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RESEARCH ARTICLE

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Previous exposure to myxoma virus reduces survival of European rabbits during outbreaks of rabbit haemorrhagic disease

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Abstract

1. Exploiting synergies among diseases or parasites could increase the efficacy of biological control of invasive species. In Australia, two viruses were introduced to control European rabbits *Oryctolagus cuniculus*: myxoma virus in 1950 and rabbit haemorrhagic disease virus in 1995. While these biological controls caused initial declines of >95% in affected populations, and despite recurring outbreaks of both diseases, rabbits remain a problem in many areas.
2. We used 18 years of capture–mark–recapture, dead recovery, and antibody assay data from a sentinel population in South Australia to test whether these two diseases interact to modify the survival of individual wild rabbits. We compared four joint, multistate, dead-recovery models to test the hypotheses that rabbit haemorrhagic disease and myxoma viruses have synergistic (i.e., previous exposure to one virus affects survival during outbreaks of the other virus) or additive effects (i.e., previous exposure to one virus does not affect survival during outbreaks of the other virus).
3. Rabbit haemorrhagic disease outbreaks reduced the survival of individuals with no immunity by more than half during the 58-day capture-trip intervals, i.e., from 0.86–0.90 to 0.37–0.48. Myxomatosis outbreaks had a smaller effect, reducing survival to 0.74–0.82; however, myxomatosis outbreaks were more prolonged, spanning more than twice as many trips.
4. There was considerable information-theoretic support ($wAIC_c = 0.69$) for the model in which exposure to myxomatosis affected survival during rabbit haemorrhagic disease outbreaks. Rabbits previously exposed to myxoma virus had lower survival during rabbit haemorrhagic disease outbreaks than rabbits never exposed to either virus. There was negligible support for the model in which previous exposure to rabbit haemorrhagic disease affected survival in myxomatosis outbreaks ($wAIC_c < 0.01$).
5. *Synthesis and applications*. Our results indicate that biological control agents can have a greater impact than single-pathogen challenge studies might suggest. Introducing additional biological control agents might therefore increase the mortality of rabbits beyond the additive effects of individual biological controls.

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Furthermore, our results show that by understanding and exploiting disease synergies, managers could increase the efficacy of biological controls for other invasive animals.

KEYWORDS

biological control, disease synergies, host–pathogen interactions, invasive species, multistate capture–mark–recapture, myxoma virus, *Oryctolagus cuniculus*, RHDV

1 | INTRODUCTION

Disease synergies occur when simultaneous or sequential infection with two or more pathogens has a greater impact on the host than the additive effect of independent infections (Jolles, Ezenwa, Etienne, Turner, & Olff, 2008; Telfer et al., 2010; Thumbi et al., 2013). Disease synergies can affect host susceptibility, duration of infection, severity of symptoms, and risk of pathogen transmission (Lass et al., 2013; Thumbi et al., 2013; Vaumourin, Vourc'h, Gasqui, & Vayssier-Taussat, 2015). For example, in African buffalo *Syncerus caffer*, a gastrointestinal nematode causes immune suppression, facilitating infection with *Mycobacterium bovis*, the causative agent of bovine tuberculosis (Ezenwa, Etienne, Luikart, Beja-Pereira, & Jolles, 2010; Jolles et al., 2008). Similarly, experimental co-infection of laboratory mice with gastrointestinal helminths *Heligmosomoides polygyrus* and respiratory bacteria *Bordetella bronchiseptica* resulted in higher bacterial loads, increased shedding of helminth eggs, and higher mortality compared to individuals with single infections (Lass et al., 2013). In humans, infection with herpes simplex virus (HSV-1 or HSV-2) is associated with increased susceptibility to human immunodeficiency virus and a greater probability of transmission (DaPalma, Doonan, Trager, & Kasman, 2010).

In addition to their obvious importance for human health and animal conservation, disease synergies could potentially influence the efficacy of biological controls for invasive species. Pathogens such as macroparasites and viruses are often used for biological control of invasive animals and plants (McColl, Cooke, & Sunarto, 2014; van Frankenhuyzen, Lucarotti, & Lavallee, 2015), and their impacts on the invasive host can be mediated by co-infections with other pathogens (Boag, Hernandez, & Cattadori, 2013; Cattadori, Albert, & Boag, 2007). Synergies can occur between a biological control agent and (a) pathogens introduced with the invasive species, (b) pathogens that occur naturally in the invasive range, or (c) another biological control agent introduced to reduce the abundance of the same invasive species (Boag et al., 2013; Lello, Boag, & Hudson, 2005). Thus, quantifying how disease synergies operate is an important element of eco-epidemiological research aiming to improve the efficacy of biological control.

European rabbits *Oryctolagus cuniculus* are one of the most damaging alien vertebrate species in Australia's native ecosystems and agricultural areas. Since the first major releases on mainland Australia in *c*. 1859 (Peacock & Abbott, 2013), rabbits have caused extensive environmental and economic damage through grazing on native plants, competing with native herbivores, and degrading agricultural land (Cooke, 2012; Zenger, Richardson, & Vachot-Griffin, 2003). Methods to control rabbit populations in Australia included the release of myxoma virus in 1950 and the introduction of rabbit haemorrhagic disease virus (RHDV) in 1995 (Cooke & Fenner, 2002; Ratcliffe, Myers, Fennessy, & Calaby, 1952). Initially, myxomatosis (the disease caused by the myxoma virus) caused declines of 90%–99% in affected rabbit populations (Fenner, Marshall, & Woodroofe, 1953; Ratcliffe et al., 1952). Within the next 2 years, however, rapid host–virus co-evolution led to the emergence of less-virulent myxoma strains and rabbit resistance to the virus (Kerr, 2012; Marshall & Fenner, 1960). Data quantifying the extent of subsequent population recovery are lacking. However, immediately before rabbit haemorrhagic disease first reached wild rabbits in 1995, populations in some areas had reached counts of 76 individuals per spotlight kilometre (Mutze, Cooke, & Alexander, 1998), or approximately nine rabbits per hectare (Mutze, Cooke, et al., 2014). Rabbit haemorrhagic disease initially caused population declines of up to 95% (Mutze et al., 1998), but less than a decade later, many populations had recovered and stabilised at approximately half the size they were prior to RHDV release (Mutze, Bird, et al., 2014). Today, rabbits persist in most temperate and semi-arid parts of Australia, despite annual or biannual outbreaks of myxomatosis and rabbit haemorrhagic disease (Mutze, Bird, Cooke, & Henzell, 2008).

While the dynamics of myxomatosis and rabbit haemorrhagic disease in Australia have each been investigated extensively, their impacts on the survival of individual wild rabbits with different exposure histories to both diseases remain unknown. Quantifying how myxomatosis and rabbit haemorrhagic disease affect individual survival might be used to guide more effective virus-release programmes to reduce rabbit abundance and minimise their associated environmental and economic damage. For example, if previous exposure to one virus affects mortality during outbreaks of the other virus (i.e., myxomatosis and rabbit haemorrhagic disease have a synergistic effect on rabbit mortality), would it be possible to increase the efficacy of control actions by manipulating the timing of outbreaks so that rabbit haemorrhagic disease outbreaks occur immediately following myxomatosis outbreaks, or vice versa? Furthermore, estimating how individual survival is affected by current biological controls will allow managers to predict more accurately the potential impact of future biocontrols on Australia's rabbit populations.

We tested two competing hypotheses that could explain how myxoma and RHDV affect rabbit mortality: (**1**) Rabbit haemorrhagic

disease and myxoma viruses have a synergistic effect on rabbit mortality. This could manifest in three different ways: (*a*) Both the effect of myxomatosis outbreaks on survival is greater for rabbits that have been exposed to RHDV than those never exposed to either virus, and the effect of rabbit haemorrhagic disease outbreaks on survival is greater for rabbits previously exposed to myxoma virus than those never exposed to either virus. (*b*) The effect of myxomatosis outbreaks on survival is greater for rabbits that have been exposed to rabbit haemorrhagic disease than rabbits never exposed to either virus. (*c*) The effect of rabbit haemorrhagic disease outbreaks on survival is greater for rabbits that have been exposed to myxoma virus than those never exposed to either virus. The null hypothesis is that (2) the effects of rabbit haemorrhagic disease and myxoma virus on mortality are strictly additive. Here, exposure to one virus does not affect survival during a subsequent outbreak of the other virus.

2 | MATERIALS AND METHODS

2.1 | Data collection

We modelled individual survival of rabbits using 18 years (1998–2015) of capture–mark–recapture, carcass recovery, and antibody assay data collected from a long-term monitoring site at Turretfield, South Australia. Data were collected by the Department of Primary Industries and Regions (South Australia) and included 107 trapping sessions and 4,236 caught rabbits. Trapping sessions were done once every 58 days on average. Upon each capture, rabbits were weighed, tagged if new, and a blood sample was collected (Peacock & Sinclair, 2009) to test for antibodies to rabbit haemorrhagic disease (Capucci, Nardin, & Lavazza, 1997; Kerr, 1997) and myxoma viruses (Cooke, Robinson, Merchant, Nardin, & Capucci, 2000; Kerr, 1997). The immunity state of rabbits was classified as: no immunity (N), immune to myxoma virus only (M), immune to RHDV only (R), or immune to both viruses (B). We considered "immune" rabbits fully protected against further infection.

During periods when disease activity is most common at this site (Mutze, Sinclair, et al., 2014), the area was frequently searched for rabbit carcasses and rabbits with signs of clinical myxomatosis, i.e., partial or complete blindness, swollen face and/or genitalia, and conjunctival discharge (Fenner & Woodroofe, 1953). If carcasses that showed no signs of clinical myxomatosis were found, a subset of carcasses was tested to confirm the presence of RHDV. If rabbit haemorrhagic disease carcasses were recovered during a trapping session, trapping was immediately cut short to prevent interfering with the natural spread of the virus. Trapping sessions that were <30 days before or included a period during which four or more rabbit haemorrhagic disease carcasses were retrieved were classified as "outbreaks" of rabbit haemorrhagic disease. We based this definition of rabbit haemorrhagic disease outbreaks on predicted dates of death (based on carcass decomposition factors), as well as data that show RHDV is present in flies up to one month before carcasses were found (Amy Iannella, unpubl. data, University of Adelaide, 2014). We classified myxomatosis "outbreaks" as trapping sessions where at least one rabbit was found with signs of clinical myxomatosis, or more than two rabbits per trapping day had developed immunity to myxoma virus since the previous trapping session.

2.2 | Capture–mark–recapture models

We constructed detailed individual capture histories with the data described above, and constructed mark–recapture models in the R programming language (R Core Team, 2017), using the "RMark" interface to run program MARK (Laake & Rexstad, 2016; Cooch & White, 2016). All code and data are available on the Dryad Digital Repository (Barnett et al., 2018).

We set initial stages based on weight at first capture: *kittens* were individuals weighing <600 g, and *subadults/adults* were those weighing >600 g. We defined and applied two stages because rabbits >600 g are unlikely to have residual maternal immunity to rabbit haemorrhagic disease (Robinson, So, Müller, Cooke, & Capucci, 2002). Although previous studies used three age classes — kittens (<600 g), subadults (600–1,200 g), and adults (>1,200 g) (Mutze, Sinclair, et al., 2014)—our preliminary analyses indicated that multistate models incorporating three age classes were not identifiable due to the large number of parameters (transition probabilities and state-specific survival rates) required for an additional subadult class. The stages and mass of rabbits at tagging (i.e., first capture) are shown in Supporting Information Figure S1. Analysing kittens and subadults/adults in the same model allowed us to track individual survival as rabbits aged and acquired immunity. Rabbits transitioned from the kitten to the subadult/adult stage over the 58-day inter-trip interval based on the established growth rate of *c*. 10 g/day (Peacock & Sinclair, 2009). To estimate recapture probability (ρ), we created a "trapping effort" variable by multiplying the number of trapping days in a trip and the number of traps set. We scaled and mean-centred this value prior to analysis. In all models, we set immigration and emigration to zero, because the Turretfield population is isolated and untagged immigrant adult rabbits are rarely recorded (Amy Iannella, unpubl. data, University of Adelaide, 2014).

To estimate rabbit abundance (*N*) on each capture occasion, we ran a POPAN ("POPulation ANalyis") model (Schwarz & Arnason, 1996) on the live capture data, using time and capture probability (ρ) as predictors. Next, we used a joint multistate, dead-recovery model (White, Kendall, & Barker, 2006) to analyse individual survival (*S*) and the probability of transitioning between immunity states ψ. We used stage (i.e., "kitten" or "subadult/adult") and known outbreaks of myxomatosis and rabbit haemorrhagic disease to estimate survival (*S*) for different immunity states, as well as the probability of transitioning between immunity states. We set up the immunity-state transition matrix so that rabbits could not lose immunity to rabbit haemorrhagic disease or myxomatosis; for example, once a rabbit was classified as being immune to myxoma virus (M), it could either stay in the same state or move into the "immune to both" (B) category. We estimated the probability of remaining in the same stratum until the next capture occasion by subtraction (i.e., 1 − the sum of transition probabilities from that stratum) (White, et al., 2006).

To test our hypotheses, we compared four different multistate models:

(1) Rabbit haemorrhagic disease and myxomatosis have a synergistic effect on mortality:

a. *During both rabbit haemorrhagic disease outbreaks and myxomatosis outbreaks.* In this model, the effect of RHDV outbreaks on survival can differ between rabbits that have been exposed to myxoma virus (M) and those exposed to neither virus (N), and the effect of myxoma virus (MV) outbreaks on survival can differ between rabbits that have been exposed to rabbit haemorrhagic disease (R) and those never exposed to either virus (N):

$$S_M \neq S_N|\text{RHDV}; S_R \neq S_N|\text{MV}$$

b. *During myxomatosis outbreaks only.* In this model, the effect of myxomatosis outbreaks (MV) on survival can differ between rabbits that have been exposed to rabbit haemorrhagic disease (R) and those never exposed to either virus (N). We fixed the effect of rabbit haemorrhagic disease outbreaks (RHDV) on survival to be the same for rabbits with neither antibodies (N) and those exposed to myxoma virus (M):

$$S_R \neq S_N|\text{MV}; S_M = S_N|\text{RHDV}$$

c. *During rabbit haemorrhagic disease outbreaks only.* In this model, the effect of rabbit haemorrhagic disease outbreaks (RHDV) on survival can differ between rabbits that have been exposed to myxoma (M) and those never exposed to either virus (N), but we fixed the effect of myxomatosis outbreaks (MV) on survival as the same for rabbits that have been exposed to rabbit haemorrhagic disease (R) and those exposed to neither virus (N), i.e.,

$$S_M \neq S_N|\text{RHDV}; S_R = S_N|\text{MV}$$

(2) We also tested the model where rabbit haemorrhagic disease and myxomatosis have a purely additive effect on mortality. In this model, we set the effect of rabbit haemorrhagic disease outbreaks (RHDV) on survival to be the same for rabbits never exposed to either virus (N) and those previously exposed to myxoma (M), and set the effect of myxomatosis outbreaks (MV) on survival to be the same for rabbits exposed to neither virus (N) and those previously exposed to rabbit haemorrhagic disease (R):

$$S_M = S_N|\text{RHDV}; S_R = S_N|\text{MV}$$

There is currently no goodness-of-fit test available for joint multistate, dead-recovery data, so we ran a goodness-of-fit test for the live data only using the program U-CARE (Choquet, Lebreton, Gimenez, Reboulet, & Pradel, 2009).

3 | RESULTS

Of 107 trapping sessions, we classified 28 (26%) of them as myxomatosis "outbreaks," of which four outbreaks spanned two successive trapping sessions, three outbreaks spanned three successive trapping sessions, and one spanned four trapping sessions (Figure 1). Rabbit haemorrhagic disease outbreaks occurred in 13 trapping sessions (12%). Our estimates of abundance using live-recapture data were similar to the number of rabbits known to be alive at Turretfield on each capture occasion (Figure 1), confirming the closed nature of the population and the high proportion of marked individuals relative to total abundance.

3.1 | Multistate, dead-recovery model

The most parsimonious multistate model (Model **1**c, Table 1) supported the hypothesis that previous exposure to myxoma virus reduces survival in rabbit haemorrhagic disease outbreaks. This model ($S_M \neq S_N|\text{RHDV}; S_R = S_N|\text{MV}$) had substantially higher information-theoretic model support, with a sample size-corrected Akaike's information criterion weight ($wAIC_c$) of 0.69 that was larger than the additive model or those that allowed previous exposure to RHDV to affect survival in myxomatosis outbreaks (Table 1).

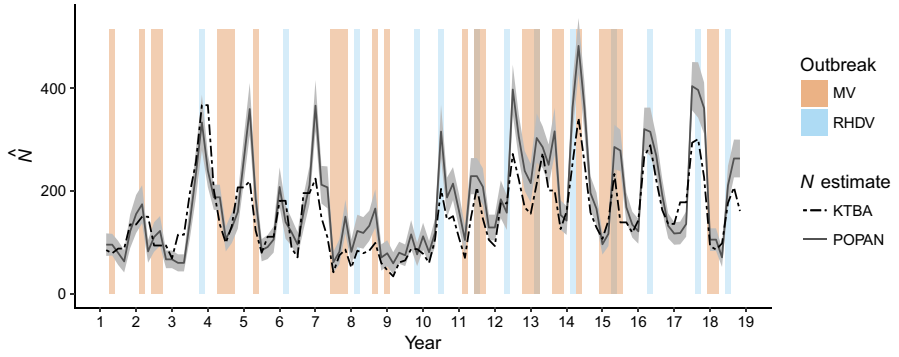

FIGURE 1 Abundance of rabbits (*N*) at Turretfield estimated using a POPAN model—solid line (± 95% confidence intervals), and the total number of rabbits known to be alive (KTBA) at each trapping occasion—dashed line. Vertical bars show timing of known outbreaks of myxoma virus (MV) and rabbit haemorrhagic disease virus (RHDV) [Colour figure can be viewed at wileyonlinelibrary.com]

TABLE 1 Comparison of joint multistate, dead-recovery models used to test whether rabbit haemorrhagic disease virus (RHDV) and myxoma virus (MV) have a synergistic effect on rabbit survival. Immunity states are denoted by the letters, N = no immunity, M = immune to myxoma, R = immune to rabbit haemorrhagic disease, and B = immune to both viruses. The model that allowed the effect of rabbit haemorrhagic disease virus outbreaks (RHDV) on survival (*S*) to vary between individuals with no immunity (N) and individuals with immunity to myxoma virus (M), was the most highly ranked according to the information-theoretic Akaike's information criterion (sample-sized corrected; AIC_c). Shown are the number of model parameters (*k*), change in AIC_c between each model and the top-ranked model (ΔAIC_c), and the model weight (~probability; $wAIC_c$)

Hypotheses		Model	*k*	AIC_c	ΔAIC_c	$wAIC_c$
1*c*	*One-way synergistic*—exposure to myxoma virus affects survival in RHDV outbreaks	$S_M \neq S_N \mid RHDV$ $S_R = S_N \mid MV$	35	38,622.8	0	0.69
1*a*	*Two-way synergistic*	$S_M \neq S_N \mid RHDV$ $S_R \neq S_N \mid MV$	36	38,624.5	1.67	0.30
2	*Additive* effect at all times	$S_M = S_N \mid RHDV$ $S_R = S_N \mid MV$	34	38,630.9	8.03	0.01
1*b*	*One-way synergistic*—previous exposure to RHD affects survival in MV outbreaks	$S_R \neq S_N \mid MV$ $S_M = S_N \mid RHDV$	35	38,632.7	9.90	<0.01

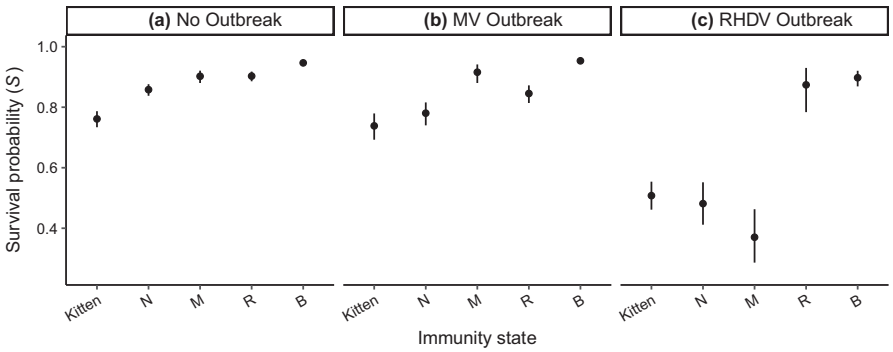

FIGURE 2 Estimated survival probability (*S*) from the AIC_c top-ranked model, in which previous exposure to myxomatosis affected survival of rabbits during outbreaks of rabbit haemorrhagic disease virus. Estimated survival is shown for kittens (across all immunity states) and subadults/adults in different immunity states (N = no immunity, M = immune to myxoma virus only, R = immune to rabbit haemorrhagic disease virus only, B = immune to both viruses) for (a) no outbreak; (b) a myxoma virus (MV) outbreak; and (c) a rabbit haemorrhagic disease virus (RHDV) outbreak. Survival is the probability of surviving from one trapping session to the next (with a mean trip interval of 58 days)

Overall, myxomatosis outbreaks had a relatively small effect, reducing survival over the 58-day intervals, and individuals with no immunity (N) had a survival rate of 0.78 during myxomatosis outbreaks. This was only 7.8% less than the estimated survival of individuals with no immunity (N) during times when there were no outbreaks (Figure 2; Supporting Information Table S1). However, myxomatosis outbreaks often spanned successive trips, and survival rates were likely to be lower over the duration of the outbreak; for example, if a myxomatosis outbreak lasted for two successive trips, the cumulative survival rate for individuals with no immunity (N) was 0.61, and if the outbreak persisted for three trips, survival dropped to 0.47.

During rabbit haemorrhagic disease outbreaks, rabbits with no immunity (N) had a survival rate of only 0.48, which made them 37.7% less likely on average to survive than when there was no outbreak (Supporting Information Table S1). Furthermore, individuals with no immunity (N) were on average 39.3% less likely to survive rabbit haemorrhagic disease outbreaks than individuals with immunity to RHDV (R), and the average survival of rabbits with immunity to myxoma virus (M) was 50% lower than that of rabbits with immunity to rabbit haemorrhagic disease (R) (Figure 2, Supporting Information Table S1). Therefore, on average, individuals previously exposed to myxoma virus had a 10% lower survival during rabbit haemorrhagic disease outbreaks than individuals that had never been exposed to either virus. Although confidence intervals in Figure 2 overlap for rabbits exposed to myxoma virus and those never exposed to either virus during rabbit haemorrhagic disease outbreaks, this figure collapses all temporal variation during rabbit haemorrhagic disease outbreaks into a single, time-invariant, average survival probability. Thus, the figure does not express the model's complexity; however, the one-way synergistic model ($S_M \neq S_N \mid RHDV$) has 69 times more information-theoretic support (evidence ratio = 0.69/0.01 = 69) than the additive model ($S_M = S_N \mid RHDV$) based on Akaike's information criterion weights (Supporting Information Table S1).

Kitten survival was lower than that of adults in all immunity states during times when there were no outbreaks, but it was similar to the survival of adults with no antibodies to either disease

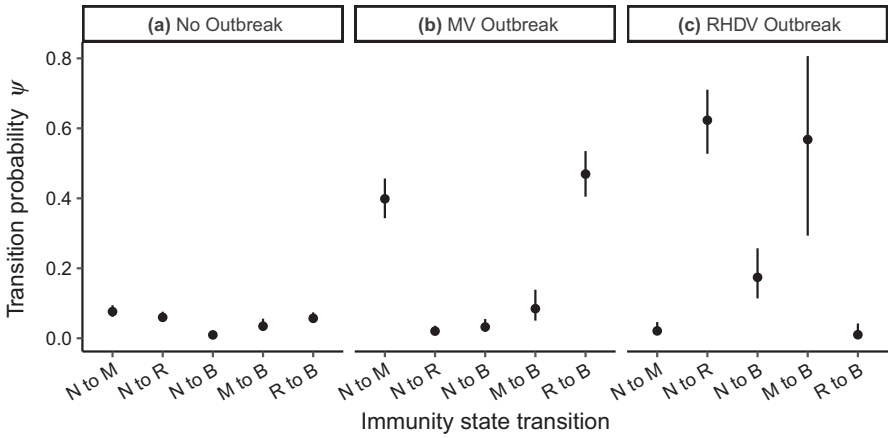

FIGURE 3 Probabilities of transitioning between immunity states ψ conditional on survival, for (a) no outbreak, (b) myxoma virus (MV) outbreak, and (c) rabbit haemorrhagic disease virus (RHDV) outbreak. N = no immunity; M = immune to myxoma virus only; R = immune to rabbit haemorrhagic disease virus only; B = immune to both viruses

during outbreaks of rabbit haemorrhagic disease and myxomatosis (Figure 2).

Estimates of immunity-state transition probabilities (conditional on survival) revealed a low probability of developing immunity to rabbit haemorrhagic disease or myxoma during times when there was no outbreak (Figure 3). Conversely, rabbits surviving myxomatosis or rabbit haemorrhagic disease outbreaks had a high probability of developing immunity to the virus responsible for the outbreak (Figure 3). Goodness-of-fit tests revealed no over-dispersion of our multistate live recapture data, with the estimated over-dispersion parameter $\hat{c} = 0.72$ for kittens and 0.80 for subadults/adults (no over-dispersion is indicated when $\hat{c} \leq 1$).

4 | DISCUSSION

Pathogens such as viruses and macroparasites are often used as biological controls to manage invasive species (Fagan, Lewis, Neubert, & Van Den Driessche, 2002; Lu et al., 2015; Pech & Hood, 1998; van Rensburg, Skinner, & van Aarde, 1987). However, synergies are common in nature, and the impact of a biological control is likely mediated by co-infection with other pathogens (Elias, Mengistu, Akuffo, & Britton, 2006; Jolles et al., 2008; Telfer et al., 2010). Using an unprecedented, long-term dataset of a closed population of wild vertebrates, we provide the first evidence of disease synergies between two biological control agents affecting survival.

We revealed a synergistic effect of myxomatosis and rabbit haemorrhagic disease on individual rabbit survival, with rabbit haemorrhagic disease outbreaks having a greater negative impact on survival of rabbits that had previously been exposed to myxoma virus than those never exposed to either virus (Table 1; Figure 2). While this is the first evidence of disease synergies between myxomatosis and rabbit haemorrhagic disease, myxomatosis is known to affect immune responses to other pathogens (Boag et al., 2013; Cattadori et al., 2007). For example, in Scotland, rabbits infected with myxoma virus had higher mean oocyst counts of the protozoan parasite *Eimeria stiedae* than rabbits not infected with myxoma (Boag et al., 2013). Similarly, myxoma virus can increase the susceptibility of rabbits to the nematode *Trichostrongylus retortaeformis* (Cattadori et al., 2007). However, both of those studies only assessed the impact of current infection with myxoma virus as shown by signs of disease. We demonstrate here that myxoma virus—or possibly another unmeasured phenomenon associated with myxoma infection—also has a protracted effect on the survival of wild rabbits beyond the period of active myxoma virus infection, as revealed by the antibody assay data measuring individual histories of virus exposure.

Results such as ours could have occurred if, during the year, myxoma spread gradually (increasing the proportion of rabbits with myxoma antibodies) and, simultaneously, the mean age of infection with RHDV increased due to another unknown factor (i.e., increased age-related lethality of RHDV infection with a higher proportion of rabbits positive to myxomatosis when infected by RHDV). However, Mutze, Sinclair et al. (2014) showed that the average age at RHDV infection declines during the latter stages of outbreaks, so our results are contrary to what would be expected if the within-year or within-outbreak changes in age of infected rabbits were influential. Another potential criticism of our study is that some older *adults* could have been misclassified as having no immunity, because antibody concentrations can wane with age (Cooke et al., 2000). However, 87.2% of all rabbits were initially tagged as kittens or subadults (Supporting Information Figure S1; Mutze, Sinclair et al., 2014), and had a known serological history from which reliable classification of seronegative adults was possible.

Our results indicate that myxomatosis outbreaks occurring before rabbit haemorrhagic disease outbreaks are increasing mortality due to rabbit haemorrhagic disease as a biological control in Australia. Rabbit haemorrhagic disease outbreaks already have a large effect on rabbit survival over the 58-day interval, reducing survival probability of individuals with no immunity (N) from 0.86 to 0.48. Following myxomatosis outbreaks, many of the survivors are likely to be immunocompromised through exposure to myxoma virus (Figure 2), reducing survival by another 10% during subsequent rabbit haemorrhagic disease outbreaks (Figure 1; Supporting Information Table S1). Therefore, manipulating the timing of outbreaks such that rabbit haemorrhagic disease outbreaks occur more

frequently after myxomatosis outbreaks could increase the efficacy of rabbit control. In practice, manipulating the timing of rabbit haemorrhagic disease outbreaks could be achieved by introducing virus-inoculated baits or blowflies after clinical myxomatosis is observed in the population (Mutze, Sinclair, Peacock, Kovaliski, & Capucci, 2010; Sharp & Saunders, 2016). Alternatively, since rabbit haemorrhagic disease outbreaks usually occurred in spring (Mutze, Sinclair, et al., 2014; Figure 1), myxoma virus could be introduced via infected fleas (Parer, Conolly, & Sobey, 1985; Robinson & Holland, 1995) prior to anticipated rabbit haemorrhagic disease outbreaks. However, more research is required to understand the duration of immunosuppression caused by the myxoma virus (Jeklova et al., 2008; Kerr et al., 2017), and population-level effects of manipulating disease timing.

A broader implication of these results for biological control of rabbits in Australia is that new pathogens have the potential for synergistic effects with existing biological control agents. New pathogens, such as the gut parasites *Eimeria* spp. (Boag et al., 2013; Henzell, Cooke, & Mutze, 2008; Hobbs, Twigg, Elliot, & Wheeler, 1999a, 1999b), could potentially reduce rabbit survival by a substantially greater margin than their individual effects measured by laboratory-challenge studies in isolation from other pathogens. On the other hand, antagonistic interactions between pathogens can also occur, and previous exposure to one pathogen might in fact enhance survival upon exposure to another (Nemeth, Bosco-lauth, & Bowen, 2009; Reich et al., 2013; Strive, Wright, Kovaliski, Botti, & Capucci, 2010; Thumbi et al., 2013). For example, in red-winged black birds *Agelaius phoeniceus*, inoculation with West Nile virus provided protection from Japanese encephalitis (Nemeth et al., 2009). In addition to laboratory trials, population modelling can highlight the potential impact of synergistic and antagonistic disease interactions prior to the introduction of new biological controls. By providing individual survival estimates for wild rabbits with different exposure histories and disease state-transition probabilities, our results will enable managers to predict the impact of potential new biological controls on rabbit populations in Australia, including or excluding possible synergistic effects.

Our work also indicates that disease synergisms could increase the efficacy of other biological control programmes. Viruses have been widely used as biological controls for insect pests (Lacey, Frutos, Kaya, & Vail, 2001), and have potential for the control of invasive vertebrates (McColl et al., 2014). For example, the feline panleukopenia virus contributed to the eradication of cats on Marion Island (Bester et al., 2002) and Jarvis Island (Rauzon, 1985), and cyprinid herpesvirus-3 is currently being considered to control invasive carp *Cyprinus carpio* in Australia (McColl, Sunarto, & Holmes, 2016; McColl et al., 2014). Investigating whether interactions between these biological controls and other pathogens can be exploited to maximise mortality of invasive species should be a focus of future research.

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AUTHORS' CONTRIBUTIONS

L.K.B., C.J.A.B., T.A.A.P., G.J.M., and D.E.P. conceived the analysis and designed the modelling methodology; R.G.S., D.E.P., G.J.M., and J.K. collected the data; L.K.B., T.A.A.P., and C.J.A.B. analysed the data; L.K.B. and C.J.A.B. led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

DATA ACCESSIBILITY

Data available from the Dryad Digital Repository https://doi.org/10.5061/dryad.j91d66c (Barnett et al., 2018).

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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