Epidemiological implications of the susceptibility to BSE of putatively resistant sheep

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The experimental infection of sheep with bovine spongiform encephalopathy (BSE) by the oral route and the likelihood that sheep were fed BSE-infected meat and bone meal has led to extensive speculation as to whether or not sheep are naturally infected with BSE. In response, the UK government has initiated the National Scrapie Plan (NSP), an ambitious £120 million per year project to create a BSE- and scrapie-resistant national sheep flock, by selectively breeding for a genotype of sheep believed to be resistant to both diseases. This genotype has recently been shown to be susceptible to BSE by intracerebral (i.c.) inoculation. Should these sheep be sufficiently susceptible to BSE via natural transmission, the NSP might fail. Here we estimate the susceptibility of this genotype to horizontal (sheep-to-sheep) transmission of BSE by comparison with more extensive oral and i.c. exposure data for other sheep genotypes. We show that a previous estimate of the risk of BSE transmission to sheep via the feedborne route remains robust. However, using a mathematical model for the within-flock transmission of BSE, we show that, while the best estimate indicates that the NSP should be successful, current data cannot exclude the failure of the NSP.

INTRODUCTION

No natural infection of a sheep with bovine spongiform encephalopathy (BSE) has ever been observed. However, it is known that sheep can be experimentally infected with BSE by the oral route (Foster et al., 1993). Further, at the same time that BSE-infected bovine material in meat and bone meal (MBM) feed was driving the BSE epidemic in UK cattle, UK sheep were being fed protein supplements that may have contained MBM. Thus a 1990s feed-borne epidemic of BSE in sheep is a distinct possibility (Kao et al., 2002), and the potential implications for human health and its real impact on international trade mean that even a hypothetical epidemic of BSE in sheep must be treated very seriously (Ferguson et al., 2002; Harrison, 2002). Scapie, a disease of sheep related to BSE in cattle and variant Creutzfeld–Jacob disease in humans, is difficult to distinguish from BSE using current technologies, and thus there is also concern that an epidemic of BSE in sheep could be masked by scrapie (Foster et al., 1996a). The susceptibility of sheep to infection by scrapie is strongly associated with amino acids encoded at positions 136, 154 and 171 of the PrP gene (Hunter, 1997). Only one (unconfirmed) case of scrapie in a sheep homozygous for alanine at 136 and arginine at 154 and 171 (ARR/ARR) has ever been found (Ikeda et al., 1995). Further, none of 20 ARR/ARR sheep fed 5 g BSE-infected material has been found to harbour BSE infectivity (Jeffrey et al., 2001), with the last group of five still alive and free of clinical disease nearly 5 years post-infection (M. Jeffrey, personal communication). Thus the prevailing hypothesis is that a national flock composed of a sufficient proportion of ARR/ARR sheep will be resistant to both scrapie and BSE, and thus selective breeding for ARR/ARR sheep is the foundation of the National Scrapie Plan or NSP (Anon., 2001). Recently, the European Union has approved a proposal requiring all member states to adopt a similar programme (Anon., 2002). A recent report has shown that, of 20 ARR/ARR sheep intracerebrally (i.c.) challenged with 0.05 g BSE-infected bovine brain homogenate, three have thus far become infected (Houston et al., 2003), all with long incubation periods (1008, 1124 and 1127 days). There is a precedent in other species for susceptibility to i.c. challenge but with no evidence for susceptibility via the oral route (Ryder et al., 2000), and thus this result does not necessarily imply that ARR/ARR sheep are susceptible to BSE. Nevertheless, this report of BSE infection in ARR/ARR sheep is alarming, and demands examination of the impact of ARR/ARR susceptibility on any potential epidemic of BSE in sheep, and on the likely success of the NSP and similar programmes in other countries.

In this report, we consider the available evidence on ARR/ARR susceptibility to BSE and construct a model that
examines the question of how much impact this would have on the NSP.

**METHODS**

There are five frequently observed alleles that are known to affect scrapie susceptibility. However, the limited data obtained following oral exposure have thus far only shown that glutamine (Q) at codon 171 influences susceptibility of sheep to BSE (Foster *et al*., 2001). Therefore we simplify the genetic structure to consider only Q or R at 171, and thus three genotypes QQ, RR and QR (sheep encoding any combination of alanine and valine at codon 136 will all be considered ‘QQ’ sheep, for example).

Data on oral exposure of RR sheep to BSE are limited; of 15 exposed RR sheep thus far examined for PrP sc, the last group were at ages 350–354 days post-exposure (Jeffrey *et al*., 2003; McLean & Bostock, 2000). Given the lack of informative data, we assume that the distribution of incubation periods in RR sheep is the same width as the distribution for the QQ sheep in the same experiment (for QQ sheep, incubation period is 554 days, SD ± 45 days). A standard approach to account for right censoring of the data is used, with likelihood function (LF)

\[
LF = \prod_{i=1}^{3} \theta_i \prod_{j=4}^{12} (1 - \Theta_j)
\]

where \(\theta_i\) is the value of the normal distribution evaluated at the incubation periods of the sheep \(i\) that developed clinical signs (total three sheep developing clinical signs), and \(\Theta_j\) is the cumulative normal distribution function, evaluated at the time the sheep \(j\) were last observed (total of seventeen with no clinical signs at time of report, including one sheep removed from the experiment due to poor health). The MLE parameters fit the incubation period and the number of sheep infected. This calculation gives an upper estimate of the number infected, as sheep very early on in the experiment have very little effect on the incubation period distribution. Then the experimental results thus far are consistent with at most 7 of 20 infected (incubation period 1208 days, 95% CI 1164–1292 days, and 95% CI of 6 to 9 of 20 infected, including one intercurrent death), compared to 17 of 19 QQ sheep i.c. challenged at the same dosage (Fig. 1) (Houston *et al*., 2003).

Interpretation of these data is confounded by two problems: the high dosage involved (0–0.5 g of brain tissue) and the unnatural route of infection. Natural transmission would most likely involve exposure of large numbers at low dose and be mimicked more closely by oral exposure. We therefore calculate the dose response using a logistic curve, with a slope estimated from titrations of scrapie and the limited data on BSE in mice (Kao *et al*., 2002; McLean & Bostock, 2000). The dose response curve for oral exposure of RR sheep is then calculated from oral and i.c. inoculation data in the better studied QQ sheep, by assuming that the differences in susceptibility between QQ and RR

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**Fig. 1.** Incubation periods of sheep exposed to BSE via i.c. inoculation. Data are infected ARQ/ARQ sheep (grey bars), infected ARR/ARR sheep (black bars) and ARR/ARR sheep either removed before infection could be determined or that are still in the experiment, at days p.i. last seen (white bars). The three curves from left to right represent MLEs of the incubation period, for ARQ/ARQ sheep, for ARR/ARR sheep assuming 7 of 20 infected and a normal distribution with the same SD as for ARQ/ARQ sheep, and finally for ARR/ARR sheep assuming all 20 infected. The latter is shown for comparison.
sheep are equivalent under i.c. and oral exposure, i.e.

$$\frac{ID_{50}^{RR,oral}}{ID_{50}^{QQ,oral}} = \frac{ID_{50}^{RR,ic}}{ID_{50}^{QQ,ic}} \times \frac{ID_{50}^{QQ,oral}}{ID_{50}^{QQ,oral}}$$  \hspace{1cm} (2)

where the superscripts indicate the genotype and route of transmission. The relative susceptibility of the two genotypes varies with the dose received; at very high dose, both genotypes would be infected nearly 100% of the time, while at very low dose, the probability of infection between the two would be very different. Dose response is then calculated from the logistic curve

$$\ln \left( \frac{p}{1-p} \right) = \omega_0 + \omega_1 \log(x)$$  \hspace{1cm} (3)

where $p$ is the probability of infection, $x$ is the dose in grams, and $\omega_0$ is the intercept. The slope $\omega_1 = 4.5$ is based on an extensive analysis of scrapie in the mouse model (McLean & Bostock, 2000). The sensitivity of equation (3) to this parameter is discussed elsewhere (Kao et al., 2002). At very low dose, RR sheep reach a limit of 14-fold less susceptible than QQ sheep (with a 95% confidence interval of 1-5 to 138-fold less susceptible). Here, we explore the range of susceptibilities from 1-5- to 138-fold less than in QQ sheep. Note that the data therefore do not preclude very high relative susceptibilities for RR sheep compared to QQ sheep.

The initial exposure of the sheep population and the majority of the initial cases (if any) would have been a spillover from the feedborne epidemic in cattle. In order to evaluate the impact of RR susceptibility on any feedborne epidemic of BSE in sheep, we modify a published difference equation model of a feedborne BSE epidemic, stratified by the segment of the sheep industry (hill, upland and lowland) and by sheep genotype (see Appendix for mathematical details).

In this model, incubation periods are based on experimental data, and are the median values of the distribution used in a previous model, i.e. 2 years for QQ sheep, and 5 years for QR sheep (Foster et al., 2001; Kao et al., 2002). For RR sheep, the limited data show only that incubation periods shorter than 5 years are inconsistent with the available data (Jeffrey et al., 2001), and so this lower limit is assumed. Vertical transmission is also considered. Epidemiological observations provide strong evidence for perinatal transmission of scrapie (Elsen et al., 1999); however, true vertical or in utero transmission is difficult to prove, and thus far no conclusive evidence has been found for transmission via germinal cells (Foster et al., 1992, 1996b, 1999; Wang et al., 2001) or for foetal infection (Andreolletti et al., 2002b; Hadlow et al., 1982). However, infectivity and PrPSc deposits have been found in the placentas of scrapie-infected ewes (Race et al., 1998; Tuo et al., 2001). Recent results indicate that placental infection is restricted mainly to foetal cells and is controlled by the PrP genotype of the foetus (Andreolletti et al., 2002b; Tuo et al., 2002). Together with the evidence for an association of scrapie with group lambing in pens (McLean et al., 1999), this supports a hypothesis of post-natal infection by contact with infectious material derived from placental contamination of the environment. While this suggests that perinatal transmission may be genotype dependent and that control measures such as changes in lambing practices might effectively reduce it, we allow for 30% vertical transmission to lambs throughout the incubation period for all genotypes. This transmission level is as examined in a previous report (Kao et al., 2002), and roughly consistent with the results of other analyses for scrapie (Woodhouse et al., 1998).

BSE-infected MBM (DNV, 1998), the model is stratified into two groups: hill/upland and lowland. Hill and upland ewes near the end of their lifespans are incorporated in large numbers into lowland flocks ('draft ewes'), and are a potential contributor to BSE in lowland flocks. However, as lambs raised for slaughter leave the flock too soon to contribute much to TSE transmission, for purposes of exploring TSE transmission we are only interested in the breeding flock, and draft ewes mainly produce lambs for slaughter. In 1997, only 0.88 million of 10.73 million breeding ewes in the lowland sector were the progeny of draft ewes (Pollott, 1998). Thus draft ewes are unlikely to have added significantly to a feed-borne BSE epidemic in lowland breeding sheep, and the equations contain no direct interaction between the stratified industries.

The series of feed bans over the late 1980s and 1990s eliminated the majority of BSE cases in cattle (Stevenson et al., 2000), and would have also ended BSE transmission to sheep in the absence of horizontal transmission. Thus the current stability of any epidemic of BSE in sheep is analysed using a within-flock transmission model that does not consider feedborne transmission. We construct a model incorporating 14 age and 14 infection stages, to allow for consideration of realistic incubation periods (Foster et al., 2001; Kao et al., 2002) and mortality rates (McLean et al., 1999). We assume that infection load increases exponentially, with maximum load achieved when the clinical stage is reached, at which point the animal dies or is removed (Matthews et al., 2001; Stringer et al., 1998). The duration of the clinical stage is taken to be on average 1 month. Incubation periods are as in the feedborne transmission model, with the same assumptions about vertical transmission. The age structure is as previously described for the breeding flock only (McLean et al., 1999); given the incubation periods involved, non-breeding lambs (i.e. those going directly to slaughter) do not remain in the flock for a sufficient time to contribute to infection. The full mathematical details are presented in the Appendix.

Evaluation of the efficacy of control is based on the calculation of the basic reproduction ratio. The standard definition of the basic reproduction ratio ($R_0$) for this system is the average number of BSE-infected sheep that would result following the introduction of a single infected sheep into a flock harbouring no existing infection (MacDonald, 1952). It is well known that if $R_0$ is less than one, a disease cannot persist, and therefore this is used as the benchmark for disease eradication policies, and provides our definition of a 'resistant flock' (Anderson & May, 1990). Here, we calculated $R_0$ based on the related next generation matrix approach (Diekmann et al., 1990). For simple systems in which there is only a single infected class, the definition is identical to the one given above, and for all systems $R_0=1$ remains the threshold for disease eradication. The next generation matrix for a reduced form of the model with only two age and two infection classes is presented in the Appendix.

Based on our estimates of genotype differences in susceptibility at low dose, we consider ranges of relative susceptibility of RR sheep to QQ sheep of 1-5 to 138, and transmission rates up to $\beta=0-1$ infections per infectious sheep per sheep per year ($R_0$ ranging up 3-6). At the upper value, for a within-flock epidemic beginning in 1995, i.e. the year by which 90% of the feedborne infections would have occurred (Kao et al., 2002), a typical flock of 450 sheep (16% RR, 36% QQ and 48% QR sheep) would have a case incidence of 30 in 2002 with 100 cases from which 90% of the feedborne infections would have occurred (Kao et al., 2002) and mortality rates (McLean et al., 1999). We assume that infection load increases exponentially, with maximum load achieved when the clinical stage is reached, at which point the animal dies or is removed (Matthews et al., 2001; Stringer et al., 1998). The duration of the clinical stage is taken to be on average 1 month. Incubation periods are as in the feedborne transmission model, with the same assumptions about vertical transmission. The age structure is as previously described for the breeding flock only (McLean et al., 1999); given the incubation periods involved, non-breeding lambs (i.e. those going directly to slaughter) do not remain in the flock for a sufficient time to contribute to infection. The full mathematical details are presented in the Appendix.

In order to evaluate the effect on the time to create a replacement flock, we calculate the replacement rate of the Q allele to be approximately
0·1 per year (based on nine fully genotyped flocks actively trying to breed for resistance; C. Chihota, unpublished data). This is remarkably consistent across all the flocks despite large differences in allele distribution (ranging from an initial frequency of the ARR allele of 0·28 to 0·57).

**RESULTS**

If RR sheep are susceptible to orally transmitted BSE, how many were likely to have been infected via the feedborne route? Using the dose-response curve calculated above and a simple age-structured model (see Methods and Appendix), Fig. 3 shows that, even under the most pessimistic scenarios (when QQ susceptibility to BSE is assumed to be high, and therefore much higher than RR susceptibility), only a few more sheep might have been infected than previously calculated and current prevalence would remain below estimates established by surveillance of scrapie cases (Gravenor et al., 2003). Of more concern, however, is the possibility that a flock composed solely of RR sheep could sustain a BSE epidemic.

Fig. 4 describes the relative effort required to reduce \( R_0 \) to below one, in terms of the percentage of the flock that is required to be resistant (Fig. 4A) and the expected length of time to produce a resistant flock (Fig. 4B). In this scenario, the best estimate of susceptibility implies that the NSP is unlikely to fail. At the best guess of 13·8 times lower susceptibility for RR sheep, 72 % of the flock would have to become RR (85 % R alleles) to result in \( R_0 < 1 \) at the highest levels of horizontal transmission considered. However, the range of susceptibility includes the possibility that the NSP could fail; at the highest transmission levels, should RR sheep be as little as three times less susceptible than QQ sheep, even flocks composed fully of RR sheep would have \( R_0 \) above one. As one would expect, the extra effort to create a resistant flock goes up if vertical transmission is considered. At the relatively high level of vertical transmission of 30 %, and the best guess of 13·8 times lower susceptibility for RR sheep, 90 % of the flock would have to become RR (95 % R alleles) to result in \( R_0 < 1 \) at the highest levels of horizontal transmission considered, and for RR susceptibility six times lower than QQ, the NSP would fail.

We use the replacement rate of 0·1 per sheep per year to calculate the additional time required to produce a resistant flock (i.e. having \( R_0 < 1 \); Fig. 4B) and show that for most parameter estimates this would be slight, with a best estimate of no more than half a year (for a typical flock containing 40 % R alleles, total time to achieve \( R_0 = 1 \) is about 5 years). As replacements are typically born once per year in the spring, a better interpretation would be one in two taking an extra year to become resistant at the flock level. Should there be high levels of vertical transmission, this could be considerably longer: an extra year per flock to achieve resistance in the 30 % vertical transmission scenario described above.

**DISCUSSION**

Our results show that the upper bound of potential RR susceptibility does not preclude the failure of the NSP for sufficiently high transmission rates (\( \beta \)); at \( \beta = 0·1 \), failure occurs when RR sheep are 3-fold less susceptible than QQ sheep, and the upper limit of RR susceptibility consistent with the data is 1·5-fold less susceptible. For the most likely estimate of susceptibility, i.e. about 14-fold less than QQ sheep, the NSP is would be successful up to \( \beta = 0·1 \) (Fig. 4). Indeed, only in rare cases would this susceptibility result in a significant delay to the achievement of flock level resistance. The replacement rate assumes that the farmer has full knowledge of this flock genotype and this is unlikely to be universal; however, whole genotyping of flocks highly affected with scrapie is a useful way to increase the efficacy of the NSP (Kao et al., 2001) and thus this replacement
rate is a relevant upper bound on the initial rate at which flocks could breed for resistance (the rate would likely increase as more RR rams become available).

The impact of vertical transmission in this model is dependent on the assumption that RR sheep would have the same level of susceptibility to vertical transmission as QQ sheep. Should the relative susceptibility of RR lambs to both horizontal and vertical transmission scale in the same fashion (i.e. RR lambs half as susceptible as QQ lambs via horizontal transmission, implying that they are half as susceptible via vertical transmission as well) then high levels of vertical transmission would make no difference to the success of the NSP.

BSE has never been recognized in sheep except under experimental conditions, and RR sheep have never been orally infected with BSE or scrapie. Thus one may question whether the risk to RR sheep is all ‘sound and fury’. We point out that the scrapie case notification data in Britain have only resulted in 276 ARQ/ARQ TSE cases being reported. Since ARQ/ARQ and ARR/ARR sheep exist in similar proportions, this implies that ARR/ARR are no more than 1% as susceptible to scrapie as ARQ/ARQ sheep but does not preclude it, and of course no equivalent data on BSE exists. Increased surveillance and genotyping across Europe (http://europa.eu.int/comm/food/fs/sc/ssc/out238_en.pdf) may result in the discovery of scrapie in putatively resistant sheep; however, at 1% susceptibility compared to ARQ/ARQ sheep, this is unlikely to result in a national flock of ARR/ARR sheep being able to maintain endemic scrapie, though danger remains from either an as yet undiscovered strain of scrapie to which ARR/ARR sheep are more susceptible, or ‘carrier’ sheep (i.e. sheep that can transmit infection but never exhibit clinical signs). However, should BSE be discovered in the UK national sheep flock, we illustrate scenarios for RR susceptibility consistent with all the available data on BSE in sheep, and show that we cannot rule out levels of RR susceptibility that would cause the failure of the NSP.

**Fig. 3.** Estimated numbers of ARR/ARR sheep infected with BSE (A) and clinical cases (B) from 1985 and 2001 for upper values of the epidemic parameters from a previous report (Kao et al., 2002), i.e. where sheep specific parameters were calculated on the basis of 37% of cattle being fed ID70’s at the height of the BSE epidemic in cattle, 10% vertical transmission and 50% less MBM fed to sheep compared to cattle. For comparison, the total numbers of infected sheep and clinical cases are shown (C and D respectively). The upper 95% CI in ARR/ARR susceptibility is assumed. The vertical dashed area represents animals age 0 to 3 and the diagonally dashed area those aged 3 and above. The solid lines are the number of vertically transmitted infections. Even under this pessimistic scenario the impact of ARR/ARR susceptibility on the overall epidemic is small.
This study highlights the need for additional data on relative infectivity of BSE in different genotypes and breeds of sheep, titration of infectivity in sheep, and the possible infectivity of tissues from sheep with very long incubation periods, or sheep that may never develop clinical infection. Experiments to study these factors are either in progress or imminent; however, the long incubation period of the disease and the expense of conducting large-scale experiments preclude definitive answers for at least several years. While an exercise of this type is by nature speculative, we have used the best current estimates to establish that ARR/ARR susceptibility to BSE is unlikely to preclude the development of a BSE-resistant national flock, typically requiring that one out of two flocks take 6 years instead of 5 to achieve TSE resistance.

**APPENDIX: MATHEMATICAL DETAILS**

**The feed-borne transmission model**

The feed-borne epidemic is described by a system of age-structured difference equations. It is assumed that infection comes solely from consumption of BSE-infected MBM, first introduced in 1986. There is no sheep-to-sheep transmission in these equations, save for a maximum of 30% vertical transmission from ewe to lamb, in the last year of the ewe incubation period, and recycling of MBM containing BSE-infected sheep protein is not considered. It is known that in some cases, interspecies infection of animals with TSEs is less efficient than for subsequent passages (the species barrier effect; Kimberlin et al., 1987). Thus, even though the bulk of protein in MBM would have...
been from cattle, a small proportion of infected sheep protein may have had an inordinate effect. However, transmission of cattle-derived BSE to sheep is not significantly less than for transmission from cattle to cattle (Kao et al., 2002), and thus the species barrier is small and the effect of MBM containing infectious BSE material from sheep is not likely to be significant. These equations have been presented previously (Kao et al., 2002) and are described here in brief.

Flocks are divided into two classes, hill and upland flocks and lowland flocks. We define variables as follows. For the genotype labelled ‘AA’, \( I_{AA}(t, \tau, a) \) is the number of animals infected for a given age cohort \( a \), infected at time \( \tau \), which survive to time \( t \). Remaining parameters are the frequency of the genotype \( f_{AA} \), the first year of the calculation \( t_0 \) (1986), and the oldest age cohort considered, \( a_f \) (9 years). The variable \( S_{AA}(t, a) \) is the number of uninfected sheep of genotype AA of age cohort \( a \) that survive to time \( t \). The total force of infection is a combination of the consumption of infected feed \( f(t) \) and vertical transmission with probability \( v \). The natural survival proportion is \( d_a \) (100% surviving to 4-7 years, then declining at a rate of 1/1 per year thereafter, to a maximum age of 9 years), and the proportion that survive disease for a time \( t-\tau \) since infection is \( \mu(\tau-\tau) \). The annual birth rate for each age cohort \( a \) is given by \( b(a) \) in general. The equations for hill and upland flocks are:

\[
\begin{align*}
(A1.a) & \quad I_{QQ}(t, \tau, a) = \sum_{a_1=1}^{a_f} \sum_{a_2=t_0}^a \{ I_{QQ}(t, \tau, a_1, a_2) \mu_{QQ}(f_{QQ} + 1/2 f_{QR}) + I_{QR}(t, \tau, a_1, a_2) \mu_{QR}(1/2 f_{QQ} + 1/2 f_{RR}) \} + I_{RR}(t, \tau, a_1, a_2) \mu_{RR}(1/2 f_{QQ} + 1/2 f_{RR}) \\
(A1.b) & \quad I_{QR}(t, \tau, a) = S_{QR}(t-1, a-1) f_{QR}(t) \quad \text{for } a > 1 \\
(A1.c) & \quad I_{RR}(t, \tau, a) = S_{RR}(t-1, a-1) f_{RR}(t) \quad \text{for } a > 1, \tau < t \\
(A1.d) & \quad I_{RR}(t, \tau, a) = \sum_{a_1=1}^{a_f} \sum_{a_2=t_0}^a \{ I_{RR}(t, \tau, a_1, a_2) \mu_{RR}(f_{RR} + 1/2 f_{QR}) + I_{QR}(t, \tau, a_1, a_2) \mu_{QR}(1/2 f_{RR} + 1/2 f_{QR}) \} + I_{RR}(t, \tau, a_1, a_2) \mu_{RR}(1/2 f_{RR} + 1/2 f_{QR}) \\
(A1.e) & \quad I_{QR}(t, \tau, a) = S_{QR}(t-1, a-1) f_{QR}(t) \quad \text{for } a > 1 \\
(A1.f) & \quad I_{RR}(t, \tau, a) = I_{RR}(t-1, a-1) d(a) \mu_{RR}(t-\tau) \quad \text{for } a > 1, \tau < t \\
(A1.g) & \quad I_{RR}(t, \tau, a) = \sum_{a_1=1}^{a_f} \sum_{a_2=t_0}^a \{ I_{RR}(t, \tau, a_1, a_2) \mu_{RR}(f_{RR} + 1/2 f_{QR}) + I_{QR}(t, \tau, a_1, a_2) \mu_{QR}(1/2 f_{RR} + 1/2 f_{QR}) \} + I_{RR}(t, \tau, a_1, a_2) \mu_{RR}(1/2 f_{RR} + 1/2 f_{QR}) \\
(A1.h) & \quad I_{RR}(t, \tau, a) = S_{RR}(t-1, a-1) f_{RR}(t) \quad \text{for } a > 1 \\
(A1.i) & \quad I_{RR}(t, \tau, a) = I_{RR}(t-1, a-1) d(a) \mu_{RR}(t-\tau) \quad \text{for } a > 1, \tau < t
\end{align*}
\]

Equations (A1.a), (A1.d) and (A1.g) are for the first age cohorts, and include vertical transmission, dependent in general on the genotype of the lamb. Equations (A1.b), (A1.e) and (A1.h) refer to the infection of all other age cohorts through exposure to feed, and equations (A1.c), (A1.f) and (A1.i) refer to the transitions of infected sheep between age and infection classes. The equations for the lowland flocks differ only in the force of infection from feed and in the genotype proportions, and are not presented.

The sheep-to-sheep transmission model

Investigation of the effect of the impact of RR susceptibility on sheep-to-sheep transmission is based on a system of age and infection stage-structured differential equations. Here \( S_{RR,i} \) are susceptible RR sheep of age class \( i \), and \( I_{RR,i,j} \) are infected RR sheep of age class \( i \) and infection stage \( j \) \((i>j)\), with similar definitions for QQ and QR sheep. Equation (A2.f) describes the rise in infectivity over time \((t)\) (to a maximum of one at the end of the incubation period, \( \tau_{\text{inc}} \)). Equation (A2.g) is the total mortality at time \( t \), which must be balanced by births and/or the buying-in of sheep in (A2.a). The genotype of offspring is described by proportionate mixing of all genotypes in the flock \([AA]\) is the proportion of AA).

Vertical transmission is incorporated as in the feed-borne transmission equations. The parameter \( v_{ij} \) represents the proportion of infected lambs of genotype \( i \) born to infected ewes at infection stage \( j \) (i.e. infection is lamb genotype dependent; Andreoletti et al., 2002a; Tuo et al., 2002). The age structure of the breeding population is as previously described (McLean et al., 1999).

\[
\begin{align*}
(A2.a) & \quad \frac{dS_{RR,i}}{dt} = \left[ \sum_{j=1}^{N_{ages}} \left( S_{RR,j,i}(t) + 0.5 S_{QR,j,i}(t) \right) \right] g(t) / N_{\text{sheep}} \\
& \quad + \left[ \sum_{j=1}^{N_{ages}} \left( 0.5 S_{QR,j,i}(t) + 0.25 S_{QR,j,i}(t) \right) \right] g(t) / N_{\text{sheep}} - \lambda S_{RR,i} - \beta_{RR,i} S_{RR,i} \sum_{j=1}^{N_{ages}} f(c_{RR}, \tau_{RR,k}) I_{RR,j,k} \\
& \quad + \left( f(c_{QR}, \tau_{QR,k}) I_{QR,j,k} + f(c_{QQ}, \tau_{QQ,k}) I_{QQ,j,k} \right) - \kappa \delta_{RR,i} \sum_{j=1}^{N_{ages}} f(c_{RR}, \tau_{RR,k}) I_{RR,j,k} \\
& \quad - \beta_{RR,i} S_{RR,i} \sum_{j=1}^{N_{ages}} f(c_{RR}, \tau_{RR,k}) I_{RR,j,k} + f(c_{QR}, \tau_{QR,k}) I_{QR,j,k} + f(c_{QQ}, \tau_{QQ,k}) I_{QQ,j,k} \right) \quad i > 1
\end{align*}
\]
The transmission rate is $\beta_{RR,i}$ to RR sheep in age class $i$ (i.e. susceptibility is allowed to vary with age; however, it does not in the final model). The remaining parameters are the transition rate between age classes ($\lambda$), the rate of increase of infectivity ($\varepsilon_{RR}$, $\varepsilon_{QR}$ or $\varepsilon_{QQ}$, equal to 4 per year in all cases), the incubation period for the disease ($t_{RR}$, $t_{QR}$ or $t_{QQ}$, equal to 5, 5 and 2 years), the non-disease-dependent mortality at age $i$ ($\kappa_i$, equal to zero up to 4-7 years, 1-1 per year thereafter), and the disease-dependent mortality for genotype XX at infection stage $j$ ($\delta_{XX,j}$ equal to zero up to the end of the incubation period, 12 per year thereafter).

The function $\delta_{ij}$ is the Kronecker delta, equal to one if $i$ equals $j$, or zero otherwise. The model is similar to previously published models of scrapie and sheep BSE, although the others have used a partial differential equation framework and the genotype range is more restricted than in models of scrapie (Ferguson et al., 2002; Stringer et al., 1998).

Simulations show that the model predictions are robust to increases above 14 age and infection classes (mean duration of 6 months in each age/infection class).

The next generation matrix

Defining the next generation matrix as $M$, element $m_{ij}$ is the average number of individuals of infectious class $i$ created when a single individual of class $j$ is introduced into a wholly susceptible, equilibrium population. For a simplified model with only two age classes, the infectious classes are $I_{RR,1,i}$, $I_{RR,2,i}$, $I_{QR,1,i}$, $I_{QR,2,i}$, $I_{QQ,1,i}$, $I_{QQ,2,i}$ and $I_{QQ,2,i}$, and $M$ is a $9 \times 9$ matrix of the form

\[
(A.2) \quad \frac{dI_{RR,i}}{dt} = \left[ \begin{array}{c} \sum_{j=1}^{N_{QRi}} f(\varepsilon_{RR},t_{RR},k)I_{RR,j,k} \\
\beta_{RR,j}S_{RR,i} \sum_{j=1}^{N_{QRi}} f(\varepsilon_{RR},t_{RR},k)I_{RR,j,k} \\
+ f(\varepsilon_{QR},t_{QR},k)I_{QR,j,k} + f(\varepsilon_{QQ},t_{QQ},k)I_{QQ,j,k} \\
-f(\varepsilon_{QR},t_{QR},k)I_{QR,j,k} - f(\varepsilon_{QQ},t_{QQ},k)I_{QQ,j,k} \\
+ \lambda I_{RR,1,i} - \kappa_1 I_{RR,1,i} - \alpha_I I_{RR,1,i} \\
\end{array} \right] \delta_{ij} + I_{RR,j,k} \\
\frac{dI_{RR,j,i}}{dt} = \lambda I_{RR,1,i} - \kappa_1 I_{RR,1,i} - \alpha_I I_{RR,1,i} \\
\frac{dI_{QR,j,i}}{dt} = \lambda I_{QR,1,i} - \kappa_1 I_{QR,1,i} - \alpha_I I_{QR,1,i} \\
\frac{dI_{QQ,j,i}}{dt} = \lambda I_{QQ,1,i} - \kappa_1 I_{QQ,1,i} - \alpha_I I_{QQ,1,i}
\]

\[
(A.5) \quad M =
\begin{pmatrix}
\frac{v_{RR,1}[RR]}{\alpha_1 + \lambda + \delta_1} + f(\varepsilon_{RR},t_{RR,1})\beta_{RR} & \frac{f(\varepsilon_{QR},t_{QR,1})\beta_{RR}}{\alpha_1 + \lambda + \delta_1} & \lambda \\
\frac{v_{RR,2}[RR]}{\alpha_2 + \delta_2} + f(\varepsilon_{RR},t_{RR,2})\beta_{RR} & \frac{f(\varepsilon_{QR},t_{QR,2})\beta_{RR}}{\alpha_2 + \delta_2} & 0 \\
\frac{v_{RR,3}[RR]}{2} + f(\varepsilon_{QR},t_{RR,1})\beta_{RR} & \frac{f(\varepsilon_{QR},t_{QR,1})\beta_{RR}}{\alpha_1 + \lambda + \delta_1} & 0 \\
\end{pmatrix}
\]
Only the upper-left and lower-right elements are shown. Parameters are as defined in the previous section. The value of $R_0$ is then $\rho(M)$, the spectral radius of $M$.

In all cases, the equations were analysed numerically using standard algorithms in Mathematica v4.0 (Wolfram, 1999) for the solution of systems of ODEs (NDSolve) and the lead eigenvalue of the next generation matrix (Eigenvalue).

REFERENCES


