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Epidemiology and control of scrapie within a sheep flock

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Mathematical models of the transmission dynamics of scrapie are used to explore the expected course of an outbreak in a sheep flock, and the potential impacts of different control measures. All models incorporate sheep demography, a long and variable scrapie incubation period, horizontal and vertical routes of transmission and genetic variation in susceptibility. Outputs are compared for models which do and do not incorporate an environmental reservoir of infectivity, and which do and do not incorporate carrier genotypes. Numerical analyses using parameter values consistent with available data indicate that, in a closed flock, scrapie outbreaks may have a duration of several decades, reduce the frequency of susceptible genotypes, and may become endemic if carrier genotypes are present. In an open flock, endemic scrapie is possible even in the absence of carriers. Control measures currently or likely to become available may reduce the incidence of cases but may be fully effective only over a period of several years.

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

1. INTRODUCTION

Scrapie is a transmissible spongiform encephalopathy that occurs naturally in sheep and causes progressive deterioration of neurological function, loss of condition, and death. The disease has been known for more than 200 years and has been reported from many different countries worldwide. Scrapie is associated with an abnormal form of the prion protein (PrP) (Caughey & Chesebro 1997). There is no cure and methods for diagnosing preclinical cases are still under development (Schreuder *et al*. 1996).

There are limited data on the numbers of cases of scrapie within the UK. The disease became notifiable in 1993 and, typically, approximately 400 cases are reported annually, corresponding to an incidence of less than one per 100 000 sheep per year. However, reported cases are believed to greatly underestimate the true incidence; for example, a questionnaire survey has indicated that scrapie cases may have occurred on over 25% of sheep farms (Morgan *et al*. 1990).

The epidemiology of scrapie has recently been reviewed by Hoinville (1996). An important feature is the long mean incubation period of the disease (possibly two years or more). Evidence from experimental infections in mice indicates that the incubation period is a function of the route of infection and the infective dose, and that levels of abnormal PrP in the tissues (especially the central nervous system) increase from the time of first infection to the appearance of clinical signs (Bruce *et al*. 1991). The mechanisms of natural transmission are incompletely understood. There is evidence for vertical transmission from ewe to lamb (Hoinville 1996), which may occur before or shortly after birth. The rapid spread and long-term persistence of scrapie in some flocks is indicative of some form of horizontal transmission, possibly involving the shedding of the infectious agent into the environment via faeces or placental tissue and its subsequent ingestion; it is not known how long infectivity may persist in the environment. Naturally infected sheep are likely to be exposed to a range of infective doses.

There is good evidence for genetic variation in susceptibility to scrapie determined largely by alleles at the PrP locus (Hunter *et al*. 1996). For example, Cheviot sheep of the $VV_{136}RR_{154}QQ_{171}$ genotype are susceptible to natural scrapie, AA_{136} sheep (regardless of genotype at codons 154 and 171) are resistant, and VA_{136} sheep show intermediate susceptibility with $VA_{136}RR_{154}QQ_{171}$ being susceptible and $VA_{136}HR_{154}QQ_{171}$ or $VA_{136}RR_{154}RQ_{171}$ sheep being resistant (Hunter *et al*. 1996). It is not known whether 'resistant' sheep cannot become infected or, by analogy with experimental infections of mice (Bruce *et al*. 1991), whether the incubation period in these sheep is so long in relation to sheep life expectancy that clinical signs are never seen. In the latter case it is possible that 'resistant' genotypes may act as carriers.

There is increasing recognition of the desirability of eliminating scrapie infection from the UK sheep flock and more broadly throughout the European Community (Royal Society 1997). Several control options have been suggested, including (i) slaughter of flocks with a history of scrapie; (ii) slaughter of lambs from ewes that

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subsequently develop scrapie; (iii) slaughter of sheep with susceptible genotypes; (iv) slaughter of sheep with preclinical infections (given a method of diagnosis); (v) breeding only from resistant genotypes; and (vi) changes in husbandry practices to reduce rates of vertical and/or horizontal transmission (given improved knowledge of transmission routes).

We have recently developed a mathematical model of the dynamics of scrapie infection within a sheep flock (Stringer *et al*. 1998). The model incorporates a long and variable incubation period, both vertical and horizontal infection, and genetic variation in susceptibility. The model is based on a set of partial differential equations representing changes over time with respect both to sheep age and to the abnormal PrP load in infected sheep. Model outputs are the age-stratified incidence of scrapie cases (which can be detected by disease surveillance), prevalence of infection (which cannot) and changes in allele frequencies (which can be monitored by genotyping).

Here we use this model (i) to explore the expected pattern of a scrapie outbreak, comparing the results with available data; (ii) to consider the consequences of two important biological uncertainties, environmental reservoirs of infection and infectious carrier genotypes; and (iii) to explore the potential impacts of different control measures.

2. METHODS

A full description of the mathematical model will be given elsewhere (Stringer *et al*. 1998) and only a brief summary is provided here. Throughout, model assumptions and parameter values are consistent with available information on scrapie in sheep (Hoinville 1996; Hunter *et al*. 1996) or in mice (Bruce *et al*. 1991). However, quantitative data on many aspects of scrapie biology are extremely limited and model outputs must be interpreted against this background.

The model assumes that genetic susceptibility to scrapie is governed by a single locus with a susceptibility allele *r* (initial frequency 0.5) and a resistance allele *R*. *RR* homozygotes are fully resistant and heterozygotes are 50% as susceptible as *rr* homozygotes. Mating is random with respect to susceptibility alleles and genotype frequencies are initially in Hardy–Weinberg equilibrium.

A truncated Weibull curve is assumed for sheep survivorship, corresponding to mean and maximum life expectancies of four and ten years, respectively. (Lambs slaughtered before they are one-year-old are ignored as these are assumed to make a minimal contribution to scrapie transmission.) Birth rates balance death rates so that the total population size is constant. The flock may be 'closed' (i.e. there is no immigration or breeding with sheep outside the flock) or 'open' (i.e. there is immigration and/or breeding outside the flock). Here, an open flock is represented by assuming that ewes from within the flock are mated with rams from outside the flock which have constant genotype frequencies corresponding to initial conditions within the flock.

Susceptibility is assumed (in the absence of evidence to the contrary) to be independent of age. Initial infection load is assumed to be 10% of the load corresponding to the appearance of clinical signs. Infection load increases exponentially from the time of infection at a rate corresponding to a mean incubation period of 2.1 years. Initial infection load varies according to a gamma distribution with standard deviation 0.045 corresponding to standard deviation in the incubation period of 0.409 years. Relative infectiousness, by both vertical and horizontal routes, is proportional to the infection load. The horizontal transmission rate is set to correspond to 0.04 infections per infected sheep per year for a sheep at the highest level of infectivity, i.e. just prior to showing clinical signs. Sheep showing clinical signs are immediately removed from the flock.

An extensive exploration of the sensitivity of model output to parameter values will be presented elsewhere (Stringer *et al*. 1998). We consider two variations of the above 'standard' model for a closed flock. In the 'reservoir' model, horizontal transmission is indirect and infectious sheep contribute to a reservoir of infectivity, which decays at an exponential rate corresponding to a mean duration of 0.01, 1 or 3 years. In the 'carrier' model, all genotypes are equally susceptible but mean incubation periods are 2.1 years in *rr* homozygotes, 10.4 years in *RR* homozygotes, and 2.1, 3.7 or 10.4 years in heterozygotes. We also compare standard model outputs for an open flock.

We explore the potential impact of control measures by introducing control 7.5 years after the introduction of the first infected sheep. We explicitly consider four options for scrapie control within a flock: (i) setting the vertical transmission rate to half the baseline value or to zero; (ii) breeding using only homozygous resistant rams; (iii) setting the horizontal transmission rate to half the baseline value or to zero; and (iv) slaughter of preclinically infected sheep at 70% and 40% of the infection load associated with the appearance of clinical signs.

For numerical solutions of the partial differential equations corresponding to the above models we employed a Lax–Wendroff finite difference method with explicit treatment of the integrals in the age and infection load dimensions calculated using Composite Simpson's rule. Stability and convergence were confirmed by mesh-refinement studies. Numerical simulations were implemented via a Fortran computer program. Initial conditions corresponded to one newly infected *Rr* sheep introduced into a population of 200.

For comparison with model outputs we use data from two sources. Records from the Ministry of Agriculture, Fisheries and Food (MAFF) give the age distribution of 1334 reported scrapie cases in the UK between 1980 and 1990. Data from a long-term study of an experimental flock of Cheviot sheep kept by the Neuropathogenesis Unit (NPU) in Edinburgh give age distributions of cases by genotype during the course of a scrapie outbreak (Hunter *et al*. 1996).

3. RESULTS

Scrapie incidence (numbers of cases per year) and prevalence (fraction infected) through time using the standard model for a closed flock with baseline parameter values are shown in figure 1*a*. There are a number of important features. The predicted duration of a scrapie outbreak is long. There is a delay of several years before incidence reaches one case per year. Prevalence rises to a peak at $t=10.8$ years and declines below 0.005 (one sheep infected) by 26.6 years. Incidence lags approximately 2 years behind, rising to a peak at $t=12.0$ years, declining below one case per year by 24.6 years. In addition, the frequency of the susceptibility allele falls from 0.50 to 0.125 by the end of the outbreak (figure 1*a*). Prevalences are higher in *rr* sheep (which are more susceptible) but incidences are higher in *Rr* sheep (which are more

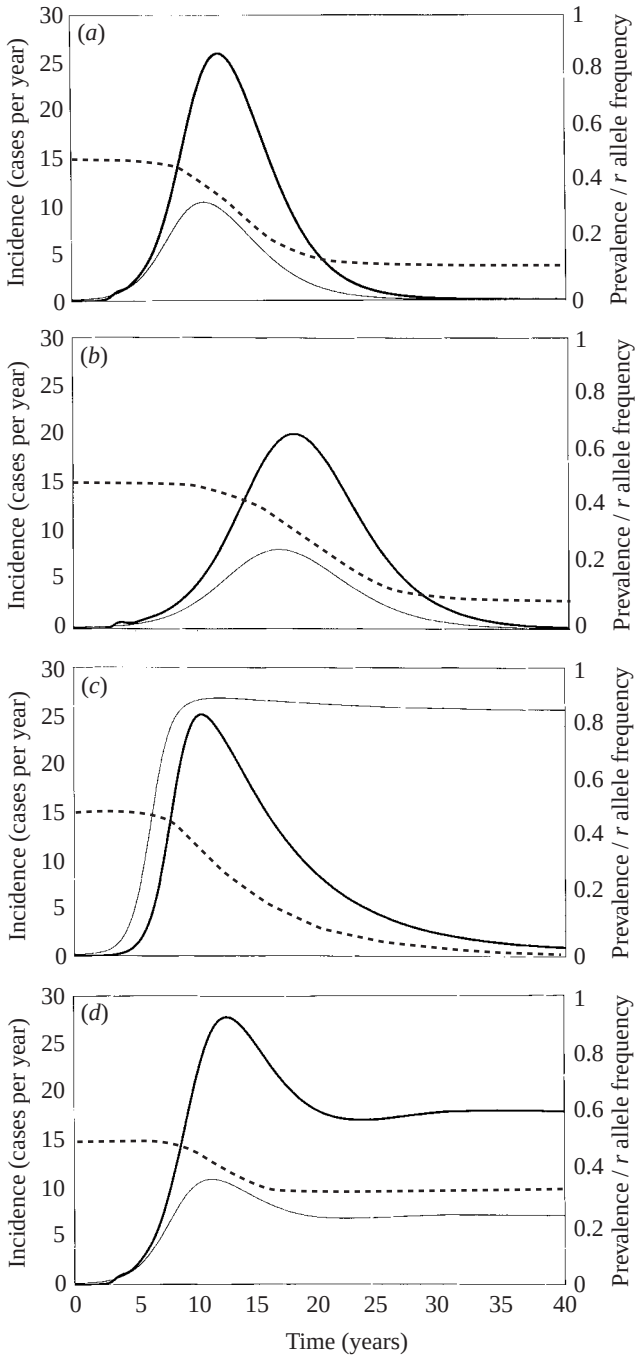

Figure 1. Comparison of model outputs: incidence of scrapie cases (bold line), prevalence of infection (narrow line) and frequency of the *r* allele (dashed line) through time. Model descriptions and parameter values are given in the text. (*a*) Standard model for a closed flock. (*b*) Reservoir model with mean duration of environmental infectivity of 1 year. (*c*) Carrier model with mean incubation periods of 2.1, 3.7 and 10.4 years for *rr*, *Rr* and *RR* genotypes, respectively. (*d*) Standard model for an open flock.

numerous), although the time-course of the outbreak is different in the two genotypes and the fraction of cases occurring in *rr* sheep declines during the course of the outbreak (not shown). Peak prevalence in *rr* sheep is high, approximately 75% (not shown). Most infections and most cases (*ca.* 80–90%) occur through horizontal transmission, and the fraction of cases attributable to horizontal infection is higher in the early stages of the outbreak (not shown).

Over the entire course of the outbreak the frequencies of cases within age classes has a similar distribution to that observed, peaking at 2–3 years with very few cases in sheep 0–1 years old or greater than 5 years old (figure 2*a*). The age of cases declines during the course of the outbreak and is greater in heterozygotes than in homozygote susceptibles, in qualitative (but not necessarily quantitative) agreement with observation (figure 2*b*).

The reservoir model predicts an outbreak of a longer time-scale (depending on the duration of reservoir infectivity: a very short-lived reservoir gives identical results to the standard model) and the *r* allele frequency is driven lower than for the standard model (figure 1*b*). There are only slight effects on the age distribution of cases. Reservoir infectivity may peak some years after peak incidence if the duration of reservoir infectivity is long (several years).

The carrier model predicts an outbreak that may have a similar time-course of scrapie incidence to the standard model but has a very different time-course of prevalence; for the parameter values used here prevalence persists at over 80% indefinitely (figure 1*c*). In the long run, the frequency of the *r* allele tends to zero (reaching 0.0025, a single copy in the population, after 45.2 years). If the heterozygotes have intermediate incubation periods then there are marked changes in the age distribution of cases (occurring more frequently in older sheep) and, as expected, there is a much greater difference in the age of cases between heterozygotes and *rr* homozygotes (not shown).

The standard model for an open flock also predicts a slow outbreak but now, for the parameter values used, there is an oscillatory approach to an endemic steady-state level of infection (figure 1*d*). The frequency of the *r* allele falls, but not to as low a level as for a closed flock.

The impacts of control measures, using the standard model for a closed flock with baseline parameter values, are shown in figure 3. There are a number of important features. All the control measures considered result in a significant decrease in the total number of cases over the course of the outbreak. However, there may be a substantial delay before there is a marked impact on incidence or prevalence. Moreover, incidence may continue to rise (control measures being introduced here before incidence has peaked) for some time (several years) after a control measure has been introduced.

For the reservoir model, essentially the same results were obtained, although the time-scale is obviously prolonged if the environmental reservoir has a long duration of infectivity (several years). For the carrier model, similar results are obtained in terms of incidence, but not in terms of prevalence. It remains possible to eliminate infection by reducing transmission rates but not (for the parameters used here) by breeding with *RR* genotypes. For the parameter values used and assumptions stated, elimination is more difficult to achieve by the slaughter of preclinical cases (here requiring diagnosis at less than 16% of the infection load corresponding to clinical signs). For the standard model with an open flock, it is possible to eliminate infection by reducing transmission rates, by breeding with *RR* genotypes, and by the slaughter of preclinical cases (here requiring diagnosis at 40% of the infection load corresponding to clinical signs).

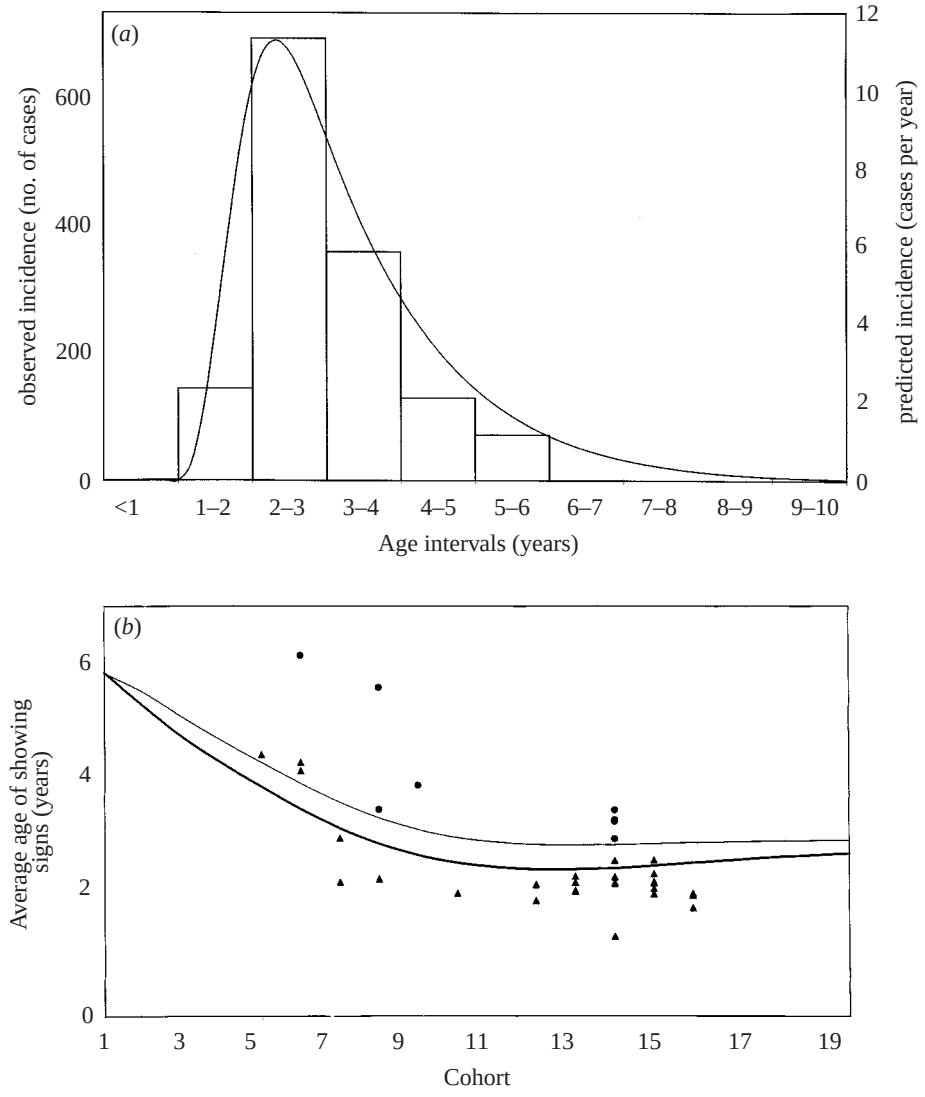

Figure 2. Comparisons of outputs of the standard model with field data. (*a*) Predicted age distribution of scrapie cases through the course of the outbreak (line) and the frequency of scrapie cases in sheep of different ages reported to MAFF in the period 1980–1990 (bars). These data include sheep of different breeds and therefore different genetics of susceptibility to scrapie. (*b*) Predicted average ages of sheep with scrapie in successive birth cohorts during the course of the outbreak compared for homozygote susceptibles (bold line) and heterozygotes (narrow line) and the ages of scrapie cases in homozygotes (triangles) and heterozygotes (circles) occurring during an outbreak of natural scrapie in the NPU flock. These are Cheviot sheep and the genetics of their susceptibility to scrapie is as described in the text.

4. DISCUSSION

An important prediction of these models is that, even in a relatively small, closed sheep flock, the time-scale for a scrapie outbreak may be as long as several decades. There are very few field data on natural scrapie outbreaks. In one flock of approximately 600 Romanov sheep in France (Elsen *et al*. 1996), 307 cases were reported in the 4 years after the first case and over 70% of sheep with the most susceptible genotypes (those with the $V_{136}R_{154}Q_{171}$ haplotype) had contracted the disease. This relatively rapid outbreak is consistent with a higher horizontal transmission rate than is assumed here. Lower transmission rates are predicted to result in outbreaks of longer duration with low incidence throughout; such outbreaks may be commonplace (Parry 1960). It should be noted that, in general, the pattern of scrapie outbreaks will be subject to stochastic effects, especially where numbers of sheep, numbers of infections and, in particular, numbers of cases are low.

A crucial question is whether or not scrapie is expected to become endemic within a sheep flock. For a closed flock this depends on the status of the 'resistant' genotype. If 'resistant' genotypes cannot become infected (the standard model), then scrapie will be eliminated from the flock as the frequency of susceptible genotypes declines. However, the susceptibility allele is not eliminated but is reduced to a threshold frequency where the density of susceptible sheep becomes too low to support endemic infection (figure 1*a*), corresponding to the basic reproduction number (the average number of secondary cases generated by one primary case introduced into a susceptible population, R_0) falling below one (Anderson & May 1991). This is analogous to the development of herd immunity, but is achieved by selection for resistant sheep rather than by the recovery of infected sheep to a resistant category. However, if 'resistant' genotypes can become infected and transmit infection while rarely or never developing clinical signs (the carrier model), then the prediction may be different. If R_0 in a 'resistant' flock is greater than one (as for the parameter values considered here), then preclinical scrapie can become endemic in the flock and selection against susceptible genotypes persists indefinitely so that these are eventually eliminated (figure 1*c*). During the Romanov outbreak the frequency of the $V_{136}R_{154}Q_{171}$ haplotype fell from 0.33 to 0.18 over three years, confirming strong selection against this haplotype, but the long-term outcome is not yet known. Again, these

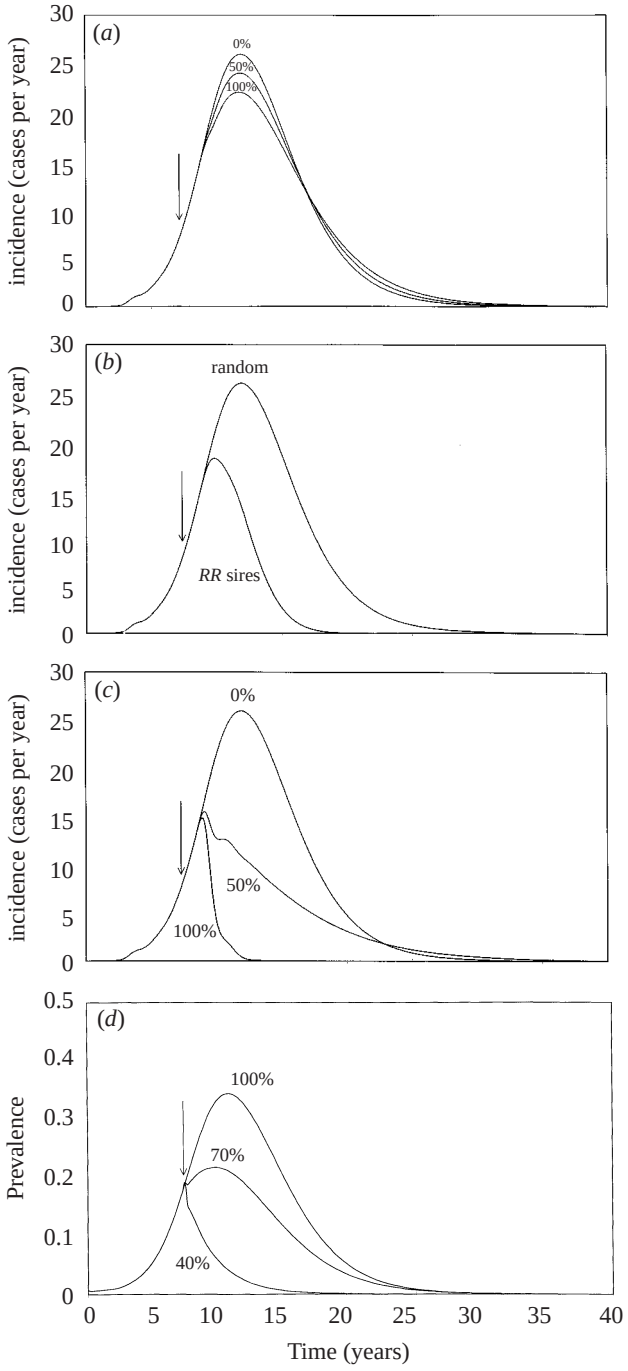

Figure 3. Impact of control measures on scrapie incidence through time using the standard model for a closed flock with control introduced 7.5 years (as indicated by arrow) after the introduction of scrapie. (*a*) Reduction of vertical transmission by 0%, 50% and 100% (as shown). (*b*) Random breeding or breeding with homozygous resistant (*RR*) rams only. (*c*) Reduction of horizontal transmission by 0%, 50% and 100% (as shown). (*d*) Slaughter of preclinically infected sheep at 100%, 70% and 40% of the infection load corresponding to the appearance of clinical signs (as shown); in this case the impact on prevalence is shown as incidence necessarily falls to zero if sheep are slaughtered before signs appear.

results are subject to stochastic effects; for example, if the frequency of the susceptibility allele is low, then elimination by genetic drift is likely, although it is also possible that drift will raise the allele frequency back above the threshold that determines whether a scrapie outbreak is possible. For an open flock the density of susceptibles may be maintained above the threshold by immigration of and/or breeding with susceptible genotypes and so endemic infection becomes possible.

The age distribution of scrapie cases depends on the mean and distribution of the age at infection, the mean and distribution of the incubation period and on sheep demography. At present, there is limited information on each of these components, although the observed age distribution of cases (figure 2*a*) is consistent with the parameter values assumed here. Also consistent with observation is the expectation of a lower age of appearance of clinical signs in homozygous susceptibles than in heterozygotes (figure 2*b*). This can arise through at least three mechanisms: (i) the different contributions of horizontal and vertical transmission because of the higher probability of a homozygous susceptible sheep being born to an infected ewe than a heterozygote (provided that homozygous 'resistant' sheep cannot become infected); (ii) the different force of infection due to horizontal transmission resulting in a greater average age of infection for heterozygotes (if these are less susceptible); and (iii) longer incubation period in heterozygotes. Conversely, observed differences in the age of cases between genotypes do not necessarily indicate differences in the incubation period, or even differences in susceptibility, between genotypes. The available data suggest that the average incubation period, for all susceptible genotypes, is unlikely to be significantly greater than 2 years.

Of the options for within-flock control of scrapie considered here, some reduction (but not by 100%) of vertical transmission may be achieved by the slaughter of lambs born to infected ewes; breeding only from 'resistant' rams can be achieved by genotyping, reduction of horizontal transmission is difficult because the mechanism is uncertain (although control by the decontamination of pasture has been attempted (Sigurdarson 1991)), and control by the slaughter of preclinically infected sheep is now becoming possible through the development of diagnostic tests (Schreuder *et al.* 1996). Our analysis suggests that reduction of vertical transmission will be relatively ineffective since most cases arise through horizontal transmission, as has been suggested (Hoinville 1996). Breeding with resistant rams is effective unless 'resistant' genotypes are carriers. Reduction of horizontal transmission is potentially effective given an effective control measure. The slaughter of preclinical cases is effective provided these can be diagnosed at low infection loads (or early in the incubation period), although the required diagnosis threshold is lower if 'resistant' genotypes are carriers. Some improvement in effectiveness is possible by combining control options.

One objective of this analysis is to illustrate aspects of scrapie epidemiology and control for biologically plausible sets of assumptions and parameter values. The detailed results are obviously sensitive to these assumptions and parameter values and it is important to emphasize that there is a great deal of uncertainty about both. However, the key conclusions appear to be robust: the time-scale of scrapie outbreaks within a sheep flock may be very long even when infection does not become endemic; and within-flock control of scrapie will, in some flocks, require

intensive effort and only be effective over a time-scale of several years.

Another objective of the analysis is to identify priorities for further research, and the results reinforce the importance of the following: (i) elucidating the mechanisms of horizontal transmission; (ii) determining the infection status of 'resistant' genotypes; (iii) further development of preclinical diagnostic tests with emphasis on the earliest possible diagnosis of infection; and (iv) the collection of field data on natural scrapie outbreaks, with emphasis on the age and genotype of affected and unaffected sheep.

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