Timing and severity of immunizing diseases in rabbits is controlled by seasonal matching of host and pathogen dynamics

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Infectious diseases can exert a strong influence on the dynamics of host populations, but it remains unclear why such disease-mediated control only occurs under particular environmental conditions. We used 16 years of detailed field data on invasive European rabbits (Oryctolagus cuniculus) in Australia, linked to individual-based stochastic models and Bayesian approximations, to test whether (i) mortality associated with rabbit haemorrhagic disease (RHD) is driven primarily by seasonal matches/mismatches between demographic rates and epidemiological dynamics and (ii) delayed infection (arising from insusceptibility and maternal antibodies in juveniles) are important factors in determining disease severity and local population persistence of rabbits. We found that both the timing of reproduction and exposure to viruses drove recurrent seasonal epidemics of RHD. Protection conferred by insusceptibility and maternal antibodies controlled seasonal disease outbreaks by delaying infection; this could have also allowed escape from disease. The persistence of local populations was a stochastic outcome of recovery rates from both RHD and myxomatosis. If susceptibility to RHD is delayed, myxomatosis will have a pronounced effect on population extirpation when the two viruses coexist. This has important implications for wildlife management, because it is likely that such seasonal interplay and disease dynamics has a strong effect on long-term population viability for many species.

1. Introduction

The perpetuation and spread of pathogens in host populations is influenced by a range of epidemiological processes and host-demographic factors. These are not independent, because birth and mortality determine the pool of individuals accessible to infections and infectious disease can significantly reduce host populations or the reproductive behaviour of individuals. Consequently, changes in either demographic or epidemiological traits in space and time can strongly influence coupled host–pathogen dynamics [1–4].

The population density and behaviour of species vary over space and time, often owing to periodic (seasonal) forcing and stochastic environmental conditions [5]. Seasonality can therefore be a prominent feature in infectious disease dynamics [6,7]. Previous studies have established the importance of seasonal variation in disease transmission rates [8] and the importance of seasonality in birth rates on disease outbreaks [9–12]. For immunizing infections (i.e. infections in which host individuals that survive infection become resistant against re-infection), recurrent patterns are primarily driven by the availability of susceptible juveniles [13–16]. Whereby, the time between birth and susceptibility can be...
delayed by natural immunity or maternally acquired antibodies [17–19]. The re-introduction rate of pathogens is assumed to be crucial for perpetuating immunizing diseases [20]. However, the role of the timing of pathogen introduction—when and for how long during an annual (seasonal) cycle—and its phenological matching with birth pulses have been rarely explored for immunizing diseases in wildlife species.

Long-term population growth of hosts affected by immunizing diseases is dependent on individuals reproducing efficiently. However, little is known about whether seasonal reproduction interacts with mechanisms of host–virus interactions such as natural immunity of juveniles or maternal antibodies (immunoactive compounds transferred to offspring) to prevent infection or to attenuate the risk of disease in juveniles [21]. In this context, it is important to distinguish between infection (invasion of host tissue by virus) and disease (pathogenic change in host body condition due to the virus). High concentrations of maternal antibodies or natural immunity of newly born individuals can, for example, delay the infection of juveniles under seasonal scenarios of virus activity if disease outbreaks are of short duration. These delays can increase the chance that juveniles survive to develop into reproducing adults before being exposed to the disease. This strategy is reliant on disease outbreaks of short duration. Alternatively, if juveniles with maternal antibodies become infected with lower risk from the disease (early in life), they may gain permanent resistance and escape disease [22]. Which of these two mechanisms is most prevalent is not clear for many organisms, but both inevitably impact fitness and long-term recruitment and may also impact disease dynamics [23]. We expect both mechanisms (infection delay or disease delay) to cause seasonally recurrent epizootics brought about by a seasonal matching of host reproduction and virus activity (figure 1).

If delay of infection is the prevalent mechanism at work, we expect the recovery rates of adults from disease to have a strong impact on long-term host population persistence, because susceptible individuals growing into adults will likely reproduce if they do not die from disease. If escape from disease is the prevalent mechanism—maternal antibodies do not prevent infection, but slow the course of disease and increase juvenile survival, allowing for permanent resistance—then individuals exposed to the disease should be predominantly juveniles with waning maternal antibodies. We might expect, in this latter situation, juvenile recovery rates would exert a strong influence on population persistence.

European rabbits, *Oryctolagus cuniculus*, are herbivorous and iteroparous mammals with a native distribution in temperate grasslands in the Iberian Peninsula (southwestern Europe), where pronounced seasonal environmental changes influence resource supply. Reproduction in this species is adapted to optimize resource use in variable environments, and such plasticity is likely to have enhanced the invasiveness of rabbits, which are now found in many temperate and Mediterranean ecosystems around the world [24]. Rabbit haemorrhagic disease virus (RHDV, an RNA calicivirus) and myxoma virus (MYXV, a DNA poxvirus) have caused significant reductions in rabbit abundances in their native range [25], and they have been used successfully as biocontrol agents in the rabbits’ exotic range [26]. Epizootics of RHD (the disease caused by RHDV) are highly seasonal in some rabbit populations, and in cooler regions of Australia, RHDV is less effective as a biocontrol agent than in drier regions [26].

Both RHDV and MYXV are associated with various arthropod vectors and induce lifelong immunity in rabbits that survive infection. Survival rates of juveniles infected with RHDV tend to be higher than those of infected adults [18],

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**Figure 1.** Illustration of two possible mechanisms by which recurrent seasonal epizootics for an immunizing disease, with maternally acquired antibodies, can be explained: via the annual matching of the timing of host reproduction (dashed grey lines) and virus introduction periods (shaded areas). Each panel represents 2 years of recurrent dynamics. Grey rabbits represent immune individuals and red ones susceptible individuals. Adults (large individuals) are left permanently immune if they survive infection, whereas juveniles (small individuals) may lose immunity because of the waning efficacy of maternal antibodies. The left panels (a,b) illustrate ‘infection delay’ scenarios: if juveniles do not become infected when they are susceptible and/or protected by maternal antibodies, they cannot develop lifelong immunity. In the absence of prolonged susceptibility and/or maternal antibody protection, only juveniles born relatively late are likely to escape the risk of dying from disease early in life (i.e. the encircled red juvenile); this increases their chance to reproduce prior to being diseased in subsequent annual cycles. The right panels (c,d) illustrate ‘disease escape’ scenarios: if juveniles with maternal antibodies do become infected, juveniles with maternal antibodies born and infected during the annual disease period may develop lifelong immunity without being diseased (i.e. the encircled grey juveniles). Whether these two suggested mechanisms impact long-term population dynamics will depend on seasonal processes, demographic rates such as survival (black lines) or reproduction (green lines) and their interaction with virus dynamics, because infection and disease escape dynamics determine birth and death processes over multiple cycles.
2. Material and methods

2.1. Study system: rabbits in Australia

European rabbits were deliberately introduced to Australia in the mid-to-late nineteenth century for hunting, but subsequently caused major adverse effects on native biodiversity and agriculture [28]. In Australia, rabbits typically reproduce with the onset of rainfall-driven increases in food supply (native and invasive grass and herb species). Rainfall varies enormously across the Australian continent, causing wide-scale spatial heterogeneity in the duration of rabbit reproduction and seasonal peaks in recruitment [29,30]. MYXV and later RHDV were released into Australia as biocontrol agents against rabbits, and both led to large reductions in rabbit populations shortly after their release (in the 1950s and 1990s, respectively). Both viruses spread quickly over large geographical areas, most likely through arthropod vectors [27,31]. While RHDV can be classified at a global scale into distinct genotypes, all genotypes identified in Australia originated from the Czech (genogroup 2) genotype [32], upon which our model is based.

Rabbits and their diseases have been intensively studied at a long-term field site in South Australia; Turretfiel (34°33′S, 138°50′E) [33,34] where they have been live-trapped for at least 4–5 consecutive days at 8–12 week intervals since 1996. All live captured animals were marked and surveyed serologically for RHDV and MYXV antibodies [32]. Additionally, regular searches were made for dead rabbits and samples were collected to determine the cause of death. Almost all cases of RHD at Turretfiel were found between calendar weeks 30 and 50 [34] (figure 2).

2.2. Coupling of individual-based epidemiological and demographic models

We linked a stochastic individual-based demographic simulation to an epidemiological model [35,36] to simulate the interaction of rabbit demography and virus epidemiology. We derived demographic and disease parameters from published values and expert knowledge (full details provided in the electronic supplementary material). In brief, we modelled a single (aspatial) rabbit population with density-dependent reproduction [37] and a carrying capacity of 500 individuals, which is the assumed maximum size at the Turretfiel site. We modelled rabbit population dynamics simultaneously as weekly time steps with the dynamics of both RHDV and MYXV operating on daily time steps. The two viruses (parameters indexed with $V$) were assumed to spread through modelled rabbit populations with transmission probabilities $\beta_V$ sampled across a range of possible (unknown) values. When host individuals encountered each other, they possibly because histo-blood group antigen expression on epithelial cells for virus binding is largely absent in very young rabbits [17]. Therefore, juveniles newly born are resistant to RHD (i.e. they do not die when exposed to the virus) and may or may not support virus replication—this period of insusceptibility lasts for approximately three weeks [21]. Disease survival results in antibodies that are passed by the mothers as maternal antibodies to the embryo, further attenuating infections and disease early in the life of juveniles [17–19]. In contrast to RHD, juveniles infected with MYXV have a lower chance of survival than infected adults [27]. This fundamental difference in age-dependent host–virus interactions for these two virus species could influence seasonality of disease outbreaks. Earlier disease susceptibility of juveniles to myxomatosis, in turn, is likely to diminish delays in infection and disease and mitigate the effect of maternal antibodies against myxomatosis. Therefore, we expect strong indirect interactions between the disease dynamics of the two viruses, because high mortality early in life induced by MYXV can reduce the pool of susceptible juveniles available to RHD during annual birth pulses.

Here, we use long-term field data, stochastic individual-based models and approximate Bayesian computation (ABC) to model the synergistic effect of seasonal demographic and disease processes (two co-circulating viruses with different epidemiological traits) on invasive rabbit population dynamics in Australia. We used sensitivity analysis to establish which conditions determine rabbit population persistence and establish the most important drivers of disease severity under complex seasonal dynamics in Australia. We also test the role of escape from infection or disease as two important yet little understood mechanisms that might explain disease severity under recurrent seasonal matching of reproduction and virus activity.

Figure 2. Patterns of host (introduced European rabbit) demography and epidemics at a long-term monitoring site (Turretfiel, South Australia) 1998–2013. The minimum number of host individuals known to be alive (MNA) were recorded during 79 captures sessions (black points). Multiple iterative regression with calendar week, year and a continuous-time vector modelled as covariates was used to generate a continuous time series of population fluctuations based on MNA (black thin line). This was also used to reveal the seasonal trend in population size using a sinusoidal model (blue line). The number of rabbits that were observed to die from rabbit haemorrhagic disease is shown as red triangles, with almost all cases recorded between calendar weeks 30 to 50. Vertical dashed lines indicate the first calendar week of each year.
caused disease state transitions (susceptible to exposed, leading subsequently from exposed to infected, then infected to resistant or dead). We assumed homogeneous mixing of host individuals within this population, because field observations have shown that rabbits frequently moved between adjacent Warrens. For both viruses, we simulated virus introduction periods (i.e. periods during which individuals were exposed to the viruses from sources other than infected individuals from the same population) during certain time windows (intperiods), for which the annual onset was defined by the first annual week of virus introduction (wkintroV). Infected rabbits were assumed to recover from disease with recovery rates (VRV) of adults (more than 90 days old) sampled across a range of possible values. We modelled recovery rates of juveniles (less than or equal to 90 days old) with logistic logit-link regression models to account for increasing susceptibility of juveniles to clinical RHD [18] and increasing recovery rates for myxomatosis [27]. For this, we assumed the decrease in juvenile recovery rates from RHD to follow a continuous decrease from 100% after the insusceptibility period towards a rate of recovery typical for adults, which we determined by sampling values of the regression slope (aRHD) of a logistic model.

For myxomatosis, we implemented a similar (inverse relationship) model, but sampled the intercept (β0VMyx) of the logistic model, which determines maximum susceptibility of juveniles after the insusceptibility period; details are provided in the electronic supplementary material, appendix A.

We modelled infection and disease resistance explicitly for an initial time period (intMV) owing to maternal antibody protection. After this period of full protection (intMV), we assumed that maternal antibody concentrations would decline linearly (1% per day), making infection of individuals with waning antibodies possible, causing juvenile recovery rates to decrease; note that the effects of αV/μV, tMV and the linear decline in maternal antibodies are additive. Owing to sparse data availability, maternal antibodies and insusceptibility are assumed to have a similar effect on survival of challenged juveniles and the effects are not fully discernible. This model does not, however, account for individual heterogeneity in infection and virus transmission rates owing to maternal antibodies; to keep our model simple (despite a large number of unknown parameters), we varied time periods of full protection (intMV) and assumed that this captured variation in infection and virus transmission rates (i.e. we assume prolonged tMV to also represent less infection probability of juveniles with waning antibodies).

Models were built using META MODEL MANAGER (http://www. vertex10.org), which allows individual age- and sex-structured demographic models (built in VORTEX v. 10.0 population viability analysis software) to be coupled to disease transition models (built in OUTBREAK v. 2.1) [36]. All demographic events and state transition dynamics are probabilistic. The introduced stochasticity represents demographic and environmental variation. Full details of the model’s structure and assumptions are given in the electronic supplementary material.

2.3. Simulation scenarios
We used the model to explore how seasonal population and disease dynamics (and their interactions) can impact emerging patterns of epidemics, disease severity and population viability. To do this, we used Latin hypercube sampling [38] to sample across an exhaustive range of demography/disease scenarios likely to be found under variable natural conditions. A total of 17 key parameters, were sampled (i) three parameters describing the extent and timing of seasonal rabbit reproduction; (ii) 12 parameters (six for each disease) modelling the annual onset, time duration and extent of virus introduction, virus transmission rate and age-structured disease recovery rates (juveniles/adults); and (iii) a parameter approximating the time window of maternal antibody protection from RHDV. Furthermore, we sampled environmental stochasticity in birth and survival rates, because temporal fluctuations in demography driven by environmental variation might influence disease dynamics (see table 1 for a summary with details in the electronic supplementary material). For derived parameters describing the seasonal matching of reproduction and virus activity (two for each disease), we calculated the proportion of the annual reproductive effort (% offspring of the total annual number) prior to any annual virus activity and after the end of the viral time window (table 1). We sampled Nsim = 10 000 different combinations of key parameters in order to cover a large range of potential parameter combinations. This approach allowed us to consider the sampled parameters as sufficiently large random samples (i.e. flat prior distributions) for investigating which simulations best fitted to field data. Each hypercube sample was treated as a different scenario and run for 10 independent iterations to account for stochastic effects [39]. We initiated all simulations with 200 rabbit individuals, of which 10 were infected randomly with RHDV and/or MYXV, respectively. We then simulated demographic and epidemiological dynamics for a total period of 10 years after burn-in periods of 5 years (to minimize the impact of initial values and transitory dynamics). This first 5-year time period of simulations were run, but not considered in the interpretation and analysis of model output. Simulations commenced in calendar week 36 when RHDV outbreaks were most frequently observed in the field at Turretfield. Preliminary inspection of results showed that 5-year initialization periods were sufficient to reach equilibria states.

2.4. Statistical analysis
We examined seasonal patterns in population counts from Turretfield, using capture–mark–recapture data [33]. We used data imputation to generate a continuous time series (weekly steps) of the minimum number of individuals known to be alive (MNA, based on all capture and carcass-recovery records) in different weeks between 1998 and 2013. To do this, we used a multiple iterative regression approach [40] with three covariates (calendar weeks, years and a continuous time vector). The imputed MNA can be expected to provide a good surrogate for population fluctuations over time, although MNA might underestimate true population size if capture probability is small [41]. We fitted to these estimates a sinusoidal model to identify key summary statistics of variation in rabbit population abundance: phase (θMNA = 43; based on calendar week) and amplitude divided by the average population size (σMNA = 0.22). We also calculated the mean (μRHD simulation = 40.52 weeks) and standard deviation (σRHD simulation = 3.26 weeks) of RHD outbreaks across the calendar weeks of field-based RHD fatality records (n = 470). We were not able to derive validation data for the seasonality in myxomatosis outbreaks, because too few carcasses with signs of myxomatosis have been recovered at Turretfield.

Similarly, we calculated these summary statistics for each simulated scenario by fitting sinusoidal models to the simulated population sizes (i.e. output from the demographic/disease model) and estimated phase (θSimulation) and amplitude divided by the average population size (σSimulation). For each simulation, we further calculated μRHD simulation, and σRHD simulation.

To compare summary statistics from field data using the outputs of the simulation model and therefore resolve the most realistic model structures and assumptions from a wide range of possibilities, we used the ABC methods. This approach allows the most likely parameter values to be approximated based on the distance between observed and simulated summary statistics [42–44]. We used the neuronet regression method in the R package abc [45] to weight the parameter values for each simulation scenario according to its match with observed summary statistics. Prediction error was minimized by determining the
Table 1. Parameter definitions and their sampled ranges for the individual-based model of seasonal demographic and epidemiological dynamics in rabbits. These are specified independently for the two studied viruses (indexed by $V$). The components of phenological matching are derived parameters for quantifying the demographic and epidemiological interplay. Full details of the model specification and software implementation are provided in the electronic supplementary material, appendix A.

<table>
<thead>
<tr>
<th>parameter</th>
<th>symbol</th>
<th>range/unit</th>
<th>description</th>
</tr>
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<tbody>
<tr>
<td>rabbit demography</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>annual reproductive effort</td>
<td>RepEff</td>
<td>50–200 (% female reproducing)</td>
<td>total reproduction quantified as proportion of reproducing females (individuals can reproduce multiple times each year)</td>
</tr>
<tr>
<td>annual peak in reproduction</td>
<td>RepPeak</td>
<td>20–50 (calendar week)</td>
<td>seasonal reproduction assumed to peak during optimal conditions, quantified as the calendar week with most reproduction (Gaussian distribution)</td>
</tr>
<tr>
<td>variation in reproduction</td>
<td>RepVar</td>
<td>2–10 (s.d. of assumed normal distribution)</td>
<td>reproductive efforts may be allocated over different time windows owing to seasonal conditions (Gaussian distribution based on RepPeak and RepVar)</td>
</tr>
<tr>
<td>environmental stochasticity</td>
<td>EV</td>
<td>0.01–0.5 (s.d. of vital rates, weekly)</td>
<td>variation in birth and survival rates reflects the stochasticity commonly encountered in natural populations</td>
</tr>
<tr>
<td>disease epidemiology</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>recovery rate</td>
<td>$\gamma_V$</td>
<td>0.2–0.9</td>
<td>recovery rate of infected adult individuals, which are at risk of dying from the disease</td>
</tr>
<tr>
<td>recovery adjustment factors (juveniles)</td>
<td>$\alpha_{\text{juv}}, \beta_{\text{juv}}$, $\mu_{\text{juv}}$</td>
<td>$\alpha_{\text{juv}, \text{RHD}}$: 0.1 to 1, $\alpha_{\text{juv}, \text{Myxo}}$: 0.13, $\mu_{\text{juv}, \text{RHD}}$: 6, $\mu_{\text{juv}, \text{Myxo}}$: 6 to 6</td>
<td>age-dependent changes in recovery rate of juveniles towards $\gamma_V$ (with opposite effects for RHD and myxomatosis); the dynamic process can be described by a logistic model with slope $\alpha_{\text{juv}}$ and intercept $\mu_{\text{juv}}$</td>
</tr>
<tr>
<td>transmission rate</td>
<td>$\beta_V$</td>
<td>0.3–0.9</td>
<td>transmission rate of virus from infection to susceptible individual during encounters</td>
</tr>
<tr>
<td>maternal antibody protection period</td>
<td>$BM_V$</td>
<td>1–50 (days)</td>
<td>progeny from females resistant to disease are protected from infection and disease, before waning maternal antibodies result in increasing disease and infection risks</td>
</tr>
<tr>
<td>virus introduction rate</td>
<td>plInt$V$</td>
<td>0–0.1 (%)</td>
<td>introduction of viruses from external sources such as arthropod vectors, carcasses or any other agents</td>
</tr>
<tr>
<td>first calendar week of virus introduction</td>
<td>wkIntro$V$</td>
<td>1–52 (calendar week)</td>
<td>first calendar week each year in which viruses are introduced/host are exposed to the virus</td>
</tr>
<tr>
<td>virus introduction period</td>
<td>IntPerio$V$</td>
<td>1–52 (weeks)</td>
<td>virus exposure may be limited to certain time windows, e.g. owing to seasonal vector activity</td>
</tr>
<tr>
<td>phenological matching (derived parameters)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>reproduction prior to virus introduction</td>
<td>RepPrec$V$</td>
<td>0–100 (%)</td>
<td>the proportion of annual reproduction prior to the time period of virus introduction</td>
</tr>
<tr>
<td>reproduction after virus introduction</td>
<td>RepLate$V$</td>
<td>0–100 (%)</td>
<td>the proportion of annual reproduction after the time period of virus introduction</td>
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Most accurate tolerance rate through a subsampling cross-validation procedure. Leave-one-out cross validation was used to evaluate the out-of-sample accuracy of parameter estimates (using a subset of 100 randomly selected simulated scenarios), with a prediction error estimated for each parameter [45]. Parameter values were calculated using a tolerance rate of 0.002, resulting in $N_{\text{post}} = 200$ samples for posterior estimates. Based on the importance weight $w(i)$ of each accepted scenario $i$ (with $i = 1, \ldots, N_{\text{post}}$), we then calculated the mean posterior estimates $\theta_i$ of all key parameters such that

$$
\theta_i = \frac{\sum_{i=1}^{N_{\text{post}}} [\theta(i)w(i)]}{\sum_{i=1}^{N_{\text{post}}} w(i)} \quad (2.1)
$$

All parameter estimates from ABC are reported as means and 95% credible intervals (CIs) of weighted posterior values.

Following model fitting and verification against the field data, we explored which simulations most impacted disease severity and led to rabbit extirpation (local extinction of a simulated population owing to demographic or diseases-induced dynamics). For this, we classified ‘disease severity’ as the average number of fatal cases for each disease per year, and we classified ‘local extinction risk’ as a binary variable of whether rabbit populations went extinct or not for each simulated scenario.

We used boosted regression trees using the gbm.step() routine in the R package dismo [46] to estimate the relative importance of key parameters on disease severity (Poisson error structure, learning
rate 0.001, tree complexity of 5, k-fold cross-validation procedure) and extinction risk (binomial error structure). Similar approaches were recently used to identify the importance of disease effects on prey availability for black-footed ferrets [47], and life-history and spatial traits on the extinction risk of vertebrates [48].

3. Results

3.1. Inference from field data

The ABC approximation results showed that only models with seasonal dynamics, which accounted for the matching between rabbit reproduction and virus introduction, were able to reproduce the patterns of abundance fluctuations and RHD epizootics observed in the field (figure 3 and table 2). The annual reproductive peak of rabbits at Turretfield occurred around calendar week 37 (CI: 29–42; RepPeak) and the first week of RHDV introduction usually occurred before this date, around calendar week 30 (CI: 8–42; wkIntroRHD). RHDV introduction periods typically lasted for approximately 23 weeks of the year (CI: 10–45; IntrPeriodRHD), indicating that continuous virus introduction and/or perpetuation throughout a year is unlikely (at least at Turretfield; figure 3). The proportion of the annual reproductive effort after the introduction of RHDV was small, with a mean estimate of only 1% (CI: 0–16; RepLateRHD, table 2), indicating that at least at Turretfield, juveniles are unlikely to escape RHD infection and disease because they are born late; rather, the cause of resistance is likely to occur through insusceptibility and/or acquiring maternal antibodies to the virus. Maternal antibodies against RHD were estimated to provide full protection from both infection and disease for approximately 30 days (CI: 7–48; tM_{RHD}). This suggests that infection delay is likely to be an important mechanism affecting rabbit population dynamics. No individuals were susceptible prior to the first week of RHDV introduction in 47% of the posterior samples. Therefore, disease escape through infection during periods of maternal antibody protection from disease is also likely to play a role in RHD dynamics. The prediction errors were high for MYXV parameters and parameters relating to recovery rates from disease (table 2).

3.2. Drivers of disease severity and rabbit extinction

In our simulations, recovery rate from myxomatosis had the largest influence on fatalities from RHD (27% relative importance weights; $g_{Myxo}$), whereas annual reproductive effort had the largest influence on fatalities from myxomatosis (24% relative importance weights; RepEff; figure 4). Recovery rates from RHD also had a considerable impact on the proportion of fatalities from both viruses, indicating further evidence of potentially strong synergistic effects between the disease dynamics of the coexisting viruses. In addition, juvenile disease susceptibility to myxomatosis ($m_{Juvi, Myxo}$) had an impact on fatalities from both diseases (figure 4), suggesting that susceptibility to myxomatosis reduces the severity of RHD by reducing the number of RHD-susceptible juveniles (figure 4). Recovery rates from both diseases exerted the largest influence on whether rabbit populations went extinct in our simulations (43% and 16% relative importance weight; $g_{Myxo}, g_{RHD}$), i.e. local extinction risk increased with decreasing recovery rates with a pronounced sensitivity to changes in $g_{Myxo}$ (figure 5). Juvenile disease susceptibility to myxomatosis also had a considerable influence on extirpation probability (14% relative importance weight; $m_{Juvi, Myxo}$); earlier disease susceptibility of juveniles to myxomatosis led to an increased likelihood of population extinction (figure 5). In contrast, the transmission rates of both diseases and time period of maternal antibody protection had negligible influences on population persistence (less than 1% relative importance weights; $\beta_{RHD}, \beta_{Myxo}, tM_{RHD}$ and $tM_{Myxo}$), whereas annual reproductive effort had a moderate impact (9% relative importance weights; RepEff). Overall, local extinction risk of rabbit
By accounting for seasonal interplay between demographic and epidemiological dynamics, our simulation study provides strong evidence that the population fluctuations of European rabbits and outbreaks of RHD in Australia are influenced by complex seasonal interactions between host reproduction and virus activity. In particular, seasonality and environmental variability appear to cause shifts in rabbit abundance, altering the intensity of biotic interactions and leading to changes in host–pathogen dynamics over multi-year cycles.

Seasonal outbreaks of RHD observed in rabbit populations in South Australia could be approximated realistically only when the virus introduction period was limited to no more than 10–45 weeks per year and rabbit reproduction took place prior or during the period of virus introduction. Delayed juvenile susceptibility to the disease along with maternal antibodies against RHD are likely to provide important protection for juveniles from infection and this could be the mechanism that leads to recurrent RHD outbreaks. However, we cannot conclude completely the competing hypothesis of escape from disease through early infection during periods of maternal antibody protection as an additional (though probably not principal) mechanism in controlling the timing and severity of recurrent outbreaks. This is because some infection of juveniles with maternal antibodies is likely. Recently, it was suggested that rabbit recruitment was primarily facilitated by increased RHDV-infection of juveniles (with maternal antibodies and age-related insusceptibility) during RHD outbreaks based on the age and time when carcasses were recovered [34]. The apparent contradiction between this conclusion and support for infection delay (opposed to a dominant role of escape from disease) based on our ABC approach warrants further investigation. In particular, more detailed data on the timing of juvenile infection during times of early resistance (as a basis for escape from disease) are needed to better understand which mechanism is most likely at work. For example, if maternal immunity prevents individuals from seroconverting (‘blocking effect’, [5]), early infected individuals become fully susceptible after their maternally acquired immunity has waned, and are likely to die if they are reinfected during the same outbreak, which is feasible if the outbreak lasts several weeks. Under this scenario, at least those individuals borne early are unlikely to escape disease. Among the factors that remained unexplored is the interannual variability in host–virus interactions, including the years of field survey without any observed outbreaks (figure 2). The force of infection may also vary within years, being high during short epizootics, allowing infection and seroconversion, but greatly reduced at other times of the year, allowing susceptible rabbits to accumulate and support recurrent RHD outbreaks. Although it is not clear what could drive a variable force of infection—seasonal effects on virus persistence outside of the host, insect behaviour and the presence of susceptible adult rabbits are all among the possibilities—it is unlikely that infection rates are temporally static.

Our sensitivity analysis revealed that the factors determining disease severity did not necessarily represent the same processes that had the largest effect on population persistence. For example, annual reproductive effort strongly influenced disease severity (typically, most individuals dying from disease are juveniles or subadults), but this did not translate to large effects on long-term population persistence, which was determined predominantly by the recovery rates from the two diseases. We suggest that disease severity and population

### Table 2. Posterior estimates and prediction error for key parameters estimated via approximate Bayesian computation (see Material and methods for details). Cross validation was used to evaluate the out-of-sample accuracy of parameter estimates, with a prediction error estimated for each parameter.

<table>
<thead>
<tr>
<th>parameter</th>
<th>posterior mean</th>
<th>95% credible interval</th>
<th>prediction error</th>
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<tbody>
<tr>
<td><strong>rabbit demography</strong></td>
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</tr>
<tr>
<td>RepEff</td>
<td>0.68</td>
<td>0.58–0.77</td>
<td>0.1</td>
</tr>
<tr>
<td>RepPeak</td>
<td>37</td>
<td>29–42</td>
<td>0.27</td>
</tr>
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<td>2–10</td>
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<tr>
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<td>0.1</td>
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<td><strong>disease epidemiology</strong></td>
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<tr>
<td>γRHD</td>
<td>0.47</td>
<td>0.38–0.56</td>
<td>0.14</td>
</tr>
<tr>
<td>αRHD</td>
<td>0.68</td>
<td>0.58–0.77</td>
<td>0.1</td>
</tr>
<tr>
<td>βRHD</td>
<td>0.68</td>
<td>0.58–0.77</td>
<td>0.1</td>
</tr>
<tr>
<td>tM_RHD</td>
<td>30</td>
<td>25–35</td>
<td>0.89</td>
</tr>
<tr>
<td>pIntrRHD</td>
<td>0.06</td>
<td>0–0.11</td>
<td>0.89</td>
</tr>
<tr>
<td>wkIntrRHD</td>
<td>30</td>
<td>25–35</td>
<td>0.89</td>
</tr>
<tr>
<td>IntrPeriod_RHD</td>
<td>23</td>
<td>15–33</td>
<td>0.9</td>
</tr>
<tr>
<td><strong>phenological matching (derived parameters)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RepPreRHD</td>
<td>33</td>
<td>25–41</td>
<td>0.87</td>
</tr>
<tr>
<td>RepLatRHD</td>
<td>1</td>
<td>0–16</td>
<td>—</td>
</tr>
<tr>
<td>RepPreMyxo</td>
<td>33</td>
<td>25–41</td>
<td>0.87</td>
</tr>
<tr>
<td>RepLatMyxo</td>
<td>1</td>
<td>0–16</td>
<td>—</td>
</tr>
</tbody>
</table>

populations from disease was moderate over the simulation, with 26% of the sampled scenarios leading to extinction within 10 years.

### 4. Discussion

The seasonal interplay of host recruitment and pathogen exposure dynamics has been given little attention in previous rabbit-disease dynamics models [49–51]. This is primarily because of a paucity of long-term and site-specific field data on RHD and myxomatosis epizootics and the absence (until recently) of modelling frameworks that are able to couple multi-disease epidemiological processes dynamically with stochastic–demographic models. However, understanding the timing of interactions between hosts and viruses, and how these interactions can vary in time and space owing to environmental conditions and global change [52] is crucial for both accurate prediction of the timing of epizootics and, subsequently, for understanding their impact on long-term population growth of hosts [4,53].

...
persistence can only be understood when accounting for the interaction between the two diseases. For example, recovery rates from myxomatosis had the largest influence on modelled fatalities from RHD by reducing the pool of juveniles that could become susceptible to RHD during or after an outbreak of myxomatosis. Therefore, the seasonal interplay of host reproduction and delay in infection may have comprised a mechanism important for the coexistence of the two viruses, i.e. earlier susceptibility of juveniles to myxomatosis could have allowed MYXV to efficiently coexist with a lower virulence compared with RHDV [54]. However, we emphasize that a prominent role of myxomatosis under current conditions in Australia is unlikely because of only moderate virulence of common circulating field genotypes of MYXV and an
overriding effect of RHD [30,55]. Importantly, the actual impact of the two diseases under natural conditions will depend on the seasonal timing of disease introduction, as a later introduction of MYXV into a host population will dampen the positive effect on disease severity and population extirpation in relation to delayed susceptibility to RHDV. Some of the impact of myxomatosis on RHD severity, however, is likely underestimated in our modelling, because we did not take immunosuppressive characteristics of MYXV [56] into account.

With a relatively low impact of myxomatosis on rabbit populations, RHDV infection and disease delay may become an important driver of disease severity and population extirpation. Because introduction of RHDV is unlikely to occur year-round (based on data from Turrefield), juvenile insusceptibility and maternal antibody protection early in life, which causes delayed infection, results in relatively more susceptible adults surviving through to subsequent years, and they then become part of the reproductive pool. If adult recovery rates during subsequent epizootics are low, the pool of reproducing individuals declines, causing population reduction.

The simulation outputs predict that populations did not persist in 26% of all sampled scenarios. This result is not consistent with evidence from field observations, which suggest that the introduction of RHDV has not caused local extinctions of Australian rabbit populations. However, the simulation model was purposefully parametrized using a large, but plausible, range of possible parameter values [57]. This included best estimates for disease parameters where empirical data were lacking.

It has been suggested that population growth may be buffered against mismatches in predator–prey interactions owing to density-dependent vital rates [58–60]. Our modelling reveals that complex host–pathogen dynamics are not only influenced by density-dependent vital rates, but are also the result of other processes such as the timing of susceptibility of juveniles to diseases (in this case, myxomatosis, and to a lesser extent RHD). If recovery rates are low and the diseases have the potential to kill a large number of individuals, seasonal mismatches are particularly likely to limit the impact of diseases on population growth. With high recovery rates, in turn, we may expect reproductive effort and disease dynamics to have equally important effects on rabbit extinction. This is because in rabbits, density-dependent population regulation is expected to disproportionately affect juvenile survival [33], and seasonally coinciding density-dependent and disease-induced mortality may counterbalance each other.

Caution should be exercised before using our results to assess the overall feasibility and cost effectiveness of using RHDV or MYXV to manage rabbit numbers in their exotic range. This is because we know that rabbit recruitment varies spatio-temporally in response to climatic variation [24], most probably conditioning host–pathogen dynamics. Although we have provided robust simulation evidence that seasonal interactions can explain RHD epidemics in Australia, future studies should target factors such as the occurrence of possible arthropod vector species, the timing of epidemics and their subsequent relationship to recruitment to further improve our predictions [26]. We therefore emphasize that parameter estimates from our ABC approach should be interpreted cautiously [61]. Furthermore, to reduce possible bias in predictions of RHD epidemics, future simulation studies should also consider variation in host–virus interaction over time and the potential interactions of RHDV with non-pathogenic Australian rabbit caliciviruses. These benign caliciviruses confer partial cross-protection to RHD [62] and reduce the pool of susceptible individuals for RHDV, affecting disease dynamics particularly in wetter regions in Australia [63]. Future studies should also explore whether the strong seasonal dynamics revealed in our study hold true in geographical areas outside of Australia with different RHDV genotypes [21], or any future scenario of new genotype appearance and rapid coevolutionary changes in host–virus interactions. Lastly, a more rigorous understanding of host–virus coevolutionary dynamics and resistance over time would provide crucial information for model improvement. In our model, we assume constant host–virus interaction over time, whereas viruses such as RHDV are likely coevolving in response to adaptation and variation in host defence mechanisms [64]. While approaches that incorporate such dynamics into population-level demographic and epidemiological models could improve understanding of coevolutionary dynamics, they require detailed information for translating the temporal scaling of molecular mechanisms of host defence and virus invasion strategies into epidemiological and demographic parameters [65,66]. Currently, we lack a sufficiently detailed understanding to translate relevant mechanisms of transmission and coevolutionary dynamics for both myxomatosis and RHD into a model. Further work is needed to better understand the potential importance of plasticity in rabbit reproduction and the coevolutionary dynamics of virulence and immune responses on host defence against infection [65].

5. Conclusion

Our models indicate that for immunizing diseases, the timing of individual infection can have fundamental consequences for disease severity and the persistence of the host population. In particular, recovery rates from myxomatosis were found to have a major influence on the severity of RHD owing to prolonged latency in disease exposure to RHD, arising from delayed juvenile susceptibility and persisting post-birth maternal antibodies. More generally, by revealing the importance of seasonal matching between rabbit recruitment and timing of disease introduction, we provide important insights into how environmental variation can influence disease-affected population dynamics. We expect these observations to be generalizable across a wide range of infectious disease systems. Advancing epidemiological models to account for the possible matching of host and pathogen dynamics under seasonal conditions might be particularly useful to anticipate possible changes in disease severity in wildlife under global change.

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