The emergence of rabbit haemorrhagic disease virus: will a non-pathogenic strain protect the UK?

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Rabbit haemorrhagic disease virus emerged in China in 1984, and has killed hundreds of millions of wild rabbits in Australia and Europe. In the UK there appears to be an endemic non-pathogenic strain, with high levels of seroprevalence being recorded, in the absence of associated mortality. Using a seasonal, age-structured model we examine the hypothesis that differences in rabbit population demography differentially affect the basic reproductive rates (R_0) of the pathogenic and non-pathogenic strains, leading to each dominating in some populations and not others. The strain with the higher R_0 excluded the other, with the dynamics depending upon the ratio of the two R_0 values. When the non-pathogenic strain dominated, the pathogenic strain caused only transient mortality, although this could be significant when the two R_0 values were similar. When the pathogenic strain dominated, repeated epidemics led to host eradication. Seroprevalence data suggest that the non-pathogenic strain may be protecting some, but not all UK populations, with half being 'at risk' from invasion by the pathogenic strain and a fifth prone to significant transient mortality. We identify key questions for empirical research to test this prediction.

Keywords: rabbit haemorrhagic disease virus; non-pathogenic strain; strain competition; mathematical model; population dynamics

1. INTRODUCTION

Emerging diseases reflect the appearance and increase in prevalence of a group of important pathogens that have provided significant threats to humans, their livestock and conservation. While a large number of these pathogens are new to science, most appear to have jumped from wildlife reservoirs as a consequence of changes in ecological factors such as changes in land use rather than major evolutionary changes in either pathogens or hosts (Schrage & Wiener 1995).

Rabbit haemorrhagic disease virus (RHDV) is a highly virulent pathogen that kills up to 95% of infected rabbits 48 hours post-infection (Barbieri *et al.* 1997; Cancellotti & Renzi 1991; Lenghaus *et al.* 1994; Mutze *et al.* 1998). It was first recorded in China in 1984, where it killed 140 million farmed rabbits, following the importation of infected rabbits from Germany (Liu *et al.* 1984). The first cases in wild rabbits in Europe were recorded from 1986, in Italy (where 64 million farmed rabbits also died), the former Czechoslovakia, Germany, Denmark and Poland (Rodak *et al.* 1991; Smid *et al.* 1989, 1991). RHDV is now found throughout Europe and the Middle East (Nowotny *et al.* 1997). In 1995 the virus was released in Australia in an attempt to control pest rabbit populations, resulting in the death of 30 million wild rabbits in just a few weeks (Mutze *et al.* 1998). In Europe there are

serious conservation concerns because rabbits are an important prey species for many predatory birds, and mammals such as the endangered Iberian lynx. In the UK, rabbits play a critical role in maintaining floral biodiversity on chalk grassland and lowland heaths and dunes.

Interestingly, there have been numerous cases in which rabbits have been found to be seropositive to RHDV without any significant mortality attributable to the disease (Capucci *et al.* 1997; Chasey *et al.* 1995; Nowotny *et al.* 1997; Rodak *et al.* 1990, 1991). This applies to samples from the former Czechoslovakia, Switzerland, Austria, Germany, Sweden and the UK, which came mostly from laboratory and farmed rabbits, where the disease would certainly have been noticed. Furthermore, the Czechoslovakian sera were collected as early as 1975 (Rodak *et al.* 1991), a decade before the discovery of RHDV. Seroprevalence in wild populations was 19% in the former Czechoslovakia, 46% in Lower Saxony and 12% in the former German Democratic Republic in 1989–1990 (Loliger & Eskens 1991; Rodak *et al.* 1990).

Several authors have proposed that this widespread immunity may be due to the presence of an endemic non-pathogenic (N) strain of RHDV (Chasey *et al.* 1995; Nowotny *et al.* 1997; Rodak *et al.* 1990, 1991; Trout *et al.* 1997b). Indeed an N-strain, which confers protective immunity to pathogenic (P) strain RHDV, was isolated from an Italian rabbit farm in 1996, where it was highly prevalent and had persisted for at least 2 years (Capucci *et al.* 1996, 1997). Since captive populations have been

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Figure 1. Seroprevalence of individuals with protective immunity to RHDV recorded in the UK between January and March 1995. (a) Sites sampled (Chasey *et al.* 1997); triangles indicate islands. (b) Boxplots of seroprevalence data. Devon and Kent are listed individually (as well as being included in the figures for England and UK) since they have had most of the recorded wild outbreaks of P-strain RHDV. Numbers in parentheses indicate number of populations sampled. Data from Chasey *et al.* (1997) and Trout *et al.* (1997b).

found to have the same P-strain sequence variants as nearby wild ones (Le Gall *et al.* 1998; Nowotny *et al.* 1997), we would expect wild populations also to have the N-strain. However, it has not been confirmed in wild populations, to date, although this may be because there has been little attempt to find it (tissue samples only tend to be taken following an outbreak of the P-strain) and it is found only at very low levels in the host, predominantly in the intestine (Capucci *et al.* 1997), which is normally not sampled.

In the UK the first outbreaks of RHDV in wild rabbits were recorded in 1994, and scattered outbreaks have since occurred country-wide (Trout *et al.* 1997a; R. C. Trout and P. J. White, unpublished data). However, it does not appear to have had any significant impact, to date, on the national population of rabbits, either in terms of the numbers of sites affected or in the total number of rabbits killed. A seroprevalence survey was performed throughout the UK in January–March 1995, when 68 populations, not known to have experienced RHDV, were sampled by shooting (Trout *et al.* 1997b). At this time of year, immediately prior to the breeding season, the mean age of the population is at its greatest and consequently we expect these seroprevalence figures to be the peak prior to a fall due to the appearance of susceptible juveniles. Unfortunately, the survey was not repeated so there are no data regarding how seroprevalence changed over the year or in subsequent years. Animals scored 'positive' had antibody titres that had been shown to be protective against experimental challenge (Chasey *et al.* 1997; Trout *et al.* 1997b). All populations sampled had 10–100% of individuals seropositive, with a mean of 64% (figure 1). There is a trend of seroprevalence increasing with latitude (general linear model (GLM) of arcsine-transformed seroprevalence data, $p < 0.003$). The majority of RHDV outbreaks have been recorded in the south, particularly in Devon and Kent, where seroprevalence was lowest. Most UK populations had much higher seroprevalence than mainland European populations, which may explain the limited impact of RHDV in the UK, if widespread immunity were preventing invasion.

Is it plausible that an N-strain may be protecting UK rabbit populations by immunizing them against P-strain RHDV? If so, then why has it not protected all UK populations, and apparently protected very few European ones? Why is seroprevalence in the UK so variable, and what level of seroprevalence is required to protect a population, to exclude the P-strain ultimately, and to prevent significant transient mortality? Can we determine if an important population, such as in a conservation area, is 'at risk' from RHDV? What proportion of UK populations is 'at risk' from the P-strain and what is the likely long-term impact of RHDV on the UK?

2. PROPERTIES OF RHDV STRAINS

RHDV is a non-enveloped RNA virus of the class Caliciviridae. Its only known host is the European rabbit (*Oryctolagus cuniculus*) (Lenghaus *et al.* 1994; Lavazza *et al.* 1996; Smid *et al.* 1991). It exhibits remarkably little amino-acid sequence variation, just 2% over the VP60 capsid protein (Capucci *et al.* 1996; Le Gall *et al.* 1998; O'Keefe *et al.* 1998). The greatest diversity of sequence variants was found in Austria, Germany, Italy and Sweden, suggesting that RHDV probably originated in central Western Europe (Nowotny *et al.* 1997), which is also where the first European outbreaks were recorded. There is no evidence for attenuation, which is in marked contrast to myxoma virus, which attenuated within a year of its release (Fenner & Myers 1978). The N-strain shares 91.5% amino-acid identity with P-strain RHDV sequence variants (Capucci *et al.* 1996), which is substantially less than the 98% identity that they share with each other.

Due to the cross-immunity between the two strains (Capucci *et al.* 1996; Trout *et al.* 1997b), the one with the lower R_0 will be excluded (Bremermann & Thieme 1989). Thus populations will be 'protected' by the putative N-strain where it is endemic and has a higher R_0 than the P-strain. In other populations the P-strain will invade and cause significant mortality, possibly even local host eradication.

The winter seroprevalence range of 10–100% indicates substantial differences in the transmission success of the

N-strain. It is important to understand the factors underlying those differences, because some of them will affect the transmission of the P- and N-strains differentially, favouring one or the other, and other factors will affect the transmission of both strains similarly and so not discriminate between them.

A number of potential factors may explain the range of seroprevalence throughout the UK, and the failure of the P-strain to do significant harm. These include differences in contact rates between rabbits within different populations, differences in population demography, the presence or absence of different vectors, differences in genetic resistance among rabbits, the presence of different N-strains of RHDV and an interaction with myxoma virus. Current data only allow examination of the first two possibilities.

It is not known what transmission routes predominate in the field, for either strain of virus. However, for both strains, inoculated rabbits infect cage-mates and virus can be detected in the airways (Capucci *et al.* 1996, 1997; Lenghaus *et al.* 1994), implicating close contact. If the strains share a transmission route then they will experience the same contact rate. Thus if most of the variation in seroprevalence were due to differences in contact rates then the majority of outbreaks of the P-strain would occur where there is the highest seroprevalence due to the N-strain. This is the opposite of what has occurred. Although high seroprevalence may prevent a sudden decline due to an epidemic, we would still expect to see a marked decline in the population, which also has not occurred.

Clearly, the P- and N-strains of RHDV have different strategies, perhaps suited to different habitats or different conditions. P-strain-infected rabbits are highly infectious for a short time, with most lost due to virus-induced mortality. Survivors of the acute phase did not infect susceptible rabbits exposed to them (Lenghaus *et al.* 1994). While pathogenic virus can be detected in infected hosts even after 10^9 -fold dilution of liver homogenate (Lenghaus *et al.* 1994), detection of the N-strain requires very sensitive techniques (Capucci *et al.* 1997). Therefore we expect those infected with the N-strain to be much less infectious. Most likely there is a trade-off between the amount of viral replication (which determines infectiousness) and virulence (the rate of killing the host) (Bull 1994). We postulate that the N-strain has a much longer infectious period and since it appears that transmission can occur even after an immune response (Capucci *et al.* 1997), this period may even be lifelong. Many viruses cause long-term infectiousness, despite the presence of circulating antibodies, including the related feline calicivirus and other RNA viruses such as *Herpes simplex* virus (Baczko *et al.* 1993; Monath 1986; Wald & Corey 1996).

The longer infectious period of the N-strain will make it more sensitive to host demography, because the natural death rate of the host population accounts for the loss of more N-strain-infected rabbits than P-strain-infected ones. The N-strain R_0 will be variable, whereas the P-strain R_0 will be similar in all populations. The degree of sensitivity of the N-strain R_0 to differences in the natural death rate depends upon the infectious period, which is not known. In this paper we assume infectiousness to be lifelong, leading to maximum sensitivity to natural death rate. We address the parsimonious hypothesis that demo-

graphic differences in UK rabbit populations underlie the different transmission success of a single N-strain in those populations, which explains both the variation in seroprevalence and why some populations are protected from the P-strain, and others not.

3. THE MODEL

(a) Overview

Rabbit populations in the UK are highly seasonal, with typically a three- to fourfold increase in numbers from the winter minimum to the midsummer peak (Cowan 1983), due to seasonal breeding with high fecundity and high juvenile mortality. There is substantial year-to-year variation in rabbit abundance on individual sites (Trout *et al.* 2000), which reflects variation in productivity, while adult mortality does not change greatly (Bell & Webb 1991; Cowan 1983; Mead-Briggs *et al.* 1975). Winter minimum population sizes remain stable from year-to-year (Cowan 1983). To capture these dynamics accurately and to investigate whether differences in the dynamics of different populations could explain the variation in the seroprevalence figures, a seasonal, age-structured model was developed. We estimate the transmission parameter for an endemic N-strain using demographic parameters for a typical UK population and the mean UK winter seroprevalence estimate. Then we investigate if the observed variation in UK seroprevalence can be explained by differences in host demographic parameters within the range recorded for British populations, specifically, differences in adult mortality and productivity. Population counts data from Dawlish Warren in Devon, a site that experienced an outbreak of RHDV in summer 1995, are used to estimate the transmission parameter for the P-strain. The impact of the introduction of the P-strain into different UK populations where the N-strain is endemic is then examined.

(b) Description of the model

The model is shown schematically in figure 2, with parameters summarized in table 1. An algebraic description is found in Appendix A. The population is divided into juvenile and adult age-classes, represented by $h\{J, A\}$. Virus strains are represented by $i\{N, P\}$. Within each rabbit age-class, there are compartments for individuals that are susceptible (S_h), infected with the N-strain (I_{hN}), infected with the P-strain (I_{hP}), and recovered (R_h). Note that individuals can only become infected with one strain, due to their immune response. Rates of recovery (σ_i) and disease-induced death (α_i) are not dependent upon age-class. The rates of loss of infected individuals (d_{hi}) are dependent upon both the strain of virus (which determines $\sigma_i + \alpha_i$) and the age-class of the host infected with it (which determines b_h).

(c) Rabbit population demographic parameters

Productivity is defined in this study as the rate at which juvenile rabbits are weaned and emerge from the warren, and is the product of the birth rate and nestling survival rate. UK birth rates of 14–22 per adult female per annum (p.a.) (Trout & Smith 1995), combined with nestling survival in the range 25–75% (Cowan 1983), give a productivity range of 1.75–8.25 young weaned per adult

Figure 2. Flow diagram of the compartmental model that incorporates two age-classes of rabbits, juveniles (J) and adults (A) and the two strains of virus, (P-strain and N-strain). Susceptible rabbits (S) may become infected (I) with either the P- or N-strain, and may then recover (R). Those infected with the P-strain incur disease-induced death at rate α_P . Individuals in all compartments incur natural death at rates b_J (juveniles) and b_A (adults) but these rates are omitted, for clarity. The annual maturation event, $m(t)$, is indicated by the broken arrows.

p.a., consistent with Bell & Webb (1991). In the model, the breeding season is represented by a step function, with five months of breeding and seven months of no breeding per year. The productivity rate used in the model ($a(t)$) is thus a function of time, taking the value zero outwith the breeding season. During the breeding season its value equals the number of juveniles weaned per adult p.a. divided by the breeding season length, in days. There is density dependence in the productivity rate, dependent upon the adult population. The density dependence is probably acting first on the quality of the nesting site (affecting nestling survival) and secondly on access to food resources (affecting the proportion of females breeding and litter size), and in both cases, adults are the main competitors. Two parameters describe the density dependence: Q , the maximum fold increase in the daily productivity rate in the absence of density-dependent constraints; and q , the mean number of adults

alive during the breeding season, in the absence of disease. Q is the analogue of the maximum growth rate in a single age-class model, and q corresponds to the reciprocal of carrying capacity, used in non-seasonal models. In the absence of density-dependent constraints, the productivity rate per adult is aQ , and declines linearly with increasing adult density, H_A . At the average adult population level over the breeding season, when $H_A = q$, the specified productivity rate is achieved. Note that q is a property of the site, reflecting nesting-site quality and resource availability and is not affected by the presence or absence of disease. In the model we assume that the maximum productivity rate is double the mean rate ($Q = 2$), which concurs with the findings of Myers *et al.* (1979) and Thompson (1994).

The adult natural daily death rate (b_A) is calculated from annual mortality, which ranges from 40 to 80% p.a. in different populations, with a mean of 60%. In the absence of disease, the juvenile natural death rate (b_J , which includes dispersal) is fitted for each combination of adult mortality and productivity, to maintain an arbitrary winter minimum population of 50 individuals from year-to-year. Thus unsurprisingly b_J is correlated with productivity. The adult population declines exponentially, to be 'topped-up' annually by recruitment of surviving juveniles, in a discrete instantaneous event that occurs immediately prior to the breeding season. In the model this is represented by the 'maturation function', $m(t)$, which transfers surviving juveniles into the adult age-class. Recruits retain their 'disease status', i.e. susceptible juveniles become susceptible adults; infected juveniles become infected adults, etc. The simulation of a disease-free, typical UK rabbit population is shown in figure 3.

(d) P- and N-strains of RHDV

The mean survival time of rabbits experimentally infected with the P-strain of RHDV is 2 days with up to 95% of infected rabbits dying and survivors becoming immune for life (Barbieri *et al.* 1997; Cancellotti & Renzi 1991; Capucci *et al.* 1996; Lenghaus *et al.* 1994; Mutze *et al.* 1998). Thus the rate of loss of experimentally infected individuals is $0.5 \text{ d}^{-1} = (\alpha_P + \sigma_P)$ and the proportion recovering is $0.05 = \sigma_P / (\alpha_P + \sigma_P)$; so $\alpha_P = 0.475 \text{ d}^{-1}$; $\sigma_P = 0.025 \text{ d}^{-1}$. The N-strain does not induce mortality ($\alpha_N = 0$) and we postulate that it causes lifelong infectiousness ($\sigma_N = 0$) as discussed above (§ 2).

Table 1. Parameter of the model.

(Further details are provided in § 3.)

$a(t)$	productivity rate per adult	outwith the breeding season, $a(t) = 0$; during the breeding season, $a(t)$ has values in the range 0.0117–0.0550 (corresponding to 1.75–8.25 weaned per adult p.a.)
Q	maximum fold increase in productivity upon removal of density-dependent constraints	2
q	mean number of adults alive during the breeding season, in the absence of disease	36.5–45.1 (corresponding to adult mortality range 40–80% p.a.)
b_h	natural death rate	b_A range 0.001 42–0.004 47 (adult mortality range 40–80% p.a.); b_J , range 0.00309–0.0106
$m(t)$	maturation function	transfers all juveniles alive at the end of the year into the adult age class of the same disease status
α_i	disease-induced death rate	$\alpha_N = 0$ (does not induce mortality); $\alpha_P = 0.475$
σ_i	rate of immune recovery from infection	$\sigma_N = 0$ (lifelong infectious period); $\sigma_P = 0.025$
β_i	transmission parameter	$\beta_N = 0.006 43$; $\beta_P = 0.812$

Figure 3. (a) Disease-free dynamics of a typical UK rabbit population, with adult mortality of 60% p.a. and productivity rate of five juveniles weaned per adult p.a. The thick line represents the total population; the thin line, adults and the broken line, juveniles. The breeding season is five months long (horizontal bars) and surviving juveniles mature into adults at the commencement of the breeding season. The winter minimum population is maintained from year-to-year at an arbitrary 50 individuals, by fitting the juvenile death rate, b_j , so the number of surviving juveniles equals the number of adults lost over the year. Seroprevalence due to the endemic N-strain is indicated. Note that the winter maximum of 64% (arrows) is reached each year. (b) Seroprevalence declines during the breeding season as susceptible juveniles enter the population and increases again, post-breeding. The model year comprises 12 months of 30 days each.

Rabbits live in social groups, each with its own territory; thus rabbit density remains roughly constant, with the area occupied by them changing in response to changes in population size. Thus the contact rate between rabbits is likely to be frequency-dependent, 'true mass action' *sensu* de Jong *et al.* (1995), which is supported by Begon *et al.* (1999).

(e) Estimation of the transmission parameter for the N-strain (β_N)

A notoriously difficult parameter to estimate in many epidemiological studies is the transmission parameter, β , which is usually fitted to data. For the N-strain, β_N was estimated by assuming that a population with typical UK population demography (adult mortality 60% p.a., five juveniles weaned per adult p.a.) will have a winter seroprevalence at the typical UK level of 64%. β_N was fitted to maintain this winter maximum seroprevalence from year-to-year ($\beta_N = 0.00643$). Note that seroprevalence declines during the breeding season as new susceptibles enter the population, and then increases, peaking at the start of the next breeding season (figure 3).

(f) Sensitivity of winter maximum seroprevalence to population demography

What range of winter maximum seroprevalences can be explained by variation in population demography?

Using the β_N value estimated above, adult mortality was varied from 40 to 80% p.a. and productivity varied independently from 1.75 to 8.25 per adult p.a. (subject to the constraint that the juvenile natural death rate (b_j) was not less than that of the adults (b_A), which meant that the minimum productivity rates were 2.34 juveniles per adult p.a. for populations with 70% adult mortality p.a., and 4.01 for 80% adult mortality p.a.). The model predicted winter maximum seroprevalences in the range 27–86%, which encompasses the figures recorded for three-quarters of the UK sites sampled (52 out of 68). Most sites with seroprevalence levels outwith this range were close to it such that a range of 27–90% encompasses 59 out of 68 (87%) of the UK sites sampled.

(g) Estimation of the transmission parameter for the P-strain (β_P)

To estimate the transmission parameter of the P-strain we used the available data of monthly counts of the rabbit population at Dawlish Warren in Devon, where an outbreak of the P-strain of RHDV began in summer 1995 (Trout *et al.* 1997a). Counting began before the outbreak and continued afterwards. In the first month of the outbreak the population declined by 63% from its peak. In the following months the population was at 31 and 11% of the summer peak. Unfortunately, there are no seroprevalence data for this site and demographic parameters are not known. However, several other sites in Devon were sampled in the winter prior to the outbreak and the mean seroprevalence from these six sites was 45% with the nearest site being 43%. Since these estimates were similar we assumed they were representative of Dawlish Warren, indicating demographic parameters of 75% adult mortality p.a., with productivity of 5.9 weaned per adult p.a. Using these demographic parameters, and with the N-strain endemic, a single adult infected with the P-strain was introduced at the end of the breeding season (day 150.5) when the population was at its peak size. The count data were then scaled so the peak count (July) equalled the peak model population size, at the end of the breeding season. Using the method of least squares, β_P was fitted to the scaled counts data for August, September and October (figure 4), giving the result $\beta_P = 0.812$. As expected the P-strain-infected host is much more infectious than the N-strain-infected host: $\beta_P/\beta_N = 126$.

(h) R_0 estimation

Due to the seasonality and constantly changing age structure of the population, R_0 cannot be calculated by a simple algebraic formula. However, for the P-strain it can be approximated by the ratio $\beta_P/(b_{avg} + \alpha_P + \sigma_P)$ where b_{avg} is the weighted mean natural death rate, estimated over the year, in the absence of disease, for each set of demographic parameter values. This gives values in the range 1.60–1.61, i.e. less than 1% variation. Because the rate of loss of infected hosts is dominated by the disease-induced death rate (α_P), which is much greater than the natural death rates of both adults and juveniles, the mean life-time of a P-strain-infected host is almost identical for adults and juveniles, so seasonality and age structure have little impact. This is not true for the N-strain, and $\beta_N/(b_{avg} + \alpha_N + \sigma_N)$ underestimates $R_{0,N}$ because the adult

Figure 4. Model fit to counts data during an outbreak at Dawlish Warren, Devon, in 1995 (Trout *et al.* 1997a). The solid line shows the population size predicted by the model with adult mortality of 75% p.a., 5.9 juveniles weaned per adult p.a. and a single adult infected with the P-strain introduced at the end of the breeding season when the population was at its peak. The broken line shows the predicted decline in the population after the end of the breeding season, in the absence of disease. Crosses indicate the mean monthly count of the six sectors of the site, scaled so that the maximum population size predicted by the model equalled the maximum mean count. Bars show standard errors.

natural death rate (b_A) is much less than b_{avg} . For the N-strain, R_0 can be estimated from the mean seroprevalence predicted when it is stably endemic in the absence of the P-strain: $R_{0,N} = 1/(1-MS)$ where MS is the mean seroprevalence. This gives estimates for $R_{0,N}$ in the range 1.16–2.95. This approach is not appropriate for the P-strain, which causes host eradication for almost all values of $R_{0,P} > 1$. These methods of R_0 estimation were validated by simulations examining criteria for invasion of a naive population ($R_0 > 1$), and for exclusion of one strain by the other (i.e. the strain with higher R_0 excludes the one with the lower R_0). The key finding is that the N-strain is much more sensitive to host demography with its maximum R_0 being 254% of its minimum, compared with the less than 1% range of the P-strain R_0 .

(i) **Impact of the introduction of the P-strain into different rabbit populations**

One of the aims of this paper is to investigate how the introduction of the P-strain affects populations with different demographic parameters. Simulations of the model were run using different adult mortality and productivity parameters and with the N-strain endemic. The P-strain was then introduced. The timing of the introduction of the P-strain affected the precise dynamics of the disease but not the mode of behaviour, i.e. transient invasion, repeated epidemics, population decline. The strain with the higher R_0 ultimately excluded the strain with the lower R_0 but different dynamics was observed, depending upon the ratio of the two R_0 values (P-strain:N-strain). When the R_0 ratio is less than 0.9 (N-strain dominant) then transient mortality will be small (<20% reduction in the following year's winter minimum population). R_0 ratios in the range 0.9 to less than 1.0 result in the P-strain causing significant short-term mortality before it is excluded. Where the R_0 ratio is

Figure 5. R_0 ratio (P-strain:N-strain) and the impact of the P-strain. Winter minimum population levels, following the introduction of the P-strain, are plotted for populations with different R_0 ratios. Adult mortality was 70% p.a., with productivity varied from 2.6 to 8.25 juveniles weaned per adult p.a. to produce R_0 ratios in the range 0.9–1.30. The maximum R_0 ratio of 1.43 corresponds to the maximum adult mortality of 80% p.a., and maximum productivity of 8.25 per adult p.a. There is only a small transient reduction in winter population size for R_0 ratios less than 0.9. In the range 0.9–1.0, a transient impact of increasing magnitude occurs. R_0 ratios greater than 1 indicate a terminal decline in the population, often with partial recovery being followed by another epidemic.

greater than 1 (P-dominant), the most common outcome was repeated epidemics, followed by a partial recovery, but ultimately declining over a number of years. At high ratios (maximum 1.43), there can be an almost continuous decline (figure 5). The rate of recovery and magnitude of transient population reduction is affected by the population dynamics, but the R_0 ratio relationships hold.

Is it possible to determine whether a population is 'at risk' from the P-strain by measuring winter seroprevalence? Within the range 44–81% a particular seroprevalence figure can result from different population dynamics, which also have different R_0 ratios (figure 6). For example, 66% winter seroprevalence can correspond to an R_0 ratio of ca. 0.89–1.08. For a given seroprevalence, populations with the higher rates of adult mortality have lower R_0 ratios and so are less likely to be at risk from the P-strain invasion. Nevertheless, it is clear from figure 6 that populations with seroprevalence less than 61% will always have an R_0 ratio greater than 1 and so will be 'at risk' from P-strain RHDV, irrespective of the underlying population dynamics. The European seroprevalence estimates of 12, 19 and 46% all fall within this 'at risk' range. If seroprevalence is greater than 73% then the R_0 ratio is less than 1 and so the P-strain will be excluded ultimately. Winter maximum seroprevalence greater than 78% indicates that there will not be great transient mortality. For seroprevalences in the range 61–73%, the outcome is unclear, due to the lack of a simple relationship between winter seroprevalence and the R_0 ratio. The status of UK populations is summarized in table 2, with about half of them being 'at risk' from P-strain invasion. One-fifth of populations are at risk from significant mortality, which may be transient or may result in host eradication.

Figure 6. Relationship between winter seroprevalence and R_0 ratio for populations with different adult mortality rates (per cent p.a.). The typical UK population has 60% adult mortality p.a., five juveniles weaned per adult p.a., and an R_0 ratio of 1.02. The R_0 range of each line is determined by the range of productivity values. Where the R_0 ratio is greater than 1, the P-strain invades and ultimately excludes the N-strain, driving the population extinct. Where R_0 ratio less than 1, the P-strain is excluded ultimately. However, it can invade transiently, reducing the following winter population by more than 20% where the R_0 ratio is greater than 0.9, indicated by the broken line.

4. DISCUSSION

There appears to be a putative N-strain of RHDV endemic in wild rabbits in the UK, because all rabbit populations have individuals with immunity to RHDV, apparently in the absence of associated mortality (Chasey *et al.* 1997; Trout *et al.* 1997b). Given the limited impact that P-strain RHDV has had in the UK, it appears that the N-strain may be protecting many, although not all, UK rabbit populations, by immunizing them against the P-strain. Consistent with this hypothesis, the north of the UK had higher seroprevalence in 1995, and has had fewer outbreaks of P-strain RHDV than the south, even though it is now found country-wide.

We have used a model to assess the plausibility of this hypothesis. The highly seasonal nature of rabbit population dynamics, which is well understood, necessitated the use of a seasonal, age-structured model because simpler models were inadequate to describe the data. The population counts data of the outbreak of P-strain RHDV will have been highly influenced by the seasonality of the population; as the outbreak occurred after the breeding season, much of the population decline would have occurred without the disease. Furthermore, it is highly likely that seroprevalence changes over the year (see figure 3), although the absence of longitudinal data means that this cannot be confirmed. Also, we have assumed endemicity of the N-strain, but since all populations sampled had some immune individuals this seems reasonable.

If the infectious period of the N-strain is sufficiently long then differences in population demography can explain most of the range of seroprevalence recorded for the UK and also why most outbreaks of P-strain RHDV have occurred in the south. The longer the infectious period, the greater the sensitivity to the natural death

Table 2. Classification of UK rabbit populations in 1995, according to the predicted effect of the introduction of the P-strain, based upon the R_0 ratio, predicted from the recorded winter seroprevalence using the relationship shown in figure 6.

status	winter seroprevalence range (%)	number of UK sites
vulnerable	< 61	32 (47%)
unclear	61 to < 73	12 (18%)
significant mortality		
possible	73 to < 78	3 (4%)
safe	78–100	21 (31%)

rates of the population. Almost all of those infected with the P-strain will die of it rapidly, so its R_0 is insensitive, changing by less than 1% across the range of parameter values. With a much longer infectious period the N-strain is much more sensitive, making its R_0 much more variable. In this paper we have assumed infectiousness to be lifelong, resulting in maximal sensitivity as the loss of infected individuals is entirely due to natural death. Under this condition, most of the variation in UK seroprevalence can be explained by differences in adult mortality and productivity, within their reported ranges. The transmission success of the N-strain is thus highly variable, whereas the P-strain would have similar success in all populations. We would predict that the P-strain will be able to invade about half of the UK populations, and be excluded from the rest by the endemic N-strain. Most of mainland Europe will be at risk, having much lower seroprevalences, all below the level required for protection. This is probably due to greater productivity, with a longer breeding season (Rogers *et al.* 1994) and lower nestling mortality.

The variability in rabbit population demography from site-to-site (Trout *et al.* 2000) means that an outbreak in one population does not mean necessarily that a neighbouring population will be 'at risk' at that time. Furthermore, year-to-year variation in productivity (Bell & Webb 1991; Cowan 1983; Mead-Briggs *et al.* 1975), coupled with the differential sensitivity of the two virus strains to host population dynamics, means that the R_0 ratio of a population will change from year-to-year. With reference to figure 6, a population could be regarded as moving along a curve determined by 'invariant' adult mortality and potentially could move from one end of the curve to the other from one year to the next. Thus a site that is 'at risk' one year may be 'protected' the next and vice versa. While populations with high adult mortality (80% p.a.) will always be 'at risk', and those with low adult mortality (40% p.a.) never so, most populations will vary from year-to-year in their 'vulnerability' to P-strain RHDV-induced mortality.

This local variability is superimposed upon a geographical trend of decreasing seroprevalence from north to south, consistent with productivity increasing from north to south, as the breeding season becomes longer and the climate becomes milder. The number of reported outbreaks increases from north to south, probably due to

there being an increasing proportion of sites 'at risk'. In terms of outbreaks, the trend in seroprevalence is probably exaggerated by spatial transmission considerations. The south has the highest density of sites with rabbit populations (Trout *et al.* 1986, 2000), facilitating transmission between them. Additionally, we presume that P-strain RHDV needs to cause a significant epidemic before it is likely to be transferred to another population. There may be a 'spatial herd immunity' effect: if a site that is 'at risk' has sufficient neighbouring sites that are 'protected' by the N-strain then it may not become exposed to the P-strain, and so be 'shielded' by them. The P-strain is now found country-wide, but we expect the majority of outbreaks causing significant mortality always to be found in the south of the UK.

Host demography cannot explain all of the seroprevalence range, even if the N-strain infectious period is life-long. Most likely contact rates have an effect, also, but as explained in §1, this cannot be the major factor in explaining the variation in seroprevalence (unless the two strains have different transmission routes). The greater the importance of contact rates within populations in explaining the variation in the N-strain transmission success, the more closely correlated will be the R_0 values of the two strains. This is because the contact rate would affect them by the same factor, leaving the R_0 ratio unchanged. Then more populations would have R_0 ratios close to unity, so more would fall into the zone of uncertainty, making the impact of P-strain RHDV much more unpredictable.

There is an urgent need to confirm or refute the presence of an N-strain in UK wild populations, and to measure its infectious period, which is a key assumption in this analysis. The predominant transmission routes of the two strains need to be determined. Also there is a need for much more quantitative data. A key area for future work is the collection of longitudinal seroprevalence data, to determine how it varies during the year and from year-to-year. We expect seroprevalence to decline during the breeding season and increase subsequently and to vary from year-to-year due to differences in productivity. Crucially, there is a severe lack of data regarding the dynamics of outbreaks of the P-strain, so we do not know how these vary and what factors may underlie that variation. Outbreaks need to be followed in detail, with frequent population counts, and ideally, seroprevalence measured before, during and after. The major difficulty here is the unpredictability in the occurrence of an outbreak and the speed with which it proceeds.

Despite important gaps in our knowledge, in many respects RHDV is a well-characterized emerging disease that has come to light because of the widespread global distribution of rabbits and the impact the disease has had on domestic and wild rabbit populations. The biological control application of the virus in Australia and New Zealand contrasts with the disease control focus in Europe and reflects the importance to us of disease in wildlife populations.

P-strain RHDV may not be a typical 'emerging disease'. In contrast to the 'ecological' changes that appear to underlie most disease emergence (Schrage & Wiener 1995), it lacks an apparent reservoir host, where it may have been residing previously. Rather, it appears to have

arisen in a relatively recent mutation event in central Western Europe. Another unusual feature is the apparently widespread, pre-existing N-strain; most emerging diseases are new to the populations they invade. However, like many emerging diseases, its rapid global spread, through international movement of rabbits and particularly rabbit meat, has occurred through access to highly susceptible populations as a consequence of the transportation opportunities of the modern world.

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APPENDIX A

(a) Model equations

$$H_h = S_h + \sum_i I_{hi} + R_h$$

$$d_{hi} = (a_i + \sigma_i + b_h).$$

(b) Juveniles

$$\frac{dS_J}{dt} = a(t)H_A \left(Q - \frac{(1-Q)H_A}{q} \right) - S_J \left[\frac{\sum_i (\beta_i \sum_h I_{hi})}{\sum_h H_h} \right] - b_J S_J - m(t)S_J$$

$$\frac{dI_{Ji}}{dt} = \beta_i S_J \left(\frac{\sum_h I_{hi}}{\sum_h H_h} \right) - d_{Ji} I_{Ji} - m(t)I_{Ji}$$

$$\frac{dR_J}{dt} = \sum_i \sigma_i I_{Ji} - b_J R_J - m(t)R_J.$$

(c) Adults

$$\frac{dS_A}{dt} = m(t)S_J - S_A \left[\frac{\sum_i (\beta_i \sum_h I_{hi})}{\sum_h H_h} \right] - b_A S_A.$$

$$\frac{dI_{Ai}}{dt} = m(t)I_{Ji} + \beta_i S_A \left(\frac{\sum_h I_{hi}}{\sum_h H_h} \right) - d_{Ai} I_{Ai}.$$

$$\frac{dR_A}{dt} = m(t)R_J + \sum_i \sigma_i I_{Ai} - b_A R_A.$$

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