Factors affecting the survival of Australian wild rabbits exposed to rabbit haemorrhagic disease

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Abstract. Rabbit haemorrhagic disease virus (RHDV) is foreign to Australia, and first entered populations of Australian wild rabbits (Oryctolagus cuniculus L.) in Australia in late 1995. Rabbits are serious environmental and agricultural pests in Australia, and RHDV, a major new pathogen, was introduced as a biological control agent to reduce their numbers. Our study evaluated some of the factors affecting survival of wild rabbits exposed to rabbit haemorrhagic disease (RHD) at 78 sites across Australia.

Our data on rabbit numbers consist of the number of rabbits per spotlight kilometre present shortly before and shortly after an RHD outbreak at each site. They are a direct measure of survival rather than mortality. By reducing the interval between the pre- and post-RHD counts to the minimum possible, we sought to minimise the influence on the analysis of other causes of change in rabbit numbers. We calculated proportional survival as the ratio (number of rabbits present after RHD)/(number present before RHD), and used regression analysis to relate it to environmental and other variables. Proportional survival was lower at higher densities of rabbits; was lower if RHDV arrived naturally at the site rather than if it was deliberately released; was lower in areas with hot, dry climates than in areas with cold, wet climates; was lower in southern, inland areas than in warm, coastal areas; and, if the outbreak occurred during summer, was lower in areas of winter rainfall than in areas of summer rainfall. Rainfall seasonality was not correlated with survival at other times of the year. Only in the last effect was there a significant interaction with the time of the year that the outbreak occurred.

Our statistical model describes correlations among the data, but does not in itself establish cause and effect. We interpret the properties of our statistical model to draw the following conclusions. First, the effectiveness of RHD is reduced in cold, wet areas and warm, coastal areas, because of the prevalence in these areas of one or more pre-existing caliciviruses in rabbits that impart year-round resistance to RHD. Second, we conclude that the poor summertime performance of RHD in areas that are wet in summer could result from poor survival of RHDV exposed to the combination of high temperature and high relative humidity, although it is also possible that during summer the effectiveness of vectors declines.

Introduction

Rabbit haemorrhagic disease virus (RHDV), also known in Australia as rabbit calicivirus, is foreign to Australia. The virus first entered populations of wild European rabbits (Oryctolagus cuniculus L.) in Australia in late 1995 after it escaped from Wardang Island off the coast of South Australia, where it was being tested as a potential biological control agent of the rabbit. It subsequently spread by natural means and deliberate release into much of the range of the rabbit in Australia (Kovaliski 1998). Rabbits are serious environmental and agricultural pests in Australia, and RHDV was introduced into Australia as a biological control agent to reduce their numbers (Williams et al. 1995; Fenner and Fantini 1999). The National Rabbit Calicivirus Disease (RCD) Monitoring and Surveillance Program monitored the spread and effect of RHDV on wild rabbit populations, and the consequential changes in the effects of rabbits on the environment, in all States and Territories. Rabbit haemorrhagic disease (RHD) greatly reduced rabbit numbers in some areas, but the extent of the reduction differed geographically. Data on population declines resulting from known or reliably inferred natural outbreaks...
of RHD, or from deliberate releases of RHDV, were collected from much of the range of the rabbit in Australia during this program, and Neave (1999) presented a statistical analysis relating the proportional decline in rabbit numbers in different areas to environmental and other factors. Lugton (1999) separately reported on the likelihood and severity of outbreaks of RHD following RHDV releases in New South Wales. Cooke (1999) summarised our present understanding of the epizootiology of RHDV and its initial spread in Australia. This paper reports the results of an extended analysis of the national data set described in Neave (1999).

The national RHD data set

Neave (1999) assembled and analysed two related sets of data that comprised the national RHD data set. The first set comprised 82 sites for which pre- and post-RHD transect counts of rabbits per spotlight kilometre were available. The second comprised 455 sites for which the decline of rabbits following RHD was categorised into one of four classes: 1, little or no decline in rabbit abundance; 2, 10–30% decline; 3, 30–65% decline; and 4, >65% decline.

The sites in the 82-site data set were all included in the National RCD Monitoring and Surveillance Program, and were regularly monitored to evaluate changes in rabbit numbers in relation to the occurrence of RHD. The data were collected by, or on behalf of, wildlife and vertebrate pest control agencies in each State and Territory.

Both of Neave’s data sets included natural outbreaks of RHD and deliberate releases of virus. A natural outbreak was included in the data sets if it was the first known occurrence of RHD in an area. Most releases were made some time after the initial spread of RHDV, and the detailed immunological status of rabbit populations at the release sites was generally unknown for the analysis. It was known that antibodies to RHD were present at some of the release sites before the deliberate release of RHDV (Cooke 1999; Neave 1999). Our data from these sites therefore represent the proportional survival of rabbits in populations that had previously been exposed to RHDV and into which RHDV was deliberately reintroduced between the pre- and post-RHD counts. RHDV was deliberately released by trapping at least 10 rabbits at the site, dosing them with RHDV in a controlled and standardised manner, and releasing them at the same site. Releases were excluded from the analysis in cases where serology or virus capture was performed and failed to demonstrate any establishment of RHDV at the site (Neave 1999).

At sites where purported RHD outbreaks included in the 82-site national data set were unsupported by serology or virus capture, it is possible that changes in population size attributed to RHD were in fact caused by confounding influences such as myxomatosis or drought. To test whether this possibility might have had a significant effect on our results, we constructed a six-level categorical variable (know) in which each level coded for a different method of demonstrating or inferring the occurrence of RHD at the site between the pre- and post-RHD transect counts. At 44 of the 82 sites, the occurrence of RHD at the site between these counts was confirmed by serological tests for the presence of antibodies to RHD in samples of surviving rabbits and/or by the presence of RHDV in liver and spleen samples taken from dead rabbits at the site (know = 1). At these sites, serology also indicated that RHD had not occurred previously. The South Arm release site in Tasmania is included here, because although serology was not done before the release, at the time the release was made there was no record of RHD outside the north-west of the island (180 km to the north at the closest); serology conducted shortly after the release confirmed that RHDV had established. At some of these 44 sites, rabbits for serological testing were shot several kilometres away from the spotlight transect to minimise the influence of shooting on the spotlight counts; rabbit carcasses tested for the presence of RHDV were collected on the spotlight transects or where rabbits were shot. Some 33 of these outbreaks were natural arrivals, and 11 were deliberate releases. At a further eight release sites, the serological status of the rabbits shortly before or after release was not known to us (know = 2). At 12 release sites, serological analysis of samples collected before the release showed that RHD had already occurred at the site, and the serological status of the rabbits shortly after release was not known to us (know = 3). At five release sites, the rabbits were serologically negative before release, and the serological status of the rabbits shortly after release was not known to us (know = 4). At all the release sites where the serological status of the rabbits shortly after release was not known to us, we inferred that an outbreak of RHD had occurred because RHDV was known to have been released into the population. The remaining 13 cases in the national data set were natural arrivals, but it was not possible to collect specimens for RHD testing on site owing to low numbers of rabbits after RHD and to the decay of carcasses or their removal by scavengers. In these cases, the occurrence of RHD was inferred from one or more similarities between the rabbit mortality event at the site and mortality events associated with confirmed RHD outbreaks elsewhere. These similarities included the following: (1) the appearance of large numbers of dead rabbits and smelly warrens that could not be attributed to other causes, such as myxomatosis or drought (rabbits dying of myxomatosis show distinctive symptoms such as swollen and closed eyes; Fenner and Fantini 1999); (2) the lack of obvious external signs of pathology in most of the dead rabbits (Fenner and Fantini 1999) and their apparently good body condition (Lenghaus et al. 2001) (rabbits dying from myxomatosis or drought are often emaciated and may show other distinctive symptoms); and (3) the geographical and temporal conformity of the outbreak to the well-documented pattern of RHD spread as confirmed by serology or RHDV...
capture (Kovaliski 1998) or from the presence of a contemporaneous outbreak of RHD within 20 km of the site that was confirmed by serology or virus capture. At four sites fitting this pattern, a known outbreak of RHD occurred within 20 km \((\text{known} = 5)\), and at the remaining nine sites the outbreaks conformed to the known pattern of RHD spread \((\text{known} = 6)\).

Mortality due to an RHD outbreak can be viewed as a step function superimposed on other more gradual changes taking place in the population caused by breeding, mortality due to other causes, immigration and emigration. Our aim was to measure the size of the step and the factors influencing it. We had no measures of the more gradual processes, and we sought to minimise their influence by reducing the time span of our observations to the minimum possible, namely the transect count conducted immediately before the outbreak and that conducted immediately after.

Both data sets include values for 35 bioclimatic variables calculated from BIOCLIM (Nix 1986).

**Neave’s analysis**

The 35 bioclimatic variables were simplified to two principal components owing to co-linearity among them. These were \(p1\text{HD}\), representing variation along an axis ranging from hot–dry (positive end of \(p1\text{HD}\)) to cold–wet (negative end), and \(p2\text{WR}\), representing variation along a winter rainfall (positive end of \(p2\text{WR}\)) to summer rainfall (negative end) axis (Appendix 1). Neave’s statistical analysis of the two data sets utilised some of the bioclimatic variables derived from BIOCLIM as well as the two principal components (see Neave 1999 for more information on the potential explanatory variables used in her analysis).

From the analysis of the first data set (82 sites), Neave concluded that survival was generally lower where RHD occurred naturally than where RHDV was deliberately released. Survival after releases was significantly reduced if the area experienced low temperatures during the coldest month of the year or during the wettest 3 months of the year. However, these same climatic factors were not significantly correlated with survival after natural occurrences. Survival after release was as low as that after natural occurrence in areas experiencing low temperatures during the coldest month of the year or during the wettest 3 months of the year; elsewhere it was higher. It also appeared that whether rabbits used warrens or lived above ground influenced survival, but there were only three sites where rabbits predominantly used aboveground cover, and the statistics relating to this effect were imprecise and difficult to interpret.

Neave’s analysis of the categorical survival data (455 sites) showed that natural outbreaks of RHD generally resulted in lower survival in hot, dry areas than in cool, wet areas. However, for deliberate releases, survival was generally lower in cool, wet areas. Neave interpreted this reversal in the geographical effectiveness of RHDV in terms of the probable prior natural occurrence of RHD at some release sites and the presence there of immune rabbits when releases were made. There was an interaction between the rabbits’ use of harbour and \(p2\text{WR}\) (see Neave 1999 for more details).

In this paper we explore in detail the relationships between the proportional survival of rabbits during RHD outbreaks, initial population density, climate and other variables, including the third bioclimatic principal component (denoted \(p3\text{SI}\)), using Neave’s first data set. These relationships, and in particular whether survival is lower at higher pre-RHD population densities, have an important bearing on the capacity of RHD to regulate the density of wild rabbit populations.

The complete data set is available on request from the editor of *Wildlife Research*.

**Statistical model**

It seems reasonable to suppose that rabbit density after RHD will be proportional to rabbit density before RHD. Neave (1999) assumed direct proportionality for simplicity and so the response measure was proportional survival. In this paper we check this assumption. As multiplicative effects are likely to apply, we transformed the data on transect counts of rabbits per spotlight kilometre by taking natural logarithms, after adding 0.1 to avoid arithmetic problems (some sites recorded no rabbits after RHD). We denoted the response variable by \(l\text{postRN}\) and the explanatory variable for pre-RHD rabbit numbers by \(l\text{preRN}\). Other explanatory variables considered are described in Neave (1999).

Thus our initial plausible model for the mean number of rabbits surviving will be of the form:

\[
l\text{postRN} = \text{constant} + \text{effect due to } l\text{preRN} + \text{mode of arrival effects (arrival: natural or release)} + \text{climate effects (} p1\text{HD}, p2\text{WR}, p3\text{SI}) + \text{season of outbreak effects (season)} + \text{harbour effects (warren)} + \text{possible interaction effects} + \text{random error}
\]

This model can easily be re-expressed in terms of proportional survival by incorporating \(l\text{preRN}\) in the model both as an offset and also as another explanatory variable. In this case, the offset \(l\text{preRN}\) is subtracted from \(l\text{postRN}\) before the model is fitted, and the effective response variable is \(\ln(p\text{postRHDnumbers}/p\text{preRHDnumbers})\). The value of the regression parameter estimate for the \(l\text{preRN}\) variable measures the departure from direct proportionality.

Assuming the data are independent, this model is a standard regression model and estimation is by ordinary least squares. However, it is possible that neighbouring sites will have similar conditions. If the explanatory variables do not fully reflect these conditions, which may affect survival, the
residuals from the model will exhibit spatial autocorrelation. Ignoring such dependence will tend to underestimate the variances so that the standard errors of the regression coefficients will be underestimated, thus giving a spurious impression of significance. We model spatial dependence by fitting a covariance model that depends on distance. The particular form we have used is an isotropic, exponential correlation model described by Diggle et al. (1994). We used restricted maximum likelihood estimation to fit this model.

The error term incorporates changes in rabbit numbers resulting from variation in factors not included elsewhere in the model, such as births, deaths from causes other than RHD, immigration, emigration, and variations in the behaviour or visibility of rabbits during spotlight counts. The intervals between the pre- and post-RHD counts were kept as short as possible to minimise these confounding effects. The unexpected escape of RHDV from Wardang Island precluded the establishment of paired sites, with the site without RHD acting as an experimental control for the other site with RHD. Our inferences regarding the factors affecting the proportional survival of rabbits exposed to RHD are therefore observational and based on statistically significant correlations between apparent proportional survival following known or inferred RHD outbreaks and other continuous and categorical variables.

We added the six-level categorical variable know to the final model to test whether there was any difference in survival between sites where RHD was known to have occurred, as demonstrated by serology or virus capture (know = 1), and the sites where its occurrence was inferred by other means (know > 1).

Results

Evidence for spatial dependence in our data was weak. However, ignoring spatial autocorrelation showed negligible change in parameter estimates and their standard errors, and so for simplicity in subsequent modelling we chose to ignore this feature of our data.

There was some evidence that the survival of rabbits was higher at sites where RHDV arrived naturally than at sites where the virus was deliberately released if rabbits predominantly used aboveground cover rather than warrens (three sites only). Other interaction effects involving the variable warren could be substituted for this interaction effect, but these were all difficult to interpret. Thus for simplicity we chose to exclude the three sites where rabbits mainly used aboveground cover. The season of release was unknown for one site, which was also excluded. This left a sample of 78 sites.

Modelling indicated that survival did not differ significantly between winter, spring and autumn, but that these three seasons differed from summer. For simplicity our model therefore presents seasonal effects as a contrast between summer and the other three seasons combined.

The period (in months) between the pre-RHD rabbit count and the post-RHD count was related to the proportional survival of rabbits (the parameter estimate was –0.0898 ± 0.0336 (s.e.); estimates for the other parameters were not greatly changed by the addition of this term). The longer the interval, the lower the proportional survival. However, the interval between the outbreak itself and the post-RHD rabbit count was not significantly related to proportional survival. An explanation of the difference between the effects of the two variables is not obvious, and the second result called into question the validity of the first. The intervals were subject to considerable measurement error because in many cases only the month was known, not the day of the month. Taking the difference between two such measurements doubles the measurement error. We therefore omitted these intervals from the model.

The addition of the six-level categorical variable know to the final model was not significant ($F_{6,55} = 0.74$). Evidence of non-linearity in the relationships between the response and continuous explanatory variables could not be found.

The parameter estimate for lpreRN was significantly less than 1 ($P < 0.001$), indicating that proportional survival decreased at higher rabbit densities. To make this clear we have included lpreRN in the model as an offset variable. The parameter estimates and standard errors for significant ($P < 0.05$) explanatory variables in the model including the offset variable are shown in Table 1. In this form our model can be directly interpreted in terms of additive effects on $\ln(postRHDnumbers/preRHDnumbers)$. This implies that the effects of explanatory variables were multiplicative on the natural scale (that is, the proportion of rabbits surviving). For example, the parameter estimate for the variable $p1HD$ is –0.0783, which means a reduction in $\ln(postRHDnumbers/preRHDnumbers)$ of 0.078 units per unit of $p1HD$; this converts to an 8% reduction in the proportion of rabbits surviving per unit increase in $p1HD$.

The proportion of rabbits surviving an RHD outbreak was markedly reduced ($P < 0.001$) if many rabbits were present before the outbreak ($lpreRN$); was lower ($P < 0.001$) at sites where RHDV arrived naturally than where it was deliberately released (arrival); decreased ($P < 0.001$) as climate changed from cold and wet to hot and dry ($p1HD$, which is bioclimatic principal component score 1); was higher ($P = 0.002$) if the outbreak occurred in summer in areas that experience aseasonal or summer rainfall (season = summer v. autumn, winter and spring seasons combined; note the interaction of season with $p2WR$, the bioclimatic principal component score 2); was lower if the outbreak occurred in summer in winter-rainfall areas (the $p2WR \times season$ interaction again); and also was higher ($P = 0.018$) if the outbreak occurred in warm, coastal areas ($p3SI$, the bioclimatic principal component score 3).

The relationships between the significant explanatory variables and the proportion of rabbits surviving an RHD
outbreak are shown in Fig. 1. The bioclimatic principal component scores for each site are given in Appendix 1.

The values of the bioclimatic principal component scores for the different sites are displayed in Figs 2–4; the larger the circle, the more negative the score. On average a decrease of one unit in p1HD, p2WR and p3SI results in an increase in survival of 8%, 2% (in autumn, winter and spring; 35% in summer), and 13%, respectively, and these effects are approximately additive on the percentage scale.

Properties of the model

Effect of population density on survival

The proportional survival of rabbits decreased by approximately 0.27% (95% CI = 0.13–0.41%) for each 1% increase in the initial population size (Fig. 1a). Pre-RHD spotlight counts per kilometre varied from 0.1 to 106.

Effect of mode of arrival of RHDV on survival

We estimated that the proportional survival of rabbits after a natural outbreak was about 45% of that following a deliberate release of the virus (Fig. 1b).

Effect of climate and season of RHD outbreak on survival

The statistically significant, negative regression coefficient for p1HD indicates that survival of rabbits is lower in hot, dry areas (high p1HD) than in cold, wet areas (Figs 1c, 2). We added p1HD \times sea to the above model to test whether there was a significant interaction between p1HD and the season of the year in which the RHD outbreak occurred (sea was a categorical variable that took a different value for each of the four seasons). This addition to the model was not significant ($F_{3,67} = 1.52$).

The significant, positive estimate for the factor season-summer indicates that survival tends to be higher for summer outbreaks, although this effect is modified by the negative interaction between season-summer and p2WR (Figs 1d, 3). The interaction reflects a gradient in survival between winter-rainfall (high p2WR) and summer-rainfall areas that is much more pronounced during summer than at other times of the year. Compared with other seasons, survival during summer is low in winter-rainfall areas and high in summer-rainfall areas. This trend is also present at other times of the year, but the effect is small and may not be real (the negative regression coefficient for p2WR is not statistically significant, and p2WR is included in the model because of its significant interaction with season).

The significant, negative relationship between survival and p3SI indicates that survival increases along a gradient between southern, inland areas (high p3SI) and warm, coastal areas (Figs 1e, 4). The gradient in p3SI is very steep near the eastern and western seabords of mainland Australia, and p3SI primarily highlights the contrast between these areas and the rest of Australia. Values of p3SI are highest in the Flinders Ranges and inland New South Wales (including the Australian Capital Territory) (Appendix 1).

Discussion

Interpretation of the statistical model

Population density

The proportion of rabbits surviving an RHD outbreak was inversely proportional to the population density, as would be expected for an infectious disease like RHD.

The influence of population density on morbidity and mortality among wild rabbits exposed to RHDV is unknown. An outbreak in a high-density wild population in the Flinders Ranges of South Australia resulted in morbidity of 98% and mortality of 97% (Mutze et al. 1998). Morbidity was up to 80% among farmed rabbits kept close together in large groups, and below 30% in small groups (Mitro and Krauss 1993). Morbidity should therefore be higher in high-density wild populations. Mortality among farmed rabbits rose in unhygienic conditions, and as stress levels and parasite loads increased (Mitro and Krauss 1993); these factors might be expected to increase mortality in high-density wild populations. Stress and parasite loads may contribute to the
recurrence of RHD in previously infected wild rabbit populations (Moriarty et al. 2000).

**Mode of arrival of RHDV**

In the statistical analysis, there were no clear interactions between arrival and other explanatory variables, and in the final model the only difference between releases and natural outbreaks was that the former were uniformly less effective than the latter.

Natural occurrences of RHD during its initial spread from Wardang Island would have occurred in immunologically naïve populations, and would be expected to have caused maximum mortality. However, RHDV had already arrived naturally at some of the sites where deliberate releases were made (Cooke 1999; Neave 1999), and in these cases releases would have been made into populations that contained the immune survivors of earlier natural outbreaks. Pre-existing immunity would be expected to increase proportional survival in the population as a whole, and this could account for the higher survival seen at release sites. In addition, the susceptible component of the population facing the return of RHD might have a different age structure from populations that had not been previously exposed. As rabbits younger than 8 weeks are less susceptible to RHD than old rabbits, this too could affect survival in the population as a whole (Cooke 1999).

Outbreaks of RHD occurring after the initial outbreak in an area might be expected to result in proportional survival
comparable to natural arrivals in our model if the population is composed mainly of immunologically naïve rabbits. Such a situation might occur if there was a long interval between successive outbreaks or if breeding activity was high. Breeding, and the loss of antibodies through time, both contribute to a build-up of susceptible rabbits in a population that may lead to new RHD outbreaks, but their pattern of occurrence may be complex (Cooke 1999). Populations

Fig. 2. Values of $p1HD$ for the 82 sites.

Fig. 3. Values of $p2WR$ for the 82 sites.

Fig. 4. Values of $p3SI$ for the 82 sites.
containing a significant proportion of rabbits that are immune and/or very young (and hence resistant to RHD) would be expected to behave more like those at release sites.

Effect of p1HD

Survival of rabbits after an RHD outbreak increased along a gradient between hot, dry areas and cold, wet areas. However, this climatic effect did not interact significantly with the season in which the outbreak actually occurred: rabbits in cold, wet areas appeared to survive summer outbreaks just as well as winter outbreaks. A significant interaction would have been expected if the effect of climate on transmission or persistence of RHDV caused the higher survival in cold, wet areas: under these circumstances, we would have expected to find higher survival during winter than summer outbreaks, as this would mirror the climatic effect on a seasonal scale. This result suggests that the factor underlying the correlation between p1HD and survival in our model is not climate per se (or the weather at the time of the outbreak, which might be expected to be broadly correlated with climate), but something with which climate is correlated and that does not change appreciably during the year. One candidate for such an effect is the pre-existing benign calicivirus present in Australia before RHDV was introduced in 1995 (Nagesha et al. 2000; Cooke et al. 2002). Rabbits infected with the pre-existing calicivirus show increased resistance to RHD, and this virus is more prevalent in rabbits in cooler, wetter areas. Antibodies to this pre-existing calicivirus might be expected to be present in previously infected rabbits throughout the year, and thereby to increase proportional survival across all seasons. However, the epizootiology of the pre-existing calicivirus is poorly understood and more work is needed to establish the plausibility of this suggestion.

Alternatively, the higher proportional survival of rabbits following RHD outbreaks in cool, wet areas compared with hot, dry areas could be spurious. Mortality caused by RHD may have been swamped by higher recruitment in cool, wet areas (Gilbert et al. 1987), giving the appearance of higher survival. However, rabbit breeding possessed a seasonal component at all of the locations examined by Gilbert et al. (1987), and if this explanation were correct it should manifest itself in our model as an interaction between p1HD and season, but this interaction was not significant ($F_{3,69} = 1.52$, NS, for the test of p1HD $\times$ seas; and $F_{1,69} = 3.45$, NS, for the test of p1HD $\times$ season; where seas was a factor taking a different level for each season and season was a factor coding for a contrast between summer, and the other three seasons combined). In addition, this mechanism should result in a higher proportion of young rabbits in populations in cool, wet areas relative to hot, dry areas. At the Coorong and the Flinders Ranges, two areas in South Australia well separated along the p1HD axis, it appears that the proportion of subadult rabbits weighing 800–1200 g in the populations was about the same (Mutze et al. 2002).

Effect of p2WR, and RHD activity during summer

The effect of the interaction between season and p2WR can be seen in Fig. 1d. During autumn–winter–spring there was little difference in survival between sites with high and low values of p2WR. In summer the outcome was a combination of the effect of the negative regression coefficient for p2WR ($-0.020 - 0.282 = -0.302$) and the positive parameter value for the factor season-summer. Proportional survival in summer increased along a gradient between winter-rainfall areas (high, positive p2WR) and summer-rainfall areas (negative p2WR). RHD outbreaks appear to be largely ineffective in summer rainfall areas during summer (Fig. 1d), although the wide confidence interval indicates considerable variability due to small numbers of sites.

Relatively few outbreaks of RHD are observed in summer (Kovaliski 1998; Lugton 1999), and it has been suggested that high temperatures may prevent RHDV being active at that time (Xu and Chen 1989; Smyth et al. 1997; Cooke 1999). RHDV survives poorly at high temperatures (Rodak et al. 1991; Cooke 1996a, 1999). However, RHDV was active on Eyre Peninsula during the 1995–96 summer in immunologically naïve rabbit populations (Kovaliski 1998). Two outbreaks near Elliston on Eyre Peninsula during the 1995–96 summer are included in our data set, and our model fits them well. These two winter-rainfall sites have high values of p2WR (Appendix 1). At that time RHDV had not spread to Western Australia (the south-west of which also experiences winter rainfall). If the two Elliston points are removed from the fit, the model does not change appreciably. The Elliston points are therefore not particularly influential, and the pattern of low survival during summer in winter-rainfall areas appears to be well embedded in the data.

Winter-rainfall areas can be very hot in summer, yet our modelling indicates that summer RHD outbreaks in these areas result in low survival of rabbits. RHD continues to occur during summer on Eyre Peninsula (G. Mutze, Animal and Plant Control Commission, personal communication 2000). This suggests that although high temperatures are inimical to RHDV, high summer temperatures alone do not prevent virulent outbreaks. Cooke (1996b) concluded that variation in the survival of RHDV at different temperatures is a relatively minor factor in the field epizootiology of RHD. Our modelling therefore suggests that the high post-RHD survival of rabbits in summer in areas experiencing summer rainfall is not due solely to high temperatures, but rather to the seasonal coincidence in these areas of high temperatures and high rainfall. It is the seasonality of the moisture regime, rather than temperature per se, that is the main determinant of p2WR (Appendix 1). The high survival of rabbits in summer in summer-rainfall areas could be due to poor
survival of RHDV exposed to the combination of high temperature and high relative humidity (RH).

The effect of high RH on RHDV has not been investigated experimentally, but several other viruses survive poorly at RHs of about 80% (Schulman and Kilbourne 1967; Sattar et al. 1984, 1987; Ijaz et al. 1985b, 1994). In other cases where interactions between temperature and RH were investigated, survival of some viruses was poorest if high RH was combined with high temperature (Ijaz et al. 1985a; Schoenbaum et al. 1990; Mbithi et al. 1991). However, some other viruses survive well at high RH (Moe and Shirley 1982; Ijaz et al. 1985b; Sattar et al. 1988; Abad et al. 1994). The suggestion that RHDV survives poorly at high RHs is plausible, but needs to be tested. RH might be expected to be relatively high in summer in summer-rainfall areas (especially in rabbit warrens), but estimates of RH are not produced by BIOCLIM. BIOCLIM does produce estimates of the precipitation in the warmest quarter and the mean moisture index of the warmest quarter; these would be correlated with RH, and both are large contributors to p2WR (Appendix 1).

The conspicuously large effect of p2WR on the estimated survival of rabbits exposed to RHD during summer (Fig. 1d) suggests an intimate connection between p2WR and the biology of the rabbit and/or the epizootiology of RHD. Inspection of the bioclimatic contributors to p2WR (Appendix 1) leads to the inference that during summer, soil moisture levels (but not necessarily soil temperatures) in summer-rainfall areas would be higher than in winter-rainfall areas. The air inside rabbit warrens (and, to a lesser extent, near the surface of the ground outside the warrens) would be humidified by this relatively moist soil. During summer in summer-rainfall areas, RH would be more consistently high inside rabbit warrens than outside. In winter-rainfall areas, the soil would dry out during summer and RH would fall. These considerations, taken together with the dominating influence of p2WR on survival during summer, make warren humidity our preferred candidate to mediate this effect. A plausible mechanism to explain the effect is that high RH inside the rabbit warren and/or near the ground surface where the rabbits are grazing, acting in combination with high temperatures, inactivates RHDV. This effect would become more pronounced during late summer, with the progressive warming of the soil, and hence the warren, at greater depths. Our conclusions regarding the RH of air inside warrens are generally consistent with the limited data available (Hayward 1961; Hall and Myers 1978; Cooke 1990).

RHDV present in soil in warrens could possibly be transmitted to rabbits through skin lesions, by conjunctival or oral contact, or by inhalation. Insect vectors feeding on infected carcasses might deposit RHDV in flyspots on pasture, which might then be eaten by rabbits (Asgari et al. 1998).

We therefore suggest that in summer-rainfall areas the source of RHDV responsible for widespread, virulent RHD outbreaks during spring (and earlier seasons) is progressively destroyed during summer by high warren humidity and temperature (Appendix 2), leading to a reduced number of less virulent outbreaks. The effect appears to be less pronounced during summer in winter-rainfall areas because the annual cycles of warren temperature and RH are out of phase.

Of the 16 outbreaks that occurred during summer, the four summer-rainfall sites with the lowest values of p2WR were all higher than 500 m elevation. It is possible that RHD was able to occur at these summer-rainfall sites during summer only because their high elevation resulted in lower temperatures, and this reduced the degradation of RHDV by high humidity.

For our conclusions to be supported, it will be necessary to show not only that high RH results in the inactivation of RHDV, but also that the effect operates only, or at least is most pronounced, at high temperature. Our conclusions could readily be tested by field studies measuring RHDV survival in different microhabitats and climatic regions of Australia during different seasons, and by laboratory studies measuring RHDV survival as an aerosol and in different media (soil, rabbit carcasses, nasal secretions, flyspots, urine and faeces) maintained at different temperatures and RHs.

Resumption of RHD activity during autumn

We hypothesise that the resumption of RHD activity in autumn (Appendix 2; Kovaliski 1998) requires a replenishment of RHDV from some source, at least in summer-rainfall areas. Virus may have survived in the soil deep in warrens, where high temperature and/or high RH did not penetrate. This source of virus might be exposed and brought to the surface the following autumn by soil disturbance associated with the cleaning out and renovation of warrens in preparation for breeding (Myers and Poole 1961), and RHD could recur as a result. This mechanism would also provide a source of virus for outbreaks in new areas. Alternatively, it is possible that RHDV is shed by carriers, and that shed virus is able to persist longer in the more favourable physical environment of autumn (it is not known whether there are carriers of RHDV). Yet another possibility is that high temperature and high RH precipitate a switch to a predominance of less effective vectors in the areas experiencing these conditions, and this switch is reversed only during autumn.

Possible role of vectors

A variety of insect vectors may be involved in the epizootiology of RHD, and the species involved might change with the seasons (Asgari et al. 1998; Cooke 1999). The blowfly Calliphora dubia is implicated, and is most abundant during spring and autumn (Cooke 1999). However,
there are many other candidate vectors, with different patterns of seasonal activity, and it is not possible to draw firm conclusions regarding variations in their collective effectiveness. Nevertheless, flying insects are very active during summer in summer-rainfall areas. On the basis of present knowledge, we therefore suggest that the effect of RH on RHDV survival discussed above is the more likely explanation for the large effect of p2WR on the survival of rabbits exposed to RHDV during summer.

**Effect of p3SI**

RHD appears to be less effective near the warm western and eastern coastlines of mainland Australia. This effect is also present but much less pronounced along parts of the cool southern coastline, in Tasmania, and at low latitudes in central Australia. RHD’s effectiveness increases in inland areas in the southern mainland. The extremely low values of p3SI along the eastern and western seaboard might be related to the presence of warm ocean currents flowing from north to south along these coasts.

RHD releases at Gingin and Cattai provide two independent indicators of the reality of p3SI’s effect. Gingin is very close to the coast north of Perth, and Cattai is on the coastal plain close to Sydney. These two locations are on opposite sides of the continent, so physical proximity is not an issue. Gingin has a low value of p3SI (~5.66), and although we have not calculated the value of p3SI for Cattai, it would also be low (other eastern seaboard sites close to the coast have low p3SlS – see Fig. 4). Gingin was excluded from our analysis because we did not know the season of release. At Gingin, the post-RHD rabbit count was double the pre-RHD count. Cattai was not included in the data set because despite three separate releases of RHDV it could never be demonstrated that the virus established (Neave 1999), which we again interpret as meaning that RHD was ineffective.

The effect of p3SI is independent of season ($F_{3,60} = 0.78$, NS), like that of $p1HD$. The possible explanation might be the same: a pre-existing calicivirus (Cooke et al. 2002). If this is true, the influence of the pre-existing calicivirus is present in parts of southern mainland Australia, in Tasmania, and in narrow strips along part of the eastern and western coastlines. There is possibly a very weak effect attributable to p3SI at low latitudes in central Australia, but this is unsupported by other evidence. However, the difference in the respective bioclimatic contributors to $p1HD$ and $p3SI$ (Appendix 1) suggests that a different agent (such as a third virus) may be involved. The involvement of a third virus is plausible, as it appears that the New Zealand benign rabbit calicivirus is different from the European benign rabbit calicivirus (Lough 2000).

**Other model properties**

Our modelling suggests that under some circumstances the number of rabbits counted after an outbreak was actually higher than the number counted before. Measurement error is likely to have contributed to this phenomenon. Also, in many cases several months elapsed between an outbreak and the post-RHD count. Breeding could have occurred in this interval and contributed to the increase, although there may also have been further mortality from RHD or other causes.

Our model explains 52% of the variance in the proportional survival of rabbits following an RHD outbreak, and 84% of the variance in $\text{ln} \text{postRN}$. The unexplained variance derives from sources of variation not included in our model. These could relate to the influence of weather on RHD outbreaks, rabbit mortality or the visibility of rabbits during spotlight counts; to the occurrence of myxomatosis; to differences in breeding activity between sites not accommodated in our model; to a mismatch between our explanatory variables and the actual causes of variation in the effectiveness of RHD outbreaks; and to differences in methodology between observers in different parts of Australia – although the methodology was standardised as far as possible (Neave 1999).

The proportional survival of rabbits exposed to RHDV varies considerably, and our model cannot explain a substantial component of this variation. The standard error of the post-RHD counts estimated from the model is 0.67 (expressed as a natural logarithm), which is equivalent to a doubling or halving of the actual number of rabbits estimated to be present. Our model should be seen as a general indicator of expected survival rather than a precise predictor. Its main value will be to predict general variations in mean proportional survival due to differences in population densities, geographic location (and hence climate), and season. The model can be used to predict proportional survival for any one outbreak, but the high standard error of the estimate indicates that the prediction will be of interest as much for the extent to which reality departs from expectation as for the expectation itself. Further exploration of such departures may lead to a fuller understanding of the epizootiology of RHD, and to a refinement of our model.

**Experimental design, and the value of observational studies**

The absence of experimental controls from our observational study weakens our power to infer cause and effect, and may also have contributed to the high residual standard error of the post-RHD counts. This deficiency, together with difficulties in standardising methods of data collection across all 82 sites, may have led us to commit type I and type II errors. Where we have detected statistically significant correlations in the data (or, in the case of some seasonal effects, their absence), we have attempted to infer the causal mechanisms that may have underlain them and to establish their plausibility on the basis of other knowledge. Despite these weaknesses in Neave’s (1999) data set, these data, collected from across Australia and involving the cooperation of all States and Territories, provide a unique...
Survival of rabbits exposed to RHD

The opportunity to study regional variation in survival among rabbits exposed to a major new pathogen, an opportunity that is unlikely to be repeated. We believe that the results of our study, when appropriately qualified, justify the means used to conduct it. The interpretation of our model presented elsewhere in this discussion suggests several promising lines for further study and properly controlled experimentation, and ultimately such studies will support or refute our interpretations. In addition, our study allows observations made in one part of Australia to be viewed in a national context (e.g. the results described elsewhere in this volume, and Saunders et al. 1999).

The addition to the model of known, the categorical variable coding for the different methods used to determine that RHD had occurred at the site, was not significant, suggesting that all our methods of determination were equivalent. One of our methods was serology or virus capture performed on samples collected before, and during or shortly after, the purported outbreak, which indicated that RHD had occurred in the population for the first time, and we consider this method to be reliable. (Its reliability would have been increased by detailed epidemiological studies involving serology, virus capture, and the measurement of population size and morbidity and mortality among tagged rabbits, in addition to studies of other processes affecting population size, but this was not feasible at most sites, nor did we have access to these data where they existed.) We interpret the statistical equivalence of our six methods to mean that all were reliable.

Although we are confident RHD occurred at our sites between our pre- and post-RHD counts, our lack of experimental controls means that we cannot be so confident that factors such as breeding, drought, stress or parasite loads did not contribute, together with RHD, to the changes in population size we observed. However, from the results of our statistical modelling we can infer that some potentially confounding effects probably did not significantly influence our results. The two main potentially confounding effects are seasonal drought (especially during summer and autumn in winter-rainfall areas, and during winter and spring in summer-rainfall areas), and breeding. For example, it is possible that most rabbits survive RHD outbreaks during summer, and the population declines we observed (and attributed to RHD) during summer in winter-rainfall areas (Fig. 1d) were caused instead by the regular summer drought reducing the quantity and quality of feed in these areas. But if the summer drought in winter-rainfall areas had made a major contribution to the population declines we observed in summer in these areas, we should have observed a comparable interaction between season and p2WR in summer-rainfall areas in a season following summer, to reflect the drying out of pastures after the summer rains, or possibly the influence of parasites or stress. The interaction might be expected to be smaller, because the seasonality of the moisture regime is most marked in winter-rainfall areas with high values of p2WR (Appendix 1), and the seasonal effect could therefore be expected to be most marked there. The interactions between p2WR and autumn, winter and spring were not significant (so we pooled these three seasons in our final analysis). The inference we draw is that although regular seasonal drought causes mortality among rabbit populations, it was not a significant influence on our results in any season. Remember that we sought to reduce the influence of confounding effects by minimising the interval between the pre- and post-RHD counts. It is also possible that the two processes (starvation as pastures dry off, and mortality due to RHD) interact if they occur at about the same time, so that if RHD occurs first, starvation is reduced (because there is less competition for the small amount of better-quality forage remaining), and if starvation occurs first, mortality due to RHD is reduced (because the pre-RHD density of rabbits is reduced). Studies using appropriate experimental controls would be necessary to separate the two processes. See the discussion of the effect of p1HD, above, and of Saunders et al. (1999), below, for a consideration of breeding as a potentially confounding effect. A further discussion of potentially confounding factors can be found in Edwards et al. (2002).

Role of weather versus climate

The general scarcity of RHD outbreaks during summer in winter-rainfall areas may not be due to seasonally high temperatures per se, since our modelling suggests the outbreaks that do occur during summer can cause high mortality. Some other inhibitory factor must also be involved in order to explain this paradox. Temporal variation in weather during summer might lead to a general climate apparently unfavourable to RHDV but still provide some opportunities for the virus to cause disease. Factors that might vary in line with short-term weather include the level and diurnal pattern of vector activity; the level of inactivation of RHDV by heat and/or high humidity; the level of rabbit-to-rabbit transmission of the virus; variation in direct cutaneous, oral, conjunctival and respiratory intake of dried RHDV by rabbits in warrens; and the susceptibility of the rabbits themselves. The paradox would be resolved if the high mortality seen during the relatively few outbreaks that do occur during summer in winter-rainfall areas resulted from the temporary amelioration in one or more of the factors suppressing RHD during summer. Winter-rainfall areas tend to be near the coast, and experience marked cool changes during summer. It is possible, for example, that effective outbreaks during summer in these areas are propagated by a large, but ephemeral and possibly localised, amelioration in one or more aspects of the virus–vector–host system, and that this is associated with the passage of low pressure systems. This explanation begs the question of why such occurrences seem to be possible in areas with a
Mediterranean climate, but are apparently less likely in other coastal areas. The explanation for this discrepancy must be sought in other factors that differ between these areas, such as generally lower levels of soil moisture during summer in areas with a Mediterranean climate (see above), or in terms of other coastal effects due to the separate influence of $p3SI$. Our model deals with long-term climatic averages, not with the actual weather before, during and after an RHD outbreak. Weather is, of course, broadly correlated with seasonal climate. The separate influence of weather would be expected to be significant, as shown by Smyth et al. (1997). We do not know whether the summer outbreaks in our data set occurred during cool changes, but the data in Smyth et al. (1997) show that the outbreaks could have occurred when daily maximum temperatures were very high (>35°C). The only relevant measure of weather in our data set was the rainfall decile for the month in which RHDV arrived or was released at each site, and this was not significant in our model.

The escape of RHDV from Wardang Island to the north-east of South Australia in October 1995 was associated with weather arguably favourable for the long-distance movement of potential insect vectors (Wardhaugh and Rochester 1996). The correspondence between the areas of the outbreaks in October 1995 near Yunta and in the Flinders Ranges of South Australia shown in Fig. 1 in Kovaliski (1998), and the areas of exceptionally high rainfall for the same month (Queensland DNR 2000), is striking. Further investigation is warranted to see whether comparable rainfall events were associated with other long-distance movements of RHDV documented by Kovaliski (1998), and whether environmental conditions were also favourable for the long distance movement of insects from areas where RHDV was already present. But even if such a mechanism can be demonstrated, it would not explain the RHD outbreaks on Eyre Peninsula during the 1995–96 summer, as these required a source of virus, and, furthermore, the area received below-average rainfall that summer (Queensland DNR 2000).

Comparison with other studies

Study by Neave (1999)

The inclusion of pre-RHD rabbit density as an explanatory variable in our model has provided a readily interpretable alternative model to that presented in Neave (1999) for the same data set (82 sites). Neave’s analysis of her large data set (455 sites), where survival was measured as a categorical variable, produced a model with several features in common with ours, although the two models differed in the interaction terms. Our modelling revealed a positive interaction between arrival and $pIHD$, but it was not statistically significant ($F_{1,69} = 3.48$), and the term was eliminated from the final model. This interaction was significant in Neave’s analysis, indicating that releases were relatively more effective than natural outbreaks in cold, wet areas, and, conversely, relatively less effective in hot, dry areas. Neave (1999) interpreted this result in terms of the prior arrival of RHDV at some of the release sites, and the presumed presence of more immune rabbits in hot, dry areas than in cold, wet areas when releases were made. Our analysis incorporates the effect of population density on proportional survival, which Neave’s analysis of the categorical survival data set could not, and might be expected to be more powerful for that reason. However, Neave’s analysis had the advantage of a larger sample size (455 observations vs. our 78), which might explain our failure to detect a significant interaction. The role of the pre-existing benign calicivirus in this context is unclear, but it too may have contributed to our failure to detect a significant interaction.

Study by Lugton (1999)

Much of New South Wales experiences aseasonal or predominantly summer rainfall. In one of his analyses, Lugton (1999) found that the season of the outbreak did not influence the extent of RHD-induced population declines in New South Wales, whereas our modelling suggests that during summer it did. Lugton’s objective, which was different from ours, was to determine the circumstances conducive to effective releases of RHDV. He excluded from the analysis referred to above outbreaks that did not result in a population decline, and it appears that about 40% of the 457 observations were excluded on these grounds. It appears likely that a disproportionate number of these observations were during summer, and it is possible that this bias explains the difference between his result and ours. If we exclude from our analysis those outbreaks that did not result in a population decline, the seasonal effect disappears. We conclude that there is no contradiction between his results and ours with respect to the seasonality of RHD’s effectiveness.

Study by Saunders et al. (1999)

For three sites in central western New South Wales, Saunders et al. (1999) reported that rabbit population declines following RHD outbreaks were lower in summer ($n = 1$) than at other times of the year ($n = 2$), and in fact their post-RHD count for the summer outbreak was higher than their pre-RHD count. Data from these three summer-rainfall sites were included in our data set, and we were able to demonstrate the statistical significance of this effect for our entire data set. Saunders et al. suggested that the effect could be due to a higher proportional survival of rabbits exposed to RHDV in summer or to compensatory breeding in summer that negated initial mortalities due to RHD. Gilbert et al. (1987) have shown that breeding occurs 2–3 months earlier in winter-rainfall areas than in summer-rainfall areas, and if compensatory breeding were involved we would have
expected our modelling to reveal an equivalent statistical interaction between season and proportional survival in spring in winter-rainfall areas. No such interaction was observed. Our modelling therefore suggests that the major contributor to the effect observed by Saunders et al. (1999) was higher survival among rabbit populations exposed to RHDV in summer.

High survival during summer outbreaks in the study area of Saunders et al. (1999) could result in recruitment being higher than mortality during the summer breeding season, leading to an increase in the post-RHD population size whether compensatory breeding occurred or not. Breeding may not produce a comparable increase in numbers during the breeding season in other areas (and during seasons other than summer in summer-rainfall areas) because RHD kills many of the young rabbits. This is consistent with our conclusion that RHDV is inactivated during summer in summer-rainfall areas.

**Study by Smyth et al. (1997)**

Data presented in Smyth et al. (1997) appear to support the conclusions we have drawn from our data, although the authors interpret their data differently (see Appendix 2 for an appraisal of their data). We believe their data show that, within the range of daily maximum temperatures experienced at their sites (10–41°C), temperature extremes (>35°C) are not necessarily inimical to RHD outbreaks, provided the minimum daily RH is low. The combination of high temperature and high RH appears to suppress RHD outbreaks. This is consistent with the results of our modelling, which suggest that in summer-rainfall areas RHD outbreaks are inhibited only during summer, when temperature and RH both tend to be generally high or very high. Another indication from our modelling is that RHD can produce large population declines during summer in winter-rainfall areas, where high maximum temperatures in summer tend to be associated with low RH, and this also appears to be consistent with the data of Smyth et al. on the prevalence of outbreaks.

**Epizootiological implications**

Australian rabbit populations were immunologically naïve when first exposed to RHDV, and the seasonal pattern of occurrence of the initial RHD outbreaks in an area would have depended on the timing of RHDV’s arrival. The seasonal pattern of subsequent RHD outbreaks might differ from this initial pattern because the rabbit populations are no longer immunologically naïve, and because virus might persist in the area or nearby. Persistent virus could give rise to outbreaks at different times of the year from the initial outbreak of RHDV, and these might preclude further outbreaks at other times until a number of susceptible rabbits sufficient to support another outbreak is present in the population.

In the long term, our model might be able to be used to demonstrate increasing resistance (or its absence) in field populations. Adjustment of the population density to allow for the immune and very young components of the population, and a clarification of the separate roles of weather and climate, will be necessary before this can be achieved.

The Australia-wide generalisation regarding poor spread of RHDV during summer needs to be re-examined in areas with a Mediterranean climate, where RHD appears capable of causing large population declines in summer. Correlations between the likelihood of an outbreak and morbidity and mortality are poorly documented.

The apparent effectiveness of the RHD outbreaks that do occur during summer in winter-rainfall areas may depend on the prevalence of low humidity and dry soil in these areas during summer. These conditions may reduce or prevent the hypothesised inactivation of RHDV by the combination of high temperature and high humidity. The strength of this effect would be expected to be less pronounced in winter-rainfall areas if the summer was unusually wet, and we note that the 1995–96 summer – during which several outbreaks were observed on the Eyre Peninsula in South Australia (Kovaliski 1998) – was unusually dry. Variation at the local level might also be expected, and the effect might be less pronounced in warrens in low-lying areas and other places where the soil remained relatively moist during summer (Hall and Myers 1978). Further consideration of these climatic and edaphic factors is beyond the scope of this paper, but they would be fruitful areas for further work.

The differences in survival observed in our study, and their relationship to climatic and other factors, should allow epidemiological models of RHD in Australia to be refined and applied at a regional and seasonal level. The generality and realism of these models have to date been constrained by the paucity of data on the survival of rabbits exposed to RHD, among other things (e.g. Barlow and Kean 1998; Pech and Hood 1998). Our statistical model indicates that the parameter values in epidemiological models should vary geographically. Also, Barlow and Kean (1998) assumed that model parameters other than those related to rabbit reproduction did not vary seasonally, whereas we interpret the statistically significant interaction between season and \( pWR \) in our model to mean that they do (particularly Barlow and Kean’s virus decay rate, \( m \)). Pech and Hood (1998) assumed that the proportion of rabbits surviving a naturally occurring RHD outbreak varied from 0.05 to 0.35, whereas our modelling indicates that it may be substantially higher under certain circumstances (especially at low population densities at sites with low values of \( pHD \) or \( p3SI \), or during summer at sites with low values of \( p2WR \)).

In summary, the main implications of this study for RHD epizootiology are as follows:
RHD outbreaks are less effective at low densities of susceptible rabbits. Given the magnitude of the differences in proportional survival between high- and low-density rabbit populations, RHD could play an important density-dependent role in regulating population size at levels lower than those prevailing before RHDV arrived in Australia.

RHD outbreaks appear to be relatively ineffective in seasonal and summer-rainfall areas during summer, possibly because the virus survives poorly when exposed to high temperature and high RH and/or because vectors are ineffective. RHD is effective at other times of the year in these areas.

Given that an RHD outbreak has occurred, the disease appears capable of causing high mortality in winter-rainfall areas and hot, dry areas throughout the year. However, the likelihood of an outbreak occurring may be lower during summer than during other seasons.

RHD outbreaks are relatively ineffective in cold, wet areas, and along the eastern and western coastlines of mainland Australia, possibly because of the presence of one or more pre-existing benign caliciviruses in Australian wild rabbit populations. However, even in these areas, proportional survival can be low at high densities of susceptible rabbits, and under these circumstances RHD may provide effective control.

Acknowledgments

John Kovaliski prepared the maps of Australia showing the values of the bioclimatic principal components and provided intersite distances for the analysis of spatial dependence. Brian Cooke, Greg Mutze and Ron Sinclair participated in fruitful discussions regarding the interpretation of our statistical model, and Greg Mutze made constructive comments on the manuscript. Many people contributed to the collection and assimilation of the data in the national RHD database, and we owe them all a considerable debt of gratitude.

References


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Appendix 1. Principal component analysis of 35 bioclimatic variables derived by BIOCLIM (Nix 1986)

Latent roots were as follows: p1HD, 21.18; p2WR, 6.49; p3SI, 3.17. p1HD explained 60.52%, p2WR explained 18.55%, and p3SI explained 9.07% of the multidimensional variance in the 35 bioclimatic variables. The loadings of the bioclimatic variables are shown in Appendix Table 1. The spikes in Appendix Fig. 1 show the magnitude of the loadings of p1HD, p2WR and p3SI associated with each of the 35 original (bioclimatic) variables.

We interpret loading 1 (p1HD) as representing primarily an axis from hot–dry to cold–wet, and loading 2 (p2WR) primarily an axis from winter rainfall to summer rainfall. For example, high values of the annual mean moisture index (bioclimatic variable 28) make p1HD more negative, while high annual mean temperatures (bioclimatic variable 1) make p1HD more positive. Similarly, high summer rainfall makes p2WR more negative (bioclimatic variable 18). Winter rainfall sites would have a high moisture index seasonality (coefficient of variation) (bioclimatic variable 31) because rainfall is high when evaporation is low, and vice versa; this would lead to a large range in monthly soil moistures and hence to a high value of bioclimatic variable 31 and p2WR. The converse argument applies to summer-rainfall areas.

We interpret loading 3 (p3SI) as representing primarily an axis ranging from southern, inland areas with variable temperatures to uniformly warm coastal areas. Highly negative values of p3SI are associated with low temperature seasonality (bioclimatic variables 4 and 7), high temperature of the coldest time of the year (variables 6 and 11), high rainfall seasonality (variables 13, 14, 15 and 16), low radiation seasonality (variable 23), and low radiation of the warmest quarter (variable 26). These factors indicate primarily warm, coastal climates with marked rainfall seasonality (but with the season of highest rainfall unspecified). This interpretation is supported by an inspection of Appendix Table 2.

Reference

Appendix Table 1. Bioclimatic variables and their loadings derived from the principal components analysis

Loading 1 is denoted by $p_{1\text{HD}}$, loading 2 by $p_{2\text{WR}}$ and loading 3 by $p_{3\text{SI}}$

<table>
<thead>
<tr>
<th>No.</th>
<th>Bioclimatic variable</th>
<th>$p_{1\text{HD}}$</th>
<th>$p_{2\text{WR}}$</th>
<th>$p_{3\text{SI}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Annual mean temperature</td>
<td>0.19833</td>
<td>-0.02525</td>
<td>-0.19262</td>
</tr>
<tr>
<td>2</td>
<td>Mean diurnal range (mean (period max. – min.))</td>
<td>0.17834</td>
<td>-0.13607</td>
<td>0.04907</td>
</tr>
<tr>
<td>3</td>
<td>Isothermality (= 2/7)</td>
<td>-0.06978</td>
<td>0.15031</td>
<td>-0.21321</td>
</tr>
<tr>
<td>4</td>
<td>Temperature seasonality (coefficient of variation)</td>
<td>0.16558</td>
<td>-0.17228</td>
<td>0.15211</td>
</tr>
<tr>
<td>5</td>
<td>Maximum temperature of warmest month</td>
<td>0.20837</td>
<td>-0.04441</td>
<td>-0.04067</td>
</tr>
<tr>
<td>6</td>
<td>Minimum temperature of coldest month</td>
<td>0.08450</td>
<td>0.23297</td>
<td>-0.28766</td>
</tr>
<tr>
<td>7</td>
<td>Temperature annual range (= 5 – 6)</td>
<td>0.17869</td>
<td>-0.16014</td>
<td>0.09832</td>
</tr>
<tr>
<td>8</td>
<td>Mean temperature of wettest quarter</td>
<td>0.15585</td>
<td>-0.23800</td>
<td>-0.11670</td>
</tr>
<tr>
<td>9</td>
<td>Mean temperature of driest quarter</td>
<td>0.05229</td>
<td>0.32242</td>
<td>-0.13549</td>
</tr>
<tr>
<td>10</td>
<td>Mean temperature of warmest quarter</td>
<td>0.20572</td>
<td>-0.05676</td>
<td>-0.10783</td>
</tr>
<tr>
<td>11</td>
<td>Mean temperature of coldest quarter</td>
<td>0.17035</td>
<td>0.05834</td>
<td>-0.29479</td>
</tr>
<tr>
<td>12</td>
<td>Annual precipitation</td>
<td>-0.19635</td>
<td>-0.10601</td>
<td>-0.16053</td>
</tr>
<tr>
<td>13</td>
<td>Precipitation of wettest month</td>
<td>-0.15396</td>
<td>-0.06298</td>
<td>-0.34606</td>
</tr>
<tr>
<td>14</td>
<td>Precipitation of driest month</td>
<td>-0.19782</td>
<td>-0.10836</td>
<td>0.10186</td>
</tr>
<tr>
<td>15</td>
<td>Precipitation seasonality (coefficient of variation)</td>
<td>0.12052</td>
<td>0.05328</td>
<td>-0.39144</td>
</tr>
<tr>
<td>16</td>
<td>Precipitation of wettest quarter</td>
<td>-0.15749</td>
<td>-0.07050</td>
<td>-0.34056</td>
</tr>
<tr>
<td>17</td>
<td>Precipitation of driest quarter</td>
<td>-0.19904</td>
<td>-0.12848</td>
<td>0.06635</td>
</tr>
<tr>
<td>18</td>
<td>Precipitation of warmest quarter</td>
<td>-0.11621</td>
<td>-0.27337</td>
<td>-0.19492</td>
</tr>
<tr>
<td>19</td>
<td>Precipitation of coldest quarter</td>
<td>-0.18424</td>
<td>0.09430</td>
<td>-0.15868</td>
</tr>
<tr>
<td>20</td>
<td>Annual mean radiation</td>
<td>0.20678</td>
<td>-0.07080</td>
<td>-0.08365</td>
</tr>
<tr>
<td>21</td>
<td>Highest monthly radiation</td>
<td>0.16379</td>
<td>0.20133</td>
<td>0.06883</td>
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<td>Lowest monthly radiation</td>
<td>0.18967</td>
<td>-0.14365</td>
<td>-0.14734</td>
</tr>
<tr>
<td>23</td>
<td>Radiation seasonality (coefficient of variation)</td>
<td>-0.16150</td>
<td>0.21818</td>
<td>0.16691</td>
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<tr>
<td>24</td>
<td>Radiation of wettest quarter</td>
<td>0.10550</td>
<td>-0.30529</td>
<td>0.05642</td>
</tr>
<tr>
<td>25</td>
<td>Radiation of driest quarter</td>
<td>0.05892</td>
<td>0.30399</td>
<td>-0.16467</td>
</tr>
<tr>
<td>26</td>
<td>Radiation of warmest quarter</td>
<td>0.17754</td>
<td>0.10461</td>
<td>0.16675</td>
</tr>
<tr>
<td>27</td>
<td>Radiation of coldest quarter</td>
<td>0.18800</td>
<td>-0.14878</td>
<td>-0.15209</td>
</tr>
<tr>
<td>28</td>
<td>Annual mean moisture index</td>
<td>-0.21319</td>
<td>-0.02450</td>
<td>-0.03772</td>
</tr>
<tr>
<td>29</td>
<td>Highest monthly moisture index</td>
<td>-0.20424</td>
<td>0.06216</td>
<td>-0.01614</td>
</tr>
<tr>
<td>30</td>
<td>Lowest monthly moisture index</td>
<td>-0.19387</td>
<td>-0.15476</td>
<td>-0.02023</td>
</tr>
<tr>
<td>31</td>
<td>Moisture index seasonality (coefficient of variation)</td>
<td>0.02055</td>
<td>0.33656</td>
<td>0.05157</td>
</tr>
<tr>
<td>32</td>
<td>Mean moisture index of highest quarter</td>
<td>-0.20572</td>
<td>0.05837</td>
<td>-0.01601</td>
</tr>
<tr>
<td>33</td>
<td>Mean moisture index of lowest quarter</td>
<td>-0.19031</td>
<td>-0.16445</td>
<td>-0.05028</td>
</tr>
<tr>
<td>34</td>
<td>Mean moisture index of warmest quarter</td>
<td>-0.16952</td>
<td>-0.18830</td>
<td>-0.15673</td>
</tr>
<tr>
<td>35</td>
<td>Mean moisture index of coldest quarter</td>
<td>-0.20587</td>
<td>0.06710</td>
<td>-0.00190</td>
</tr>
</tbody>
</table>
Appendix Fig. 1. Pairwise plots of loadings of the three principal components derived from the 35 original (bioclimatic) variables. The spikes show the magnitude of the loadings associated with each variable. Loading 1 is $p1HD$, loading 2 is $p2WR$, and loading 3 is $p3SI$. 
### Appendix Table 2. Values of the bioclimatic principal components for the sites used in the statistical analysis

RLPB, Rural Lands Protection Board

<table>
<thead>
<tr>
<th>State</th>
<th>Site name</th>
<th>Longitude</th>
<th>Latitude</th>
<th>p1HD</th>
<th>p2WR</th>
<th>p3SI</th>
</tr>
</thead>
<tbody>
<tr>
<td>NSW</td>
<td>Central Tablelands – Bathurst 2</td>
<td>149.4560</td>
<td>−33.4580</td>
<td>−4.364</td>
<td>−2.855</td>
<td>0.918</td>
</tr>
<tr>
<td>NSW</td>
<td>Central Tablelands – Euchareena</td>
<td>149.2250</td>
<td>−32.9830</td>
<td>−3.220</td>
<td>−2.649</td>
<td>0.775</td>
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<tr>
<td>NSW</td>
<td>Lake Burrendong – 100% fox removal</td>
<td>149.1670</td>
<td>−32.6330</td>
<td>−1.987</td>
<td>−2.752</td>
<td>0.738</td>
</tr>
<tr>
<td>NSW</td>
<td>Lake Burrendong – no treatment (a)</td>
<td>149.2000</td>
<td>−32.7000</td>
<td>−2.302</td>
<td>−2.584</td>
<td>0.643</td>
</tr>
<tr>
<td>NSW</td>
<td>Lake Burrendong – no treatment (b)</td>
<td>149.1670</td>
<td>−32.6670</td>
<td>−2.140</td>
<td>−2.534</td>
<td>0.634</td>
</tr>
<tr>
<td>NSW</td>
<td>Lake Burrendong – 50–75% fox removal</td>
<td>149.2000</td>
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<td>−3.084</td>
<td>−2.661</td>
<td>0.791</td>
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<tr>
<td>NT</td>
<td>Erldunda – new tank (ripped pre-RHD)</td>
<td>133.1790</td>
<td>−25.4150</td>
<td>6.143</td>
<td>−1.455</td>
<td>0.105</td>
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<tr>
<td>NT</td>
<td>Erldunda – Knob Dam (ripped post-RHD)</td>
<td>133.1630</td>
<td>−25.4540</td>
<td>6.136</td>
<td>−1.439</td>
<td>0.092</td>
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<tr>
<td>NT</td>
<td>Erldunda – No. 7 Bore (unripped)</td>
<td>133.1780</td>
<td>−25.6060</td>
<td>5.931</td>
<td>−1.417</td>
<td>0.220</td>
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<tr>
<td>QLD</td>
<td>Munrooie Lakes</td>
<td>138.6336</td>
<td>−32.2557</td>
<td>−8.644</td>
<td>−2.275</td>
<td>−4.679</td>
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<tr>
<td>WA</td>
<td>Stirling Ranges – remnant vegetation/agricultural</td>
<td>118.3667</td>
<td>−34.5333</td>
<td>−2.870</td>
<td>4.534</td>
<td>−2.019</td>
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<tr>
<td>TAS</td>
<td>North Tasmania</td>
<td>146.9667</td>
<td>−41.5000</td>
<td>−7.186</td>
<td>1.622</td>
<td>−0.373</td>
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<tr>
<td>SA</td>
<td>Strzelecki</td>
<td>140.3330</td>
<td>−31.0300</td>
<td>4.400</td>
<td>−0.792</td>
<td>2.204</td>
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<tr>
<td>ACT</td>
<td>Boboyan</td>
<td>149.0150</td>
<td>−35.8700</td>
<td>−5.073</td>
<td>−2.619</td>
<td>2.634</td>
</tr>
<tr>
<td>ACT</td>
<td>Glendale</td>
<td>149.9550</td>
<td>−35.6290</td>
<td>−6.677</td>
<td>−3.071</td>
<td>1.542</td>
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<td>ACT</td>
<td>Googong Foreshores</td>
<td>148.9770</td>
<td>−35.8460</td>
<td>−5.878</td>
<td>−2.852</td>
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<tr>
<td>ACT</td>
<td>Mt Clear</td>
<td>149.2450</td>
<td>−35.4150</td>
<td>−3.804</td>
<td>−2.409</td>
<td>1.998</td>
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<tr>
<td>NT</td>
<td>Mount Wedge Station – Yelabra Bore (unripped)</td>
<td>131.6010</td>
<td>−22.9060</td>
<td>6.527</td>
<td>−1.064</td>
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<tr>
<td>NT</td>
<td>De Rose Hill – Fish Tank (ripped pre-RHD)</td>
<td>133.2430</td>
<td>−26.4410</td>
<td>5.685</td>
<td>−1.243</td>
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<tr>
<td>NT</td>
<td>De Rose Hill – Ant well (ripped post-RHD)</td>
<td>133.2070</td>
<td>−26.5690</td>
<td>5.658</td>
<td>−1.223</td>
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<td>NT</td>
<td>De Rose Hill – Kangaroo well (unripped)</td>
<td>133.1640</td>
<td>−26.6520</td>
<td>5.671</td>
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<td>NT</td>
<td>Mt Riddock – Eric’s Dam (to be ripped post-RHD)</td>
<td>134.8490</td>
<td>−22.7490</td>
<td>6.019</td>
<td>−1.694</td>
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<td>NT</td>
<td>Owen Springs – Station Dam (ripped post-RHD)</td>
<td>133.5190</td>
<td>−23.9020</td>
<td>5.772</td>
<td>−1.973</td>
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<td>NT</td>
<td>Owen Springs – No. 1 Dam (unripped)</td>
<td>133.6270</td>
<td>−23.8620</td>
<td>5.744</td>
<td>−1.778</td>
<td>−0.585</td>
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<td>NT</td>
<td>Simpson Desert – south transect (rabbits present)</td>
<td>136.1480</td>
<td>−25.0290</td>
<td>6.987</td>
<td>−1.014</td>
<td>−0.604</td>
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<td>NT</td>
<td>The Garden/Armidalmond – Cavenagh’s Well (ripped pre-RHD)</td>
<td>134.4620</td>
<td>−23.3050</td>
<td>5.613</td>
<td>−2.012</td>
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<td>NT</td>
<td>The Garden/Armidalmond – Ulgnamba Bore (ripped post-RHD)</td>
<td>134.6180</td>
<td>−23.3370</td>
<td>5.694</td>
<td>−2.007</td>
<td>−1.169</td>
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<tr>
<td>NT</td>
<td>The Garden/Armidalmond – Coxs’s well (unripped)</td>
<td>134.5530</td>
<td>−23.3270</td>
<td>5.642</td>
<td>−2.012</td>
<td>−1.148</td>
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<td>Benandre</td>
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<td>−29.1060</td>
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<td>QLD</td>
<td>Whetstone</td>
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<td>SA</td>
<td>Elliston – Sheringa</td>
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<td>SA</td>
<td>Merna Mora</td>
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<td>3.698</td>
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<td>1.567</td>
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<tr>
<td>SA</td>
<td>Morialpa</td>
<td>139.7920</td>
<td>−32.2000</td>
<td>3.233</td>
<td>0.018</td>
<td>2.324</td>
</tr>
</tbody>
</table>
Appendix 2. Appraisal of the data presented in Smyth et al. (1997)

Smyth et al. (1997) used daily weather records to infer the factors promoting the spread and number of naturally occurring outbreaks of RHD in south-eastern Australia from the time RHDV escaped off Wardang Island in October 1995 until June 1996. They concluded that the effective spread of RHDV depended primarily on temperature, with the disease doing best at moderate temperatures (around 24°C). They considered its influence on the behaviour of RHD to be subordinate to the disease doing best at moderate temperatures (around 24°C). They concluded that outbreaks that do occur on the other, are interrelated in practice.

Our interpretation of their data is based first on a comparison of the tails of their frequency distributions: that of maximum daily temperature for occasions when RHD occurred and that of the equivalent distribution for all occasions (that is, when RHD occurred and also when it did not) (Figs 20 and 21 in Smyth et al. 1997). The former data are a subset of the latter. The comparison suggests that RHD occurred disproportionately at both very high temperatures and very low temperatures. This suggests that, within the range of daily maximum temperatures experienced at their sites (10–41°C), extremes are not necessarily inimical to RHD; if anything, the reverse is the case.

Further, we interpret Fig. 6 in Smyth et al. (1997) (see the central, temperature-contoured section of this figure) as showing that RHD occurred relatively frequently during the late spring and early summer of 1995–96 on days and at sites where daily maximum temperatures exceeded 35°C, but that these occurrences were not necessarily unfavourable to the occurrence of RHD outbreaks.

At that time (late spring and early summer), RHD also occurred relatively frequently at sites experiencing maximum temperatures of 25–30°C and minimum RH of 40%. Later that summer, and early the following autumn, temperatures over the study area fell. However, RHD outbreaks...
continued to be rare despite the return of lower maximum temperatures (of 25–30°C) at sites with RH of 40–80%, circumstances under which RHD had been relatively common the previous spring. Although the data presented in Fig. 6 of Smyth et al. are complex, it appears that an explanation for the failure of RHD to reappear must include factors other than, or in addition to, the prevalence of temperatures of 25–30°C and RH of 40–80% at that time (mid to late summer).

In the main text we hypothesised that a source of virus responsible for the outbreaks in late spring and early summer (at temperatures of 25–30°C and RH of 40–80%) was destroyed by the spell of weather that brought high temperatures (30–35°C) and moderately high RH (40–80%). High levels of RHD did not return until the following autumn, when temperatures generally fell below 25°C. In the main text we suggest that the supply of virus may have been replenished at that time by soil disturbance associated with the preparation of warrens for breeding.

We believe that the data presented in Smyth et al. (1997) are consistent with the results of our modelling, which suggest that in summer-rainfall areas RHD is inhibited during summer, when temperatures and RH would both tend to be high. Another indication from our modelling is that RHD can produce large population declines during summer in winter-rainfall areas, where summer RH is usually low when temperatures are high, and this appears also to be consistent with data of Smyth et al. on the prevalence of outbreaks.

**Reference**


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