THE ROYAL SOCIETY

Population dynamics of scrapie in a sheep flock

M. E. J. Woolhouse[1*], L. Matthews[1], P. Coen[1], S. M. Stringer[2], J. D. Foster[3] and N. Hunter[3]

[1]*Centre for Tropical Veterinary Medicine, University of Edinburgh, Easter Bush, Roslin, Midlothian EH25 9RG, UK*
[2]*Department of Experimental Psychology, University of Oxford, South Parks Road, Oxford OX1 3PS, UK*
[3]*Institute for Animal Health, Neuropathogenesis Unit, Ogston Building, West Mains Road, Edinburgh EH9 3JF, UK*

A detailed analysis of an outbreak of natural scrapie in a flock of Cheviot sheep is described. A total of 137 cases was reported over 13 years among 1307 sheep born into the flock. The epidemiology of scrapie can only be understood with reference to sheep demography, the population genetics of susceptibility to scrapie, pathogenesis during a long incubation period, and the rate of transmission (by both vertical and horizontal routes), all of which interact in complex ways. A mathematical model incorporating these features is described, parameter values and model inputs are derived from available information from the flock and from independent sources, and model outputs are compared with the field data. The model is able to reproduce key features of the outbreak, including its long duration and the ages of cases. The analysis supports earlier work suggesting that many infected sheep do not survive to show clinical signs, that most cases arise through horizontal tranmission, and that there is strong selection against susceptible genotypes. However, important aspects of scrapie epidemiology remain poorly understood, including the possible role of carrier genotypes and of an environmental reservoir of infectivity, and the mechanisms maintaining alleles giving susceptibility to scrapie in the sheep population.

Keywords: demography; genetics; incubation period; susceptibility; transmissible spongiform encephalopathy; transmission dynamics

1. INTRODUCTION

Scrapie is a progressive, fatal, neuropathological disease that occurs naturally in sheep. It is a transmissible spongiform encephalopathy (a category that includes bovine spongiform encephalopathy in cattle and new variant Creutzfeldt–Jakob disease in humans), and is associated with an abnormal form of the prion protein (PrP) (Caughey & Chesebro 1997). No immune responses to infection have been identified and there is no cure.

The epidemiology of scrapie in general has been reviewed by Hoinville (1996) and there have been several detailed studies of scrapie outbreaks within individual sheep flocks (Hunter *et al*. 1996, 1997; Elsen *et al*. 1996). However, many aspects of scrapie epidemiology remain incompletely understood. There are four components that must be considered in any epidemiological analysis of this disease: (i) sheep demography (the incubation period of scrapie is long with respect to sheep life expectancy); (ii) population genetics (susceptibility to scrapie is determined by alleles at the PrP locus); (iii) pathogenesis (levels of abnormal PrP are thought to increase over a period of several years before clinical signs develop); and (iv) transmission (scrapie is known to be vertically transmitted; routes of horizontal transmission are still uncertain but may be indirect, involving the shedding of the infectious agent into the environment via faeces or placental material and its subsequent ingestion). Mathematical models of the dynamics of scrapie within a flock incorporating these components have recently been developed (Woolhouse *et al*. 1998; Stringer *et al*. 1998).

Here, we report a detailed analysis of a flock of Cheviot sheep (NPU Cheviots) for which records have been kept on demography, pedigrees and scrapie cases since it was founded in 1960 (see Hunter *et al*. (1996) and references therein). From 1982 onwards information is also available on PrP genotype. The flock was maintained as a source of sheep for experimental studies of scrapie infectivity and pathogenesis (mostly with the scrapie source SSBP/1), with challenged sheep being kept isolated from the main flock. Natural scrapie infections occurred in the main flock from 1970 onwards. In this paper, we concentrate on the scrapie outbreak during the period 1970–1982; an analysis of events after 1982 will be presented elsewhere. However, data from 1982 onwards are used to provide independent estimates of several epidemiological parameters.

Building on previous work, we develop a mathematical model of this scrapie outbreak and compare model outputs with the field data. The value of mathematical models as an aid to interpreting epidemiological data has been extensively discussed (e.g. Anderson & May 1991). Mathematical models representing biological systems in any detail are inevitably complex. There are several

*Author for correspondence.

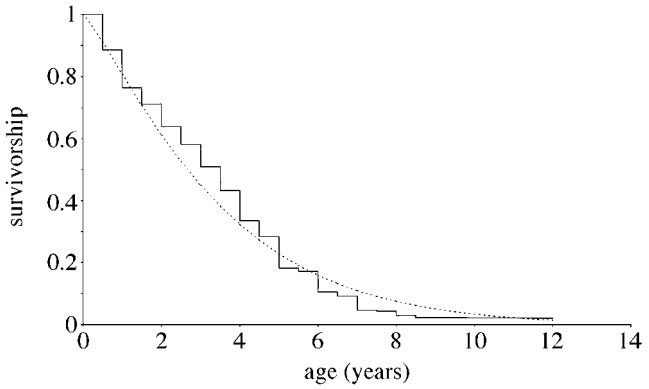

Figure 1. Pooled survivorship curve for the 1967–1978 birth cohorts. Kaplan–Meier curve with data censored for deaths due to scrapie (solid line) and truncated Weibull curve with parameters $\lambda = 0.278$ (95% confidence limits 0.263–0.293), and $\kappa = 1.209$ (1.146–1.279) fitted by maximum likelihood with maximum age 12 years (broken line). Note that the data include both deaths and emigrations from the flock. The raw data and fitted function correspond to mean life expectancies of 3.46 and 3.60 years, respectively.

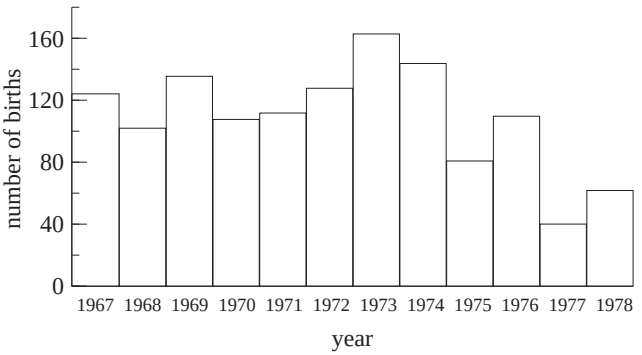

Figure 2. Numbers of sheep in birth cohorts 1967–1978.

possible approaches to dealing with this complexity. For some systems experimental data are available that provide independent information on individual components of the system (e.g. Woolhouse & Chandiwana 1990); in this case, comparison of model outputs with field data validates the model. For other systems experimental data may be lacking, but the scope and quality of the field data allow different hypotheses about individual components to be tested in some detail (e.g. Ferguson *et al*. 1997); in this case, however, the model must be validated against other, independent data sets (e.g. Ferguson *et al*. 1998). For scrapie in sheep, there is very good experimental information on some aspects of the biology of the disease, but other aspects remain poorly understood. Any mathematical model of scrapie epidemiology must therefore embody a number of hypotheses that need to be tested against data. This process is iterative: a model is developed that is consistent with current biological understanding; the model is tested against field data and refined accordingly; the refined model is then tested against further data, and so on. The major difficulty is to determine at each iteration those components of the model requiring refinement (i.e. which hypotheses have been falsified); this determination is usually made with reference to the quality and precision of the available experimental data supporting each component of the model. Here we describe part of this process for scrapie in sheep.

2. FIELD DATA

(a) *Demography*

The NPU Cheviot flock was founded by Alan Dickinson in 1960 with 16 rams (born in 1960) and 304 ewes (born 1957–1959), the latter imported from three farms believed to be scrapie-free, and there was no immigration after 1962 (Dickinson 1976). Scrapie cases occurred in cohorts of sheep born between 1967 and 1978 and this is therefore the period of interest here. Over this period flock size was maintained in the range 227–402. The sex ratio of sheep surviving more than one year was female biased. Survivorship is shown in figure 1; mean 'life expectancy' was 3.46 years (noting that sheep left the flock both through mortality and export to other flocks). Lambing took place annually between February and June, with over 75% of all lambs being born in April. However, the number of births varied substantially between years (figure 2).

(b) *Genetics*

Genetic variation in the susceptibility of sheep to scrapie is determined largely by alleles at the PrP locus, with amino-acid substitutions at codons 136, 154 and 171 being especially important (Hunter *et al*. 1996; Dawson *et al*. 1998). For this flock, genotype data are available from 1982 onwards. Four PrP alleles (defined by amino acids at codons 136, 154 and 171 in order) were present: *VRQ*, *ARQ*, *AHQ* and *ARR*. All ten possible genotypes were present. Natural scrapie has only ever been recorded in two genotypes from this flock: *VRQ*/*VRQ* and *VRQ*/*ARQ* (figure 3). We infer that the other genotypes are resistant to this scrapie strain (although certain other genotypes can be experimentally infected with SSBP/1; Goldmann *et al*. 1994).

Genotype frequencies in the founding flock are not known. However, all sheep in the flock were allocated either to a scrapie-susceptible or to a scrapie-resistant line on the basis of the susceptibility of their parents to experimental challenge with SSBP/1 (also referred to as 'positive' and 'negative' lines, respectively; see Dickinson & Outram 1988). Over the period 1962–1982, *ca*. 40% of the sheep were assigned to the resistant line and 60% to the susceptible line. Mating took place within lines, and during the 1960s there was selection within lines for resistance or susceptibility to SSBP/1 scrapie. During the 1970–1982 outbreak of natural scrapie, only one sheep in the resistant line developed natural scrapie, and we infer that the *VRQ* allele was still segregating at very low frequencies in this line. By the late 1960s, almost all sheep in the susceptible line had been born to parents that were susceptible to SSBP/1, and we infer that sheep in this line had a 50–100% chance of carrying at least one *VRQ* allele.

(c) *Pathogenesis*

Other studies have suggested that the incubation period of natural scrapie is typically of the order of two years (Hoinville 1996; Woolhouse *et al*. 1998). It is not known how the infectiousness of sheep varies during the

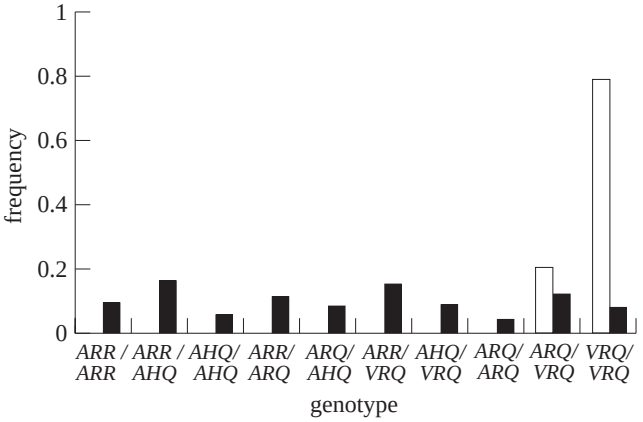

Figure 3. Frequency distributions by PrP genotype for all sheep ($n = 615$) and for confirmed scrapie cases ($n = 24$) over the period 1982–1996.

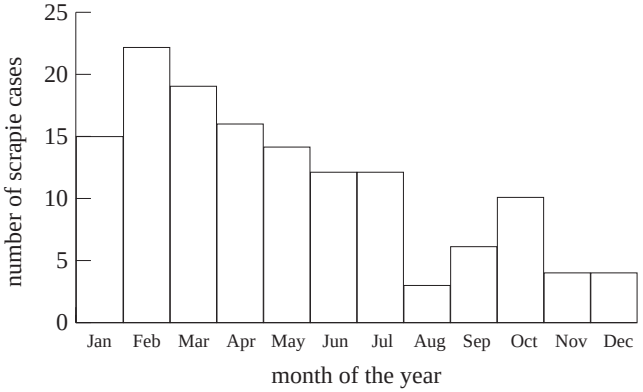

Figure 4. Annual distribution of scrapie cases over the period 1970–1982 ($n = 137$). There is a significant difference between months ($\chi^2_{11} = 37.0$, $p < 0.001$).

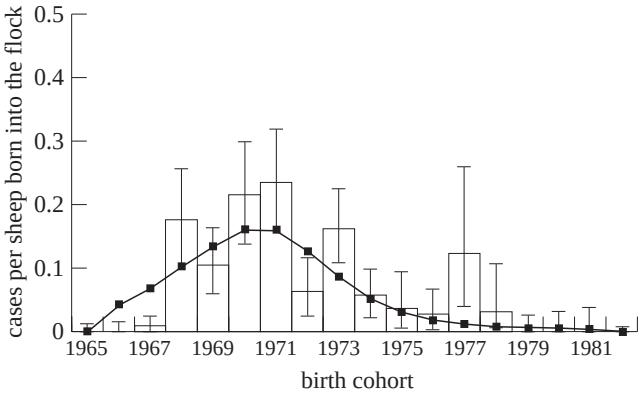

Figure 5. Incidence of scrapie cases and 95% confidence limits in birth cohorts from 1967 to 1978 ($n = 137$) (histogram). Total numbers of cases in each cohort are shown. Model output is compared (line and symbols).

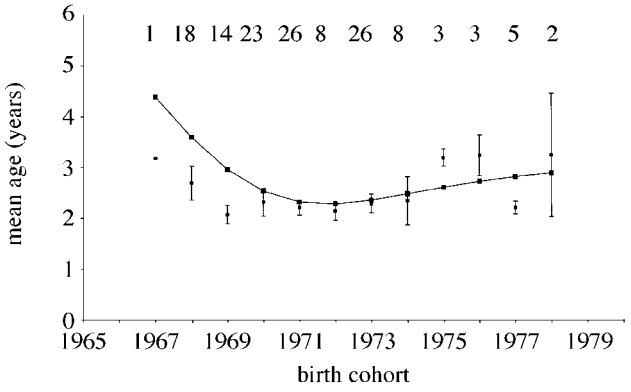

Figure 6. Mean ages of cases and 95% confidence limits for the means by birth cohort from 1967 to 1978 (numbers of cases as shown, total $n = 137$) (symbols only). Model output is compared (line and symbols).

incubation period but, in the mouse model, levels of abnormal PrP increase from the time of infection to the time of first appearance of clinical signs (Bruce *et al*. 1991).

(d) *Transmission*

The first cases of natural scrapie were reported in 1970, eight years after the flock was closed (Dickinson 1974). Between 1970 and 1982, 137 cases were diagnosed from clinical signs and confirmed by histopathological examination (Hunter *et al*. 1996). There was seasonal variation in the occurrence of scrapie cases (figure 4). The incidence of cases by birth cohort is shown in figure 5; peak incidence (23%) was recorded in the 1971 birth cohort. The mean age of cases varied with year of birth (figure 6). All affected sheep were culled within a few days of developing clinical signs. No information is available on subclinical infections.

There are very limited data on either vertical or horizontal transmission rates for scrapie. Data from one study of a mixed-breed sheep flock in Texas (Hourrigan *et al*. 1979) give an estimate of the force of infection of 0.26 per sheep per year and a minimum estimate of the probability of vertical infection of 0.09 (figure 7). Here, estimates of the horizontal transmission rate must be made by fitting the model to the data (see below). However, an estimate of the probability of vertical transmission, conditional on the ewe surviving the incubation period, can be obtained directly from the field data: from 1970 to 1982, 32 scrapie lambs were born to 80 ewes which subsequently developed scrapie, giving an upper bound to the estimated probability of 0.40 (95% confidence limits 0.28–0.52). Previous analyses have suggested that the majority of scrapie cases are horizontally rather than vertically transmitted (Hoinville 1996; Woolhouse *et al*. 1998).

The relative susceptibility of different genotypes to natural infection can be estimated from the data in figure 3, which suggest that *VRQ/ARQ* sheep are 0.28 times as susceptible as *VRQ/VRQ* sheep (there is uncertainty of up to 20% around this estimate due to the potentially different contribution of vertical transmission in the two genotypes).

3. MODEL DEVELOPMENT

A full description of the mathematical model on which this work is based is given elsewhere (Stringer *et al*. 1998); only a brief summary of the original model and an explanation of modifications introduced for this analysis is provided here. The model is deterministic and comprises a set of coupled partial differential equations representing changes through time with respect to sheep age and the infection load in infected sheep. Numerical analyses were implemented via a Fortran computer program.

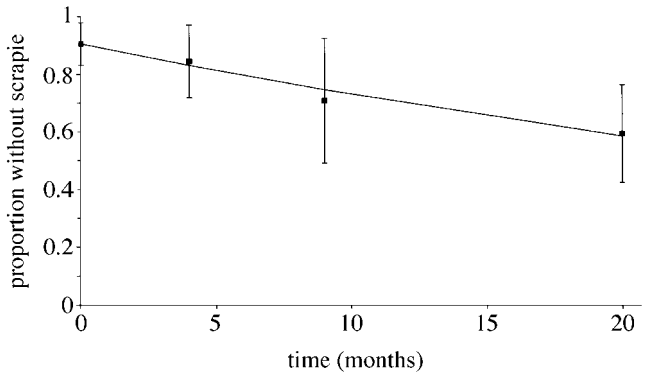

Figure 7. Estimates of force of infection with scrapie for a mixed-breed sheep flock in Texas (data from Hourrigan *et al.* (1979)). Symbols indicate the proportion of sheep removed to a scrapie-free environment at age shown that did not subsequently develop scrapie (with 95% confidence limits). The line shows the fit of a model with probability of vertical infection 0.09 (95% confidence limits 0.04–0.17) and a constant horizontal force of infection of 0.26 per sheep per year (0.12–0.40). Parameter values were estimated by maximum likelihood.

(a) *Demography*

Survivorship is represented by the truncated Weibull curve shown in figure l; this corresponds to a mean life expectancy in the absence of scrapie of 3.60 years with a maximum lifespan of 12 years. There is an annual birth pulse to which all sheep between 2 and 12 years old contribute equally. Flock size therefore varies within years; the mean flock size is set at 366, the average value during the peak of the outbreak. All numerical analyses were repeated with a continuous birth-rate function scaled to maintain a constant flock size. The initial age structure corresponded to the steady-state age structure in the absence of scrapie. The flock was closed, i.e. there was no immigration.

(b) *Genetics*

Susceptibility to scrapie is assumed to be governed by a single locus with the four alleles *VRQ*, *ARQ*, *AHQ* and *ARR*. *AHQ* and *ARR* are both dominant for resistance (and so do not need to be distinguished in numerical analyses). *ARQ* is partially dominant for resistance. Therefore, *VRQ*/*VRQ* homozygotes are fully susceptible, *VRQ*/*ARQ* heterozygotes are partially susceptible, and all other genotypes are resistant (table 1).

Mating is assortative: 40% of matings are random amongst sheep without the *VRQ* allele (the resistant line); 60% of matings are random amongst the remaining sheep (the susceptible line). Note that the allele frequencies in the two lines are, therefore, not fully independent, as appears to have been the case in practice. It is assumed throughout that genotype frequencies in rams reflect genotype frequencies in ewes.

From the arguments presented above, the initial frequency of the *VRQ* allele at the start of the outbreak was in the range 0.16–0.37; here we use the upper limit. The initial frequencies of the other alleles are estimated from their relative frequencies in other flocks (N. Hunter, unpublished data): this gives frequencies of 0.28 for *ARQ* and 0.35 for *AHQ* or *ARR*. Initial genotype frequencies were calculated taking into account the non-random mating patterns from 1962 onwards (table 1). However, there is inevitably some uncertainty regarding initial genotype frequencies; sensitivities to the results of numerical analyses to initial genotype frequencies are discussed below.

Table 1. *Differences between genotypes assumed for numerical analysis*

genotype	relative susceptibility	mean (var) incubation period (yr)	initial frequency
1. *VRQ*/*VRQ*	1	1.9 (0.14)	0.20
2. *VRQ*/*ARQ*	0.28	1.9 (0.14)	0.15
3. *VRQ*/*AHQ* or *ARR*	0	—	0.19
4. *ARQ*/*ARQ*	0	—	0.09
5. *ARQ*/*AHQ* or *ARR*	0	—	0.22
6. *AHQ* or *ARR*/*AHQ* or *ARR*	0	—	0.14

(c) *Pathogenesis*

Infection load is assumed to increase exponentially from the time of infection to a level corresponding to clinical signs, at which point an infected sheep is removed from the flock. Relative infectiousness, by both horizontal and vertical routes, is assumed to be proportional to the infection load. The mean initial infection load is set at 0.1 (corresponding to an infected animal being one-tenth as infectious at the beginning of the incubation period as at the end); an analysis of the sensitivity of model outputs to changes in the values of this parameter is presented elsewhere (Stringer *et al.* 1998). The rate of increase in infection load was set to a value consistent with data on the age of cases in the flock from 1982 onwards, and corresponds to a mean incubation period of 1.9 years; this value is slightly lower than the one used earlier (Woolhouse *et al.* 1998), the difference arising because a lower relative susceptibility of heterozygotes is assumed here. For a given rate of increase of infection load, variation in the incubation period is generated by assuming a gamma distribution for the initial infection load (Stringer *et al.* 1998). The variance of this distribution used here corresponds to a variance in the incubation period of 0.14.

(d) *Transmission*

Susceptibility varies between genotypes (as above, see table 1) but is assumed (in the absence of contrary evidence) to be independent of age and sex. The function used for the growth of infection load corresponds to a mean probability of vertical transmission over the entire incubation period of 0.40 (the maximum value consistent with the data presented above). The horizontal transmission rate was estimated as the value required to generate the observed total number of scrapie cases in 1970–1982. Initial conditions corresponded to a single *VRQ*/*ARQ* sheep newly infected with scrapie.

4. RESULTS

The incidence of scrapie by birth cohort predicted by the model is compared with the field data in figure 5. The model (with a continuous birth function) successfully reproduces the rise in incidence to a peak in 1971 and the

slow fade-out of the outbreak. The earliest date for the introduction of scrapie consistent with the observed time-course of the outbreak is 1966, i.e. four years after the flock was closed. There is an expected lag of three years from the introduction of scrapie to the incidence rising above one case per year. The outbreak has a long expected time-course, 13 years during which the incidence exceeds one case per year. The eventual fade-out occurs because of selection against the *VRQ* and *ARQ* alleles, and therefore an expected reduction in the numbers of susceptible sheep (figure 8). By 1982, the frequency of the *VRQ* allele is expected to have fallen from 0.37 to 0.12.

If births are modelled as an annual pulse in April, then the model predicts a seasonal distribution of scrapie cases, with the highest incidence occurring in February and March (cf. figure 4). This pattern is due partly to seasonality in vertical transmission and partly to seasonality in the numbers of sheep, and is affected by the mean and the variance of the incubation period. The seasonality does not greatly influence the patterns of interest for this study, and therefore only results from the model with a continuous birth function and constant flock size are reported here.

The ages of cases in each birth cohort predicted by the model are compared with the field data in figure 6. The model successfully reproduces the observed fall in mean age in the early cohorts affected and the subsequent rise in later cohorts affected but overestimates mean age early in the outbreak.

The estimated horizontal transmission rate is 0.09 per susceptible sheep per maximally infectious sheep per year. This value is a lower limit because both the vertical transmission probability and, more importantly, the initial frequency of susceptible genotypes are set at their upper bounds.

5. DISCUSSION

The model is capable of reproducing key features of the time-course and age structure of the outbreak, including the long duration of the outbreak and the mean age of scrapie cases in sheep of two to three years old (figures 5 and 6). However, comparison of observed and expected distributions of cases shows several features requiring comment. There is some indication that scrapie may have been introduced several years after the flock was closed in 1962; but the confidence limits for this interval are likely to be broad, and may well include zero, because of the importance of stochastic effects during the early phase of the outbreak when expected numbers of infections are low and especially when the expected incidence of cases is less than one per year. The initial time-course and age structure of the outbreak are least well described. This is expected given uncertainties regarding the initial conditions: for example, the lower observed than expected mean ages of the earliest cases could be due to there being more than one primary case (corresponding to a greater initial force of infection). Thereafter, the model reproduces the time-course of the outbreak well, including the fall to very low incidences in cohorts born after 1978, although there are apparently greater observed than expected year-to-year fluctuations in incidence between cohorts (figure 5).

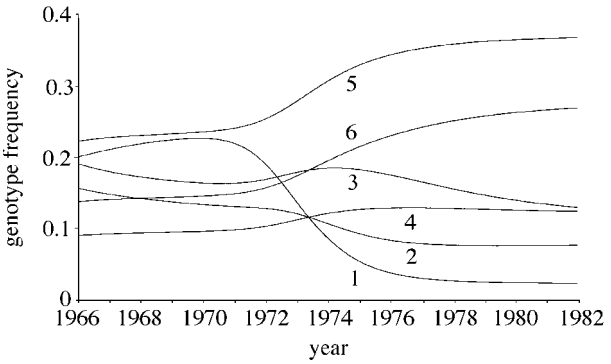

Figure 8. Predicted changes in genotype frequencies during the course of the outbreak. Genotypes numbered as in table 1. The susceptible genotypes are (1) (*VRQ*/*VRQ*) and (2) (*VRQ*/*ARQ*).

The expected time-course of an outbreak is highly sensitive to the horizontal transmission rate (Stringer *et al*. 1998). Here, the value of this parameter is estimated at its lower bound; a lower probability of vertical transmission or, especially, lower initial fractions of susceptible genotypes would both lead to a higher estimate of the horizontal transmission rate. However, it should be noted that it is difficult to reproduce the observed time-course of the outbreak with a significantly higher horizontal transmission rate. Most cases (88%) are expected to arise through horizontal transmission; this is consistent with the results of risk analyses (Hoinville 1996). The model also predicts that, over the course of the outbreak, 1.8 times as many sheep become infected as develop clinical signs: this prediction arises from the long incubation period relative to sheep life expectancy—a large fraction of infected sheep to do not survive long enough to develop clinical signs. Note that in commercial flocks, where mean life expectancy may be much less, this fraction will be significantly larger. Such predictions are, in principle, testable for contemporary outbreaks following the development of diagnostic tests for preclinical infections (Schreuder *et al*. 1996).

The model also reproduces the observed fall and rise in mean age in cohorts affected during the course of the outbreak (figure 6). This pattern arises from (i) the increase and decrease in the force of infection (and hence the decrease and increase in the average age of infection) as the outbreak waxes and wanes; (ii) changes in genotype frequencies; and (iii) changes in the fraction of cases due to horizontal transmission (Stringer *et al*. 1998).

Model predictions are also at least qualitatively consistent with data from other outbreaks for which information on scrapie genotypes is available. The model predicts that a large fraction (68%) of cases occurs in *VRQ*/*VRQ* genotype sheep (cf. figure 3) and that the mean age of these cases is younger (by 0.4 years) than that of *VRQ*/*ARQ* sheep. Both expectations are consistent with data from a later scrapie outbreak in the same flock (Hunter *et al*. 1996). The expected high prevalence of infection in the *VRQ*/*VRQ* genotype (up to 87%) and the rapid decrease in the frequency of the *VRQ* allele at the peak of the outbreak (figure 8) are consistent with data on a scrapie outbreak in a flock of Romanov sheep (which are similar to the Cheviots in their scrapie-susceptibility genetics) (Elsen *et al*. 1996).

This last result raises an important question. Scrapie has been recognized for over 200 years and is apparently endemic in the UK; yet, as described above, strong selection against alleles for susceptibility to scrapie is both expected and observed. It is unclear how these alleles persist in the sheep population in the long-term. There are three possibilities. First, there might be 'costs of resistance' such that susceptibility to scrapie is linked to traits that enhance fitness or are favoured by breeders. A possible example of the latter is the occurrence of scrapie susceptibility in a line of Suffolk sheep selected for lean-meat production (Hunter *et al*. 1997), although there may be other explanations (such as founder effects) for this pattern. Second, there might be frequency-dependent selection because different genotypes are susceptible to different scrapie strains: indeed, differential susceptibilities have been confirmed experimentally (Goldmann *et al*. 1994). Finally, it is possible that scrapie is not in fact endemic, but is epidemic with an extremely long time-scale. Supporting this possibility is the expectation that outbreaks in individual flocks may persist for more than a decade (see above), and the observation that the national flock has a metapopulation structure with *ca*. 100 000 flocks, with each flock containing an average of several hundred sheep. An 'epidemic' lasting hundreds of years may well be possible in such a system.

The analysis presented here confirms that the model structure and parameter values are broadly consistent with the dynamics of a well-documented scrapie outbreak. However, this analysis has not tested, and therefore has not refuted, other possible models of scrapie dynamics that embody alternative or additional assumptions regarding the epidemiology of the disease (see Hoinville 1996; Woolhouse *et al*. 1998). Possibilities include (i) different relationships between duration of infection and infectiousness; (ii) genotype-specific incubation periods (as is well established for the mouse scrapie model; Bruce *et al*. 1991); (iii) 'carrier' status of genotypes that do not develop clinical signs; (iv) a persistent (and variable) reservoir of environmental infectivity (Sigurdarson 1991); (v) age-specific susceptibility to infection; (vi) scrapie-induced mortality among preclinically infected sheep; and (vii) different incubation periods for horizontally and vertically transmitted infections (or for infections vertically transmitted at different stages of the incubation period). Each of these is, in principle, amenable to experimental study, and their expected epidemiological impacts can be explored using straightforward extensions of the mathematical model used here. Of those listed, the possibilities of 'carrier' genotypes and of a persistent environmental reservoir have been shown previously (Woolhouse *et al*. 1998) to have a major impact on the long-term dynamics of scrapie in a sheep flock, and are a priority for future research.

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