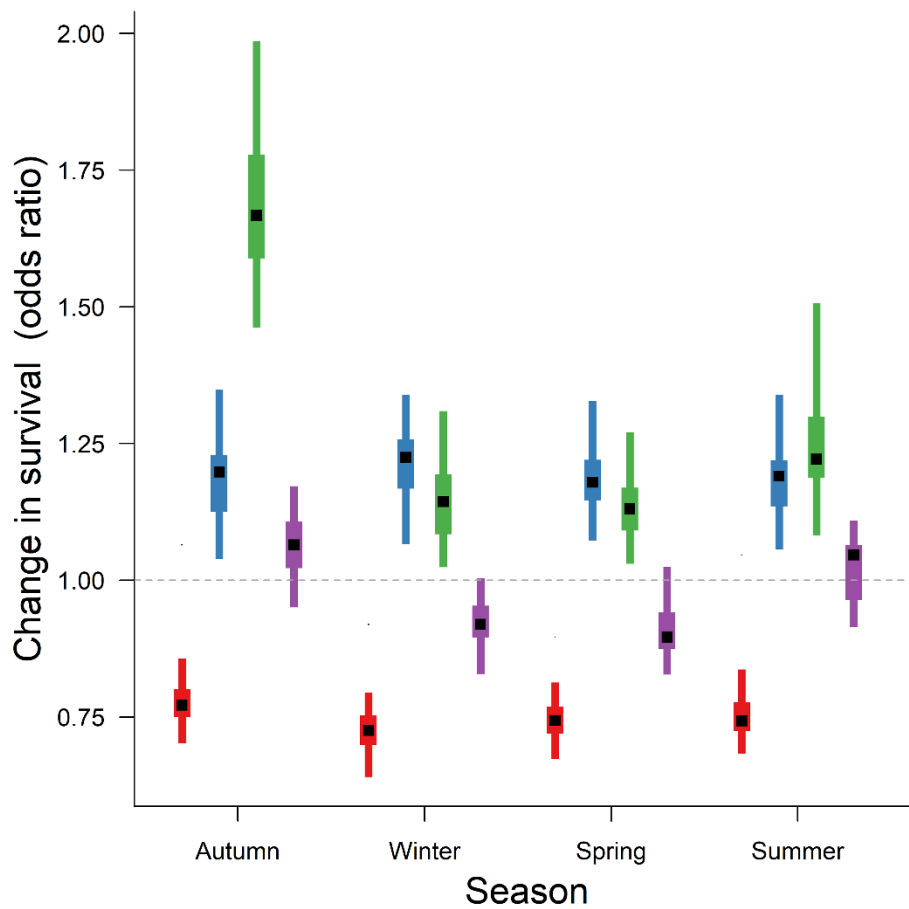


713

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 \rightarrow susceptible Ψ_{MS} , susceptible \rightarrow susceptible Ψ_{SS} , susceptible \rightarrow 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.

726

727



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

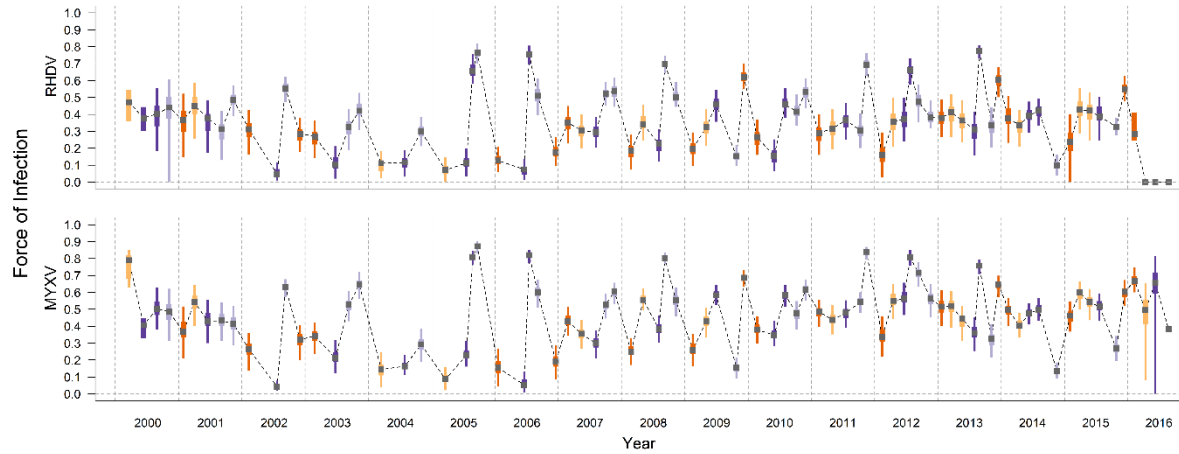
735 rabbits that survived previous infection of RHDV and MYXV (as indicated by seropositive

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).

739



740

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.