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Quantifying BSE control by calculating the basic reproduction ratio R_0 for the infection among cattle

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Abstract. The safety of using meat and bone meal (MBM) in mammal feed was studied in view of BSE, by quantifying the risk of BSE transmission through different infection routes. This risk is embodied in the basic reproduction ratio R_0 of the infection, i.e. the average number of new infections induced by one initial infection. Only when R_0 is below 1, will the disease die out with certainty and the population will become free from BSE. Unfortunately this is a slow process due to the slow progression of the disease.

We calculate R_0 explicitly from basic ingredients taking several different transmission routes into account. Several of the basic ingredients are functions of age or of infection-age. We also calculate the exponential growth rate r in terms of the same basic ingredients.

Next we quantify the ingredients from available data and compute the effects on R_0 of various scenario's for controlling BSE, with examples for the UK and the Netherlands.

1. Introduction

Enormous public attention recently focused on bovine spongiform encephalopathy (BSE) and related diseases. BSE may pose a threat for human health, as consumption of BSE infected beef may induce a new variant of Creutzfeldt-Jakob disease (vCJD) [4]. The risk for humans to contract vCJD seems to be very small, when assuming that the average incubation period is less than ten years. However, the incubation period may be much longer, in which case we have not yet reached the peak of the vCJD-epidemic and infection risks may be underestimated so far. Presently, the BSE epidemic in the UK is far below its peak of 1992 and many food-safety measures make sure that the risk of contracting new infections in the human population has decreased enormously.

The BSE epidemic in the United Kingdom was mainly due to the use of BSE infected meat and bone meal (MBM) in cattle feed [25]. In the 1980s a major part of MBM in the UK was produced at low temperatures (about 100^oC). Later it was

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shown that such conditions were insufficient for the inactivation of the infectious agent of BSE [22].

To answer questions about the efficacy of control measures and to quantify regional risk of BSE outbreaks, we calculate the basic reproduction ratio R_0, incorporating the postulated mechanisms of BSE transmission in an age-structured population of cattle. The basic reproduction ratio R_0 of an infection is the expected (average) number of new infections, caused by a typical infected individual. (See [8].) We will derive an explicit expression for R_0 of BSE in terms of basic ingredients that describe cattle demography and transmission routes of the BSE agent. In section 2 we describe the ingredients, the calculation of R_0 and of the real-time exponential growth rate r of the early part of an epidemic. In section 3 we quantify all ingredients from data. In section 4 we compute values for R_0 under various realistic combinations of control measures, and provide confidence intervals for R_0-estimates. Finally we briefly discuss implications for EU-guidelines in section 5.

2. The ingredients

2.1. Infection routes

A model of infection-host interaction intended for risk assessment by quantifying R_0 should include all characteristics of the infection and the host that may have a major impact on R_0. The characteristics that we consider important in BSE epidemiology are, firstly, the incubation time of BSE, which is extremely long, close to the average life time of cattle. Secondly, new born calves are more susceptible than adult cattle, as a result of which the average age at infection is rather low, therefore age needs to be taken into account. Thirdly, different infection routes exist.

We consider the following five infection routes. (1) Recycling of proteins in MBM that is used in cattle feed (horizontal infection). (2) Maternal infection, i.e. from mother to calf (vertical infection) probably at birth, (3) Birth related infection may also affect other cattle at close proximity during birth (diagonal infection). The afterbirth is supposed to be a risk factor. (4) Direct animal to animal transmission (horizontal). (5) Infectious material in the environment (use of MBM as fertilizer and remaining infection from feed, left in manure).

Infection routes (4) and (5) seem of minor importance, but are included in the model to allow an assessment of their impact. Given the difficulties in inactivating the BSE agent, infectious material may persist in the environment for a long time. Scrapie material is known to remain infectious for several years in the environment [3] and for BSE similar survival may be expected. Therefore, although infection route (5) may cause only a small risk at any moment in time, it is possible that new cases will arise by this route over a very long period to come. This delay effect of the environment is not incorporated into the model.

As infection routes (3), (4) and (5) can not be estimated separately from the data presently available, we clustered them into one parameter ω. During the initial stage of the epidemic in the UK, these routes were probably negligibly small compared to the feed infection route. However, when infection via the feed infection route has become small due to BSE control measures, ω may become of influence.

2.2. *Fundamental assumptions*

Two fundamental assumptions underlie the model of the BSE-cattle interaction. One assumption is very common in epidemiological models: infection is transmitted during random contacts between animals, so spatial structure is not incorporated. This implies that all infectious contacts are randomly distributed in space over the whole cattle population and the model neglects clustering of the infection in space. Generally, such a model is well suited for describing contact infections within a herd, and can be applied to a regional or national population when there is contact (direct or indirect) between all herds.

The second main assumption underlying the model is that the infectious agent of BSE behaves according to the 'single hit theory' [14]. This means that there are numerous infectious particles (in case of BSE prions) in an animal with clinical BSE and each of these particles has a very small probability of inducing infection. Present knowledge of BSE (and Transmittable Spongiform Encephalopathy's, TSEs in general) suggests that the infection does not trigger any immune reaction in the host, so multiple doses of ingested infectious material are not supposed to lead to increasing resistance.

2.3. *The parameters*

We list the parameters of our description of cattle demography and BSE-cattle interactions. Some of these are functions of age (a) and infection-age (τ, i.e. time elapsed since the animal got infected). The latter variability is introduced since susceptibility of cattle depends on age and infectiousness depends on infection-age. The population dynamics of cattle is described by age-dependent culling and age-dependent birth rate. The infectious load in an infected animal grows with the time τ since the animal was infected. All newborn animals arrive in the susceptible class, except those that already get infected maternally. For infected animals, culling can be either due to age, or due to recognition of BSE-symptoms.

The model contains parameters that can be influenced by control measures against BSE or local conditions, and parameters that cannot. The parameters that remain fixed in this study are:

- age-dependent susceptibility of cattle, $\beta(a)$
- infection-age dependent infectious load of an infected animal, $\gamma(\tau)$
- maternal transmission rate (per unit of infectious load of the mother), m
- contact infection rate via the environment, ω

The following parameters can be affected by control measures or local conditions:

- per capita culling rate for cattle (not infected), μ
- per capita birth rate, b
- the reduction of infectious load by the rendering process, k_1
- the fraction of MBM that is fed to cattle (not to other animal species), k_2
- the fraction of infectious load from a non-BSE suspect carcass that enters the rendering process, c_1

- the fraction of infectious load from a BSE suspect carcass that enters the rendering process, c_2
- the per capita culling rate of infected cattle by recognizing BSE symptoms at time τ after infection, $\nu(\tau)$.

2.4. Characterization of R_0

After naming the basic ingredients of the BSE cattle interactions, the basic reproduction ratio R_0 can be characterized. It applies to a set of models, which may include features like heterogeneity and stochasticity, which need not be specified at this point. Such model details are not essential for the methodology, but they may influence parameter estimations. A general description of the method to calculate R_0 can be found in [8]. During an epidemic, the fraction of susceptible animals decreases and due to that, the expected number of infections initiated by an infected animal also decreases. Although the basic reproduction ratio is a measure for the initial phase of an outbreak, it also supplies information about the expected number of animals that will get infected during the whole outbreak, (see also [8]).

To characterize the basic reproduction ratio R_0, we need to define the 'typical' infected individual. Reviewing the five different infection routes, it appears that there are two typical distributions for age at infection. Maternal infection takes place at birth, and thus for maternally infected animals infection age is equal to their real age. Animals infected by the other infection routes all have a distribution of age at infection, depending on the age-dependent susceptibility and age-dependent survival. For animals infected via MBM, the age distribution will also depend on the amount of MBM ingested at different ages. As data are very hard to obtain, we assume this to be a constant for all ages, thus leading to only two different groups of infected animals: a group of animals infected at birth and a group that can be described by a fixed probability distribution of age at infection. Thus we arrive at a two dimensional 'age at infection' space, spanned by a delta 'function' $\delta_a = \delta_0$ for maternal infections and the function $\beta(a)\mathcal{F}_s(a)$, for horizontal, diagonal en environmental infections.

For the two types of infected animals we separately determine the expected number of new infections that they will induce during their whole infectious period (by the two types of infection routes). We denote the expected number of infections via a type i route caused by an animal that was itself infected through a type j route as q_{ij}. Explicit formulas for q_{ij} can be derived.

A first step in formulating q_{ij} is constructing a survival function $\mathcal{F}_s(a)$, which describes the probability for a susceptible animal to survive until at least age a:

$$\mathcal{F}_s(a) = e^{-\int_0^a \mu(\alpha)d\alpha} \tag{1}$$

The infection survival function $\mathcal{F}_i(\tau)$ describes the probability of an infected cow to survive until at least infection age τ, under the condition that the animal will be culled only due to BSE signs, i.e. neglecting the possibility of normal cull.

$$\mathcal{F}_i(\tau) = e^{-\int_0^\tau \nu(\alpha)d\alpha} \tag{2}$$

Then the survival function of infected cattle under normal farming conditions (including normal cull) is represented by $\mathcal{F}_s(a+\tau)\,\mathcal{F}_i(\tau)$, where a represents the age at infection, and the true age of the animal becomes $a+\tau$.

Here q_{11} represents the expected number of new feed infected individuals from one (average) feed infected individual. Next, q_{21} represents the expected number of new maternally infected individuals from one (average) feed infected individual. One (average) feed infected animal is distributed over all possible ages at infection according to the density.

$$\frac{\beta(a)\mathcal{F}_s(a)}{\int_0^\infty \beta(\alpha)\mathcal{F}_s(\alpha)d\alpha} \tag{3}$$

Now q_{11} is calculated as the probability of cull μ at age $(a+\tau)$ and ν at infection-age τ, multiplied with the fraction of its infectious load $\gamma(\tau)$ that enters rendering (c_1 and c_2), the rendering reduction factor (k_1) and the fraction fed to cattle (k_2). Thus $c_1\mu(a+\tau)+c_2\nu(\tau)$ is the fraction of the infectious load (prions) of an infected animal entering the rendering process, and multiplication with k_1k_2 gives the fraction that survives rendering and is fed to cattle. This expression then has to be multiplied with the probability of an infected animal to survive, $\mathcal{F}_s(a+\tau)\mathcal{F}_i(\tau)/\mathcal{F}_s(a)$, and also has to be multiplied with the infectious load $\gamma(\tau)$ at infection-age τ. Accumulating this over all possible combinations of age and infection age at cull (integral over a and τ), we find the expected number of infectious doses in feed taken up by cattle. Multiplying this with the age dependent susceptibility $\beta(a)\mathcal{F}_s(a)$ yields the total number of new infections. After dividing by a factor to normalize the relevant distributions we obtain the expected number of new feed infections that will be caused by the 'average' feed infected cow of 'average' age. The ω for the other horizontal infection also needs to be incorporated for the various age groups. The calculation takes account of both the age of the infectious animal (α) and the age of a newly infected animals (a) for the feed infection route. The other three partial ratios (q_{ij}) can be derived likewise, leading to

$$q_{11} = \frac{\int_0^\infty\int_0^\infty \beta(\alpha)\left((c_1\mu(\alpha+\tau)+c_2\nu(\tau))k_1k_2+\omega\right)\mathcal{F}_s(\alpha+\tau)\mathcal{F}_i(\tau)\gamma(\tau)d\tau d\alpha}{\int_0^\infty \mathcal{F}_s(a)da} \tag{4}$$

$$q_{21} = \frac{\int_0^\infty\int_0^\infty \beta(\alpha)b(\alpha+\tau)\mathcal{F}_s(\alpha+\tau)\mathcal{F}_i(\tau)m\gamma(\tau)d\tau d\alpha}{\int_0^\infty \beta(\alpha)\mathcal{F}_s(\alpha)d\alpha} \tag{5}$$

$$q_{12} = \frac{\int_0^\infty \beta(a)\mathcal{F}_s(a)da}{\int_0^\infty \mathcal{F}_s(a)da}\int_0^\infty \left((c_1\mu(\tau)+c_2\nu(\tau))k_1k_2+\omega\right)\mathcal{F}_s(\tau)\mathcal{F}_i(\tau)\gamma(\tau)d\tau \tag{6}$$

$$q_{22} = \int_0^\infty b(\tau)\mathcal{F}_s(\tau)\mathcal{F}_i(\tau)m\gamma(\tau)d\tau \tag{7}$$

From these four partial reproduction ratio's by infection type, the overall reproduction ratio R_0 of BSE in a cattle population can be calculated as the dominant eigenvalue of the 2x2 matrix, Q. The two components of the right eigenvector show the relative importance of the feed infection route and the maternal infection route.

$$R_0 = \frac{1}{2}q_{11} + \frac{1}{2}q_{22} + \frac{1}{2}\sqrt{\left(q_{11}^2 - 2q_{11}q_{22} + q_{22}^2 + 4q_{12}q_{21}\right)} \tag{8}$$

To stop the epidemic, several measures can be taken. The rendering method can be improved, brains and spinal cord can be removed from rendering, and feeding of MBM to cattle can be minimized. In the model we can calculate the effect of these measures on R_0 by adjusting the relevant parameters, such as c_1, c_2, k_1 and k_2 (see Section 4). In this way one can draw up a set of regulations to minimize the risk of a major epidemic given the costs of following these regulations, or regulations that lead to a fast decline in the number of new infections, given that an epidemic has started (see section 2.5).

2.5. Characterization of growth rate r

Analogous to the derivation of the reproduction ratio R_0, we can derive an equation for the per capita growth rate r of the infection at the initial (exponential) phase of the epidemic. This derivation is slightly more complicated.

The real-time evolution of the infection is described by

$$\begin{aligned} I(a,t,0) &= \overline{S}(a)\beta(a)\int_0^\infty\int_0^\infty \theta_{11}(\alpha,\tau)\, I(\alpha,t-\tau,0) \\ &\quad + \theta_{12}(\alpha,\tau)I(0,t-\tau,0)d\tau d\alpha, \qquad a>0 \\ I(0,t,0) &= \int_0^\infty\int_0^\infty \theta_{21}(\alpha,\tau)\, I(\alpha,t-\tau,0) \\ &\quad + \theta_{22}(\alpha,\tau)I(0,t-\tau,0)d\tau d\alpha \end{aligned} \tag{9}$$

which is the continuous-time counterpart of the next-generation operator Q on which the expression for R_0 is based. Here, $I(a,t,0)$ denotes the feed-induced incidence of new cases with age a arising at time t (then infection age $\tau = 0$) and $I(0,t,0)$ denotes the maternally induced incidence with age $a = 0$ at time t. $\overline{S}(a) = F_s(a)/\int_0^\infty F_s(\alpha)d\alpha$ denotes the age distribution arising from the demographic steady state of the cattle population (held constant by the farmer) and $\beta(a)$ denotes the age dependent susceptibility. The transmission kernel $\Theta(a,\tau)$ is given by a

two-dimensional matrix with elements $\theta_{ij}(a,\tau)$:

$$\begin{aligned}
\theta_{11}(a,\tau) &= ((c_1\mu(a+\tau)+c_2\nu(\tau))k_1k_2+\omega)\,\frac{\mathcal{F}_s(a+\tau)}{\mathcal{F}_s(a)}\mathcal{F}_i(\tau)\gamma(\tau)\\
\theta_{21}(a,\tau) &= b(a+\tau)\frac{\mathcal{F}_s(a+\tau)}{\mathcal{F}_s(a)}\mathcal{F}_i(\tau)m\gamma(\tau)\\
\theta_{12}(a,\tau) &= (c_1\mu(\tau)+c_2\nu(\tau))k_1k_2+\omega)\,\mathcal{F}_s(\tau)\mathcal{F}_i(\tau)\gamma(\tau)\\
\theta_{22}(a,\tau) &= b(\tau)\mathcal{F}_s(\tau)\mathcal{F}_i(\tau)m\gamma(\tau)
\end{aligned} \tag{10}$$

We write $i(a,t)$ for the vector $(I(a,t,0), I(0,t,0))^{\mathrm{T}}$ and can then rewrite system (9) as

$$i(a,t) = \begin{pmatrix} \overline{S}(a)\beta(a) & 0 \\ 0 & 1 \end{pmatrix} \int_0^\infty \int_0^\infty \Theta(\alpha,\tau) i(\alpha, t-\tau) d\alpha d\tau \tag{11}$$

To derive the growth rate r, we look for exponential solutions to (11), i.e. solutions of the form:

$$i(a,t) = F(a)e^{rt} \tag{12}$$

where $F(a) = (f(a), f(0))^{\mathrm{T}}$. Substitution into equation (11) leads to a relation for the vector $F(a)$:

$$F(a) = \begin{pmatrix} \overline{S}(a)\beta(a) & 0 \\ 0 & 1 \end{pmatrix} \int_0^\infty \int_0^\infty \Theta(\alpha,\tau) F(\alpha) e^{-r\tau} d\alpha d\tau \tag{13}$$

One can show that this operator has a two-dimensional range and that we can reformulate the eigenvalue relation (see Diekmann & Heesterbeek, 2000, section 5.3.3) as stating that the value of r we are looking for should be such that the dominant eigenvalue of the following 2*2-matrix is one:

$$\begin{pmatrix} \int_0^\infty \int_0^\infty \theta_{11}(\alpha,\tau)\overline{S}(\alpha)\beta(\alpha)e^{-r\tau}d\alpha d\tau & \int_0^\infty \int_0^\infty \theta_{12}(\alpha,\tau)e^{-r\tau}d\alpha d\tau \\ \int_0^\infty \int_0^\infty \theta_{21}(\alpha,\tau)\overline{S}(\alpha)\beta(\alpha)e^{-r\tau}d\alpha d\tau & \int_0^\infty \int_0^\infty \theta_{22}(\alpha,\tau)e^{-r\tau}d\alpha d\tau \end{pmatrix}.$$

This leads to an equation for r which can be solved numerically.

From the available data (see Section 3) we find that $\theta_{12}(\alpha,\tau)$ and $\theta_{22}(\alpha,\tau)$ are small relative to $\theta_{11}(\alpha,\tau)\overline{S}(\alpha)\beta(\alpha)$, so the second component of the eigenvector (vertical transmission) will be rather small relative to the first. Therefore we restrict

ourselves to the horizontal-infection routes we get a simple relation from which r can be estimated:

$$f(a) = \overline{S}(a)\beta(a) \int_0^\infty \int_0^\infty \theta_{11}(\alpha, \tau) f(\alpha) e^{-r\tau} d\alpha d\tau$$

If we define the operator K_r by the right-hand side of this relation, then r is defined as the value for which $f(a)$ is an eigenvector of K_r corresponding to eigenvalue 1. Note that K_r has a one-dimensional range and $\overline{S}(a)\beta(a)$ is the only eigenvector corresponding to a non-zero eigenvalue. Substituting this into the eigenvalue relation leads to an implicit relation:

$$1 = \int_0^\infty \int_0^\infty \theta_{11}(\alpha, \tau) \overline{S}(\alpha)\beta(\alpha) e^{-r\tau} d\alpha d\tau \tag{14}$$

which can be shown to have a unique solution r, which can be computed by, for example, a Newton algorithm. We will make this restriction for the remainder of this paper.

3. Estimation of parameter values

3.1. Demographic parameters

In most developed countries the replacement rate of cattle older than two years is approximately 1/3 to 1/4 per year, and rather constant over ages. Young stock are submitted to higher culling rates, about 0.5 per year with peaks in the first half year and at about 18 months. Culling of young stock is normally done to control the size of a local herd, so it depends on both the culling rate of adult cattle and the birth rate.

We assume a constant per capita culling rate per year for cattle older than two years. For the Netherlands that culling rate of adult cattle is $\mu_a = 0.3$ and for the UK it is somewhat lower, i.e. $\mu_a = 0.25$. In cattle younger than two years BSE has rarely been detected (0.002%) and therefore this age group is supposed to hardly contribute to the spread of the infection. Thus, the precise shape of the survival function up to two years old is of minor importance. We simplify the culling rate of young stock to a constant, μ_y. Cattle reproduce from two years of age onwards, and produce on average one calf per cow per year ($b = 1$). Assuming a constant population size and stable age distribution, the fraction $\mathcal{F}_s(2)$ of cattle surviving until at least two years of age can be calculated as the adult culling rate (μ_a) divided by the birth rate b, since $b\mathcal{F}_s(2)/\mu_a$ should equal one, at a constant population size. Thus we estimate the culling rate of young stock (μ_y) from $\mathcal{F}_s(2) = e^{-2\mu_y} = \mu_a/b$. In summary, we use:

$$b(a) = \begin{cases} 0 \text{ if } & a < 2 \\ 1 \text{ if } & a \geq 2 \end{cases} \tag{15}$$

$$\mu(a) = \begin{cases} 0.55 \text{ if} & a < 2 \text{ in NL} \\ 0.3 \text{ if} & a \geq 2 \text{ in NL} \end{cases} \tag{16}$$

$$\mu(a) = \begin{cases} 0.6 \text{ if} & a < 2 \text{ in the UK} \\ 0.25 \text{ if} & a \geq 2 \text{ in the UK.} \end{cases} \tag{17}$$

3.2. Probability of becoming infected

Recently, various models (e.g. [18]) for the infectious behaviour of prions were developed, generally involving polymerization of prions and giving very plausible explanations of some features found in infectious load development and dose response relations. We will focus on one of the simplest models for infection, assuming that BSE infection is spread by many small infectious particles (prions or small clusters of prions), which all have an extremely low probability p to induce infection (single hit theory). This leads to the following dose-response relation: (See also [14])

$$\text{response} = 1 - (1 - p)^{\text{dose}} \tag{18}$$

Here response is defined as the probability for an animal to become infected by the dose of infectious material ingested (expressed in grams of brain material) and this is estimated from the fraction of animals that respond (get infected) in a bioassay. Because p is very small, this relation can be approximated by:

$$\text{response} = 1 - e^{-p \cdot \text{dose}} \tag{19}$$

For different amounts of infectious material ingested (dose) we visualized this relation in Figures 1 and 2. Using (18), we analyzed titration data from mouse bio-assays of Taylor [22],[21] and Schreuder et al. [20] for the effect of heating and rendering on prion survival. Generalized linear modelling (GLM, see [19]) with a binomial distribution and a complementary log-log link function is applied to the data using GENSTAT v5, which estimates the constant p and its standard error. Our results are generally the same as the original analysis with the Kärber method, which expresses the results in terms of ID50, i.e. dose that is infectious for 50% of a test group.

This dose response relation is also used to describe the transmission of the infection in the population. In the population the incidence of BSE is generally low and infectious contacts are spread widely over the population due to the processing steps of MBM-rendering, feed processing and feed distribution. Therefore, the infectious dose per individual animal will remain low. For low individual dose, the dose response relation can be linearized, which leads to a constant probability for each particle to induce infection:

$$\text{response} = 1 - e^{-p \cdot \text{dose}} \approx p \cdot \text{dose}$$

Next we define the unit of infectious dose as the amount of infectious material, that is expected to induce one new infection in a population. In the linearized model,

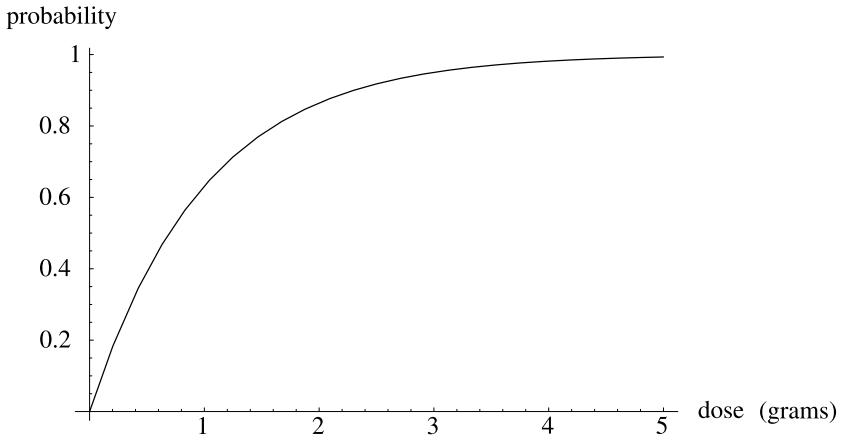

Fig. 1. Dose-response curve for TSE's.

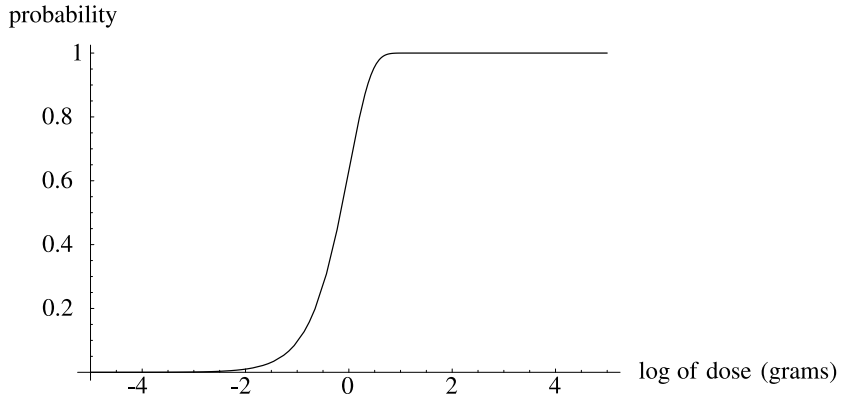

Fig. 2. Dose-response curve on a logarithmic scale (dilutions of dose).

it does not matter whether this material is spread over one herd or a hundred herds, it is still expected to induce one new infection. If such an infectious dose unit would be fed to one animal (relatively high dose), the linearization of the model is not valid any more, in that case the probability of infection for that one animal is estimated from the above to be 63%.

3.3. Infectious load in the animal

The infectious load of an infected animal is assumed to increase exponentially during the infectious period, as there is no knowledge of any inhibiting immune reaction to the BSE agent. Infectious load means the amount of the infectious agent that accumulates in the infected animal in time. Dose-incubation time analysis for cattle yields a shortening of the incubation time by 22 weeks for a 10 times higher dose, which suggests a doubling time of the infectious agent of about 6 weeks

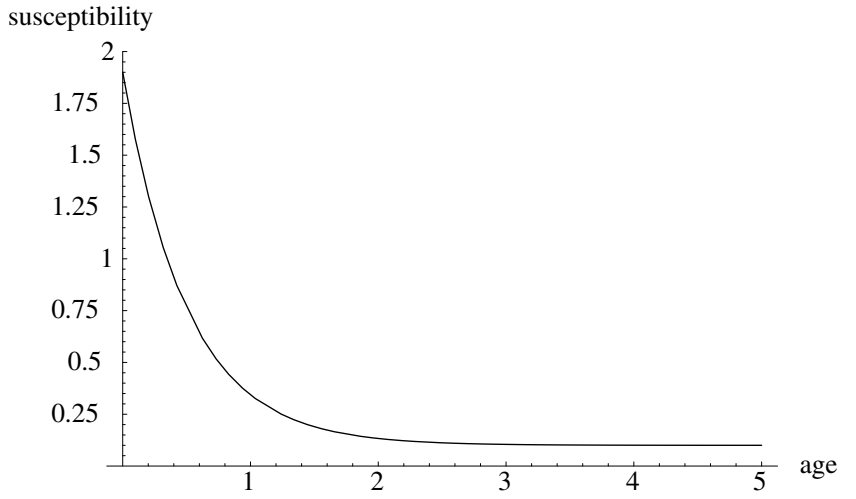

Fig. 3. Age dependent susceptibility of cattle for BSE, relative to the susceptibility of a four months old calf.

(oral exposure experiment, unpublished data from VLA, Weybridge, UK). Thus, assuming a doubling of BSE infectiousness over 6 weeks time (i.e. 0.12 year), we estimate and use a growth rate of the infectious load of 6 per year.

The oral infectious dose for cattle of 4 months old was estimated from the same oral exposure study which consists of 4 groups of 10 cattle (unpublished data, VLA), using the previously described dose response analysis. Analysis with the dose response relation as suggested above yields an estimated oral infectious dose unit of 1.9 grams of brain material of a cow with clinical BSE symptoms (equaling an ID50 of 1.3 grams). An average brain weighs 600 to 800 grams and the spinal cord weighs about 250 grams. Other parts of the carcass contain a very small infectious load compared to these parts. This leads to an estimate of the total infectious load of a BSE infected cow in the last stage (when clinical BSE signs have developed and τ can be assumed 4.5 years) of about five hundred infectious dose units.

The infectious load as a function of infection age (Figure 3), can be derived by calculating backwards from the infectious load at the time clinical symptoms show ($\tau = 4.5$ years) and using an exponential growth rate of 6 per year:

$$\gamma(\tau) = 10^{-9} e^{6\tau} \tag{20}$$

3.4. Other infection related parameters

We assume that cattle of all ages receive equal amounts of feed. The exact shape of the decreasing function that describes the age-dependent susceptibility is not known. We assume an exponential decrease to 10% of the susceptibility of 4 months old cattle and a relative rate of decrease of 2 per year. The susceptibility of calves

of 4 months old is by definition equal to 1, and the age dependent susceptibility of cattle is given by (Figure 3):

$$\beta(a) = 0.1 + 1.8 \cdot e^{-2a} \tag{21}$$

We assume that clinical signs of BSE (and thus also the infection-age dependent culling rate) increase with the infectious load of a cow. At present in the UK, farmers and veterinarians will be more experienced in recognizing BSE than before 1990. Before 2000 most other countries were as unexperienced as Britain was in the early stage of the epidemic. We estimate the average infection-age of clinically diagnosed BSE cattle (incubation period) at 4.5 years for the UK since 1989 and in other countries as well as in the UK prior to 1989, diagnosis is assumed a few months later, 4.8 years.

Given an average incubation period of 4.5 and 4.8 years, we determined the infection-age dependent culling rate (per year) as:

$$\nu(\tau) = \begin{cases} 10^{-12} e^{6\tau} & \text{UK since 1990} \\ 5 \cdot 10^{-12} e^{6\tau} & \text{otherwise (Figure 4)} \end{cases} \tag{22}$$

To model the feed infection route we have to follow the infectious material from the infected animal up to the moment of new infection and estimate the reduction of infectivity in each step in the material, by keeping track of the total infectious load. For animals which were culled without signs of a neural disease (even though they may have been infected), the normal slaughter process will be applied. The fraction of the infectious load of a cow that enters the rendering process is called c_1. For the UK before 1989, it was estimated that almost 70% of the infectious material (mainly brains and spinal cord) of a beef carcass entered the rendering process ($c_1 = 0.7$, Table 1). Animals which were culled due to signs of a neural disease, may have been treated differently. For these animals we assume a fraction c_2 of the infectious load of a cow entering the rendering process. Before 1987, BSE was not recognized as such. About 50% of cattle with neural diseases was not used for human consumption and was therefore fully rendered, so we assume c_2 to be slightly higher than c_1: 0.85 (Table 1).

The reduction of infectious load during the process of rendering, k_1, is determined in mouse bioassays. Taylor et al. ([22],[21]) tested a continuous vacuum rendering process with high fat content, which was commonly used in the UK since the 1970s. Schreuder et al. ([20]) tested several different treatments to quantify the efficacy of various rendering processes in TSE reduction. He found similar results for the atmospheric process and also quantified processes as applied in the Netherlands (see Table 1).

Next, from the total amount MBM that is produced from the rendered cattle material, a fraction of about $k_2 = 0.20$ is used in the production of cattle feed, whereas the major part is used in pig, pet and chicken feed. These species are far less susceptible to the BSE agent than cattle (there are only a few reports of cats infected with TSE), and this is considered to be a dead end route for the spread of the infectious agent among cattle.

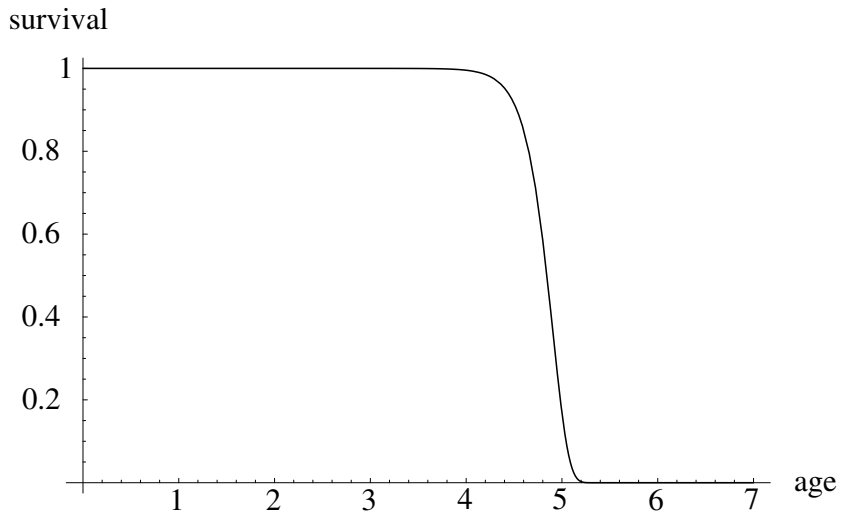

Fig. 4. survival function of BSE infected cattle, when culling will be limited to clinical BSE.

Table 1 gives an overview of the parameter values, that were separately estimated for the UK and the Netherlands. These estimates were made in four different time periods, 1986, 1991, 1995 and 1998. In 1986, BSE control was unknown, differences between countries lie only in the rendering and feeding methods: UK uses low temperature atmospheric systems whereas in the Netherlands mostly pressurized high temperature methods were applied (different value of k_1). Before 1991 both countries introduce a ban to feed MBM to ruminants (reducing k_2) and in the UK specified risk materials, SRMs, are defined and removed to be incinerated

Table 1. The parameter estimates underlying the estimate of R_0

parameter	UK 1986	UK 1991	UK 1995	UK 1998
k_1	0.1	0.1	0.1	0.1
k_2	0.2	0.02	0.005	0.002
c_1	0.7	0.05	0.05	0.01
c_2	0.85	0.05	0.05	0.01
$\nu(\tau)$	$5 \cdot 10^{-13} e^{6\tau}$	$10^{-12} e^{6\tau}$	$10^{-12} e^{6\tau}$	$10^{-12} e^{6\tau}$

parameter	NL 1986	NL 1991	NL 1995	NL 1998
k_1	0.01	0.01	0.001	0.001
k_2	0.2	0.1	0.01	0.005
c_1	0.7	0.7	0.7	0.05
c_2	0.85	0.85	0.85	0.05
$\nu(\tau)$	$5 \cdot 10^{-13} e^{6\tau}$	$5 \cdot 10^{-13} e^{6\tau}$	$5 \cdot 10^{-13} e^{6\tau}$	$5 \cdot 10^{-13} e^{6\tau}$

(reducing c_1 and c_2). In the period between 1991 and 1995, the feedban is extended and inspection for compliance with the ban follows, leading to a further reduction of k_2. Furthermore in the Netherlands rendering temperatures and pressure are slightly increased (EU regulations affecting k_1) and in the UK clinical diagnosis improves ($\nu(\tau)$). Between 1995 and 1998, especially in the UK inspection into compliance with all bans is extended and risk of cross-contamination becomes clear, leading to separate production lines and flush batches (further reducing k_2). In the Netherlands the SRM removal is introduced, reducing c_1 and c_2.

A maternal transmission study [10] gives an estimate of 10% ±5% maternal transmission when a cow gives birth at the highly infectious last stage of the infection (when the infectious load $\gamma(4.5)$ is estimated at 2000 infectious dose units, see the previous). Maternal infection is assumed to be lower for cows in earlier stages of the infection, according to the infectious load $\gamma(\tau)$ of the mother. With this information we estimate the maternal transmission rate for a calf (relative to the mothers infectiousness): $m = 0.1/\gamma(4.5) = 0.1/2000 = 0.5 \cdot 10^{-4}$.

The last parameter that remains to be estimated is very difficult to quantify. It describes the combined transmission rate for direct horizontal contact infection, ω_h, diagonal infection, ω_d and indirect infection via the environment, ω_i. These three parameters can not be estimated separately from any data presently available, so we cluster them into one parameter ω**.** The following argumentation leads to a quantification of this parameter.

During the initial stage of the outbreak in the UK, these "other" transmission routes were negligibly small compared to the feed infection route. However, when infection via the feed infection route is minimized, due to the feed and SRM bans, ω may become visible in the development of the epidemic. It can explain why it has been impossible to bring BSE transmission to a complete stop. The parameter was estimated from the observed data of infections after the feed ban (new cases during 1993 until 1997). Assuming that the feed infection route was fully closed in that period, we estimated backwards from the exponential decay rate of the epidemic at that time (using (14) and Figure 7) that ω is at most $3 \cdot 10^{-4}$.

Thus we derive a maximum estimate for ω, knowing that the feed ban was certainly not fully effective between 1990 and 1996. In the mean time we neglect a part of the environmental infection route, by only looking at short-term survival (in the order of months) of the material in the environment. Given enough time, infectious material will flow away from the soil with the rain and ground water, so this appears to be a reasonable assumption. Such infectious material may finally accumulate in lakes, seas and oceans where it is presently assumed to be harmless.

4. Quantifying R_0

4.1. Estimates

Table 2 shows estimates of the reproduction ratio R_0 for the UK and the Netherlands over the last two decades. The impact of the first control measures, such as feed and SRM bans can clearly be seen in the strong decline of R_0. Later measures had little impact. The reproduction ratio was estimated to be below one in the Netherlands even before introduction of control measures. Therefore, a BSE epidemic

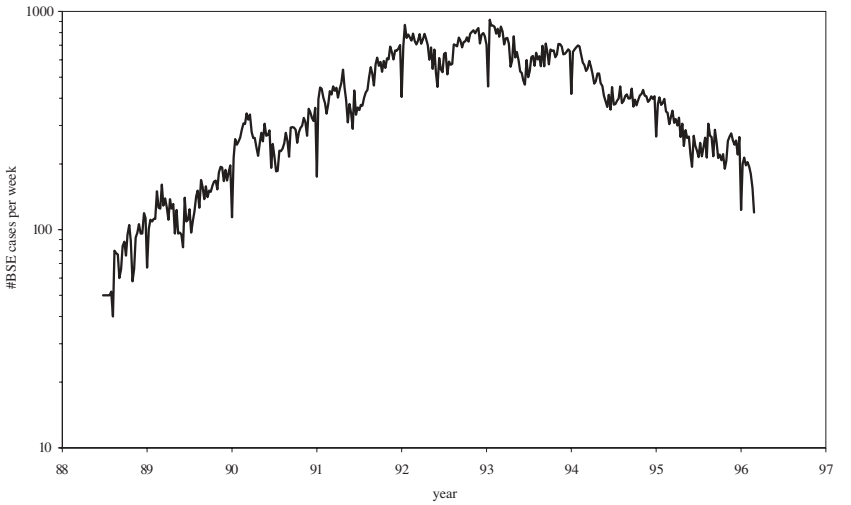

Fig. 5. Increase and decrease of the number of new BSE cases per week during the outbreak in the United Kingdom.

was not to be expected. The difference in R_0 with the UK is almost completely due to differences in the rendering processes between these countries, affecting k_1 (see also Table 1). That the Netherlands actually still finds BSE cases in the indigenous population is mainly due to large imports of risk material and live animals. Since then very slow fade out of the infection started, but total eradication is not expected within another 5 to 10 years, depending especially on the level of infection via non-feed routes.

From the model, the growth rate of the infection was estimated at $r = 0.58$ per year in the initial exponential phase of the epidemic in the UK. Using linear regression on the case data from the beginning of 1988 (when reporting levels stabilized) up to halfway 1991, we find that the growth rate $r = 0.64$. For the decreasing phase of the epidemic, the data (end 1993 to beginning 1996) lead to an estimated growth rate of $r = -0.53$ per year.

In the UK, introduction of a feed ban and SRMban (ban on the use of SRMs in the rendering process) reduced the reproduction ratio from 14 to 0.1, a 100 fold reduction. Application of an initial feed ban in the Netherlands reduced the local reproduction ratio only about 10 fold. Later extensions on the feedban led to fur-

Table 2. Estimated reproduction ratio R_0 for the United Kingdom (UK) and the Netherlands (NL), during different periods depending on the implemented control measures. Numbers between brackets are the upper boundary of the confidence interval (alpha=0.95), see section on sensitivity analysis.

R_0 (upper 95%)	1986	1991	1995	1998
UK	14 (25)	0.1 (0.3)	0.05 (0.1)	0.03 (0.1)
NL	0.7 (1.3)	0.2 (0.5)	0.08 (0.1)	0.05 (0.1)

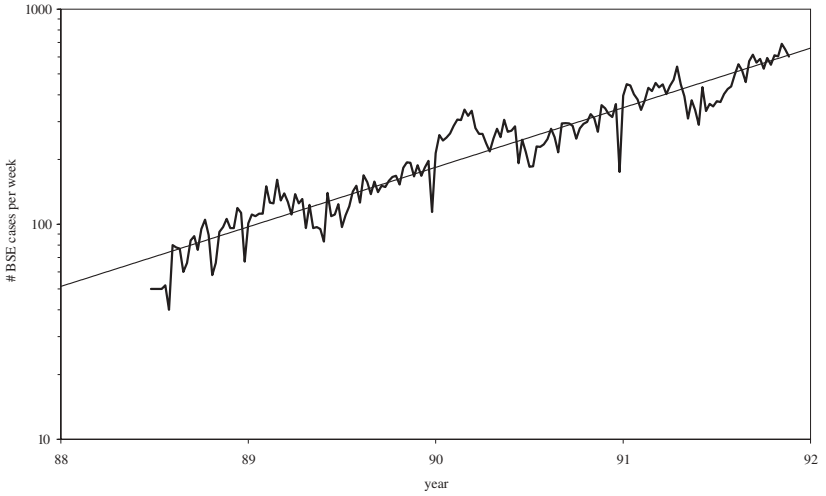

Fig. 6. Linear regression on the data of the BSE outbreak from June 1988 until December 1991.

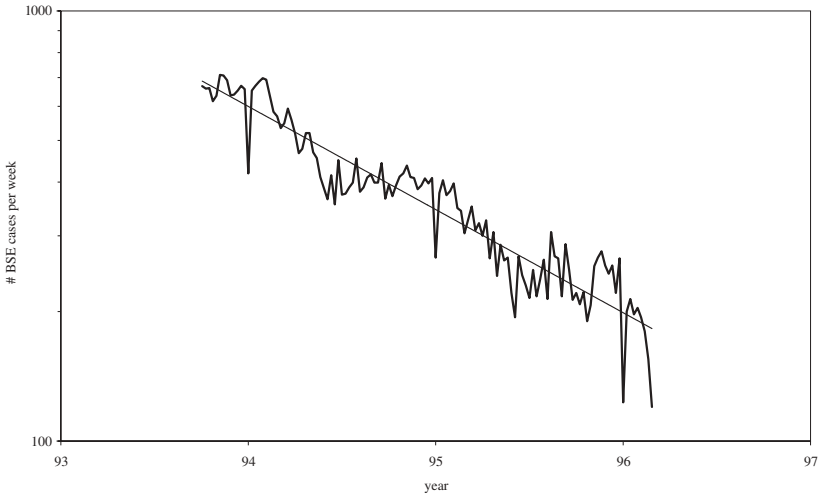

Fig. 7. Linear regression on the data of the BSE outbreak from October 1993 until march 1996.

ther decrease of R_0. In 1997 the Netherlands also introduced an SRMban, but at that point the impact was rather small because the feed infection route had already become small compared to the remaining infection routes.

4.2. Sensitivity analysis and confidence interval

The quality and accuracy of the estimate of R_0 depends heavily on the quantification of the ingredients. Whenever modelling and quantification are combined, one

of the more difficult issues to deal with is the confidence interval associated with the estimated or calculated results. Due to high uncertainty in some of the parameter estimates in this study, the ensuing uncertainty in the estimated reproduction ratio is possibly high and we need to quantify this more precisely. We determine a 95% confidence interval from the known variance of the parameters for the model with the restrictions and assumptions as mentioned before. A good choice for the distribution of the uncertainty of the parameters and of the R_0 estimate are important for the final result. In constructing methods to quantify the variance of R_0, we distinguish transmission via food, and other transmission routes.

For the feed infection route the analysis is explained in detail below. For the other infection routes, little information is available, and we restrict ourselves to determining an upper 95% confidence level, leaning on the assumption that the highest possible value of the reproduction ratio through non-feed routes is equal to the lowest reproduction ratio to be determined from the data. This is estimated at $R_0 = 0.1$, and $r = -0.5$ which fits with the negative growth rate estimated from the case data between 1994 and 1996. We therefore determine the upper boundary of the 95% confidence interval for non-feed infections at 0.1. The total variance of the estimator is the sum of the variances of the two parts, and thus, the upper confidence boundary cannot get below 0.1, but can be (much) higher if the feed transmission route is non-zero.

For the calculation of q_{11} the estimates of infectious load, $\gamma(\tau)$ and the efficacy of the rendering process, k_1 are the most uncertain ingredients. This does not imply that R_0 is most sensitive to these ingredients. One could use e.g. latin hypercube sampling to determine the contribution of all ingredients to the uncertainty in R_0. Here we have chosen to analyse uncertainty only with respect to $\gamma(\tau)$ and k_1. These two factors are estimated using generalized linear modelling on titration of infectious material in cattle ($\gamma(\tau)$) and mice bio-assays (k_1). The efficacy of the rendering process is determined by titration of material before and after treatment. The high uncertainty in these parameter estimates is due to the limitation in the number of test animals. For k_1 the material before and after a rendering process needs to be analyzed, bringing two such parameters into the equation. Those parameters derived from the bio-assays are here denoted as $\widehat{x_i}$. Given the structure of q_{11}, we assume that:

$$\widehat{R_{feed}} = f(\widehat{x_1}, \widehat{x_2}, \widehat{x_3}) = c\frac{\widehat{x_1}\widehat{x_3}}{\widehat{x_2}} \tag{23}$$

We will estimate the ensuing variance of $\widehat{R_{feed}}$, from the variance of the estimators ($\widehat{x_i}$). From the bioassay results the titre (10 log(dilution)) of the original material is determined. We assume a normal distribution for the titre with expected value μ and variance σ^2. Then the estimated infectious dose $\widehat{x_i}$ of that material follows a lognormal distribution. The expected value and variance of R_{feed} (for which we also assume a lognormal distribution) can then be analyzed as follows (see [16]):

$$E(x_i) = e^{(\mu + \frac{1}{2}\sigma^2)}$$

and

$$Var(x_i) = e^{(\mu+\frac{1}{2}\sigma^2)}(e^{\sigma^2} - 1)$$

For independent variables x_i we know that

$$Var(\ln R_{feed}) = Var(\ln x_1) + Var(\ln x_2) + Var(\ln x_3) \tag{24}$$

Now, using

$$Var(\ln x_i) \simeq \ln\left(1 + \frac{Var(x_i)}{E(x_i)^2}\right) \tag{25}$$

we derive

$$Var(\ln R_{feed}) \simeq \ln\left(1 + \frac{Var(x_1)}{E(x_1)^2}\right) + \ln\left(1 + \frac{Var(x_2)}{E(x_2)^2}\right) + \ln\left(1 + \frac{Var(x_3)}{E(x_3)^2}\right) \tag{26}$$

which can be calculated from the data. Finally using (25) and (26) we derive the variance of R_{feed}

$$Var(R_{feed}) \simeq E(R_{feed})^2 e^{(Var(\ln R_{feed})-1)} \tag{27}$$

The rules as explained above are applied to calculate the upper boundary of the 95% confidence interval as given in table 2.

4.3. Choice of functions/curves

The use of a different function for the age-dependent susceptibility may lead to very different results, especially for a cattle population with a rather unusual age distribution. We tested whether constant susceptibility over all ages would be a reasonable assumption, but from the decreasing BSE prevalence at older ages (over 7 years) it is clear that this model does not fit the observed distribution in the UK so we did not calculate the effect on R_0. Infection only at birth or very young age would fit the data rather well, if the incubation period has a very long tail to the right, but that effect on R_0 is minimal (3% at most). The assumed susceptibility curve of Formula (25) results in a good fit with the data. From the data it is not yet possible to make an accurate estimate of the susceptibility as function of age (see also Ferguson et al., [11]).

The estimated R_0 is also sensitive to the age distribution of the cattle population. Extreme age distributions (resulting from very high or very low culling rates) may lead to more than a 10 fold difference in the estimated R_0. However, for the observed cases, UK and Netherlands, realistic variation in age distributions leads to less than 1% differences.

4.4. Comparison of the estimated R_0 with field data

The results from the present model can be compared to the observed case data of the UK outbreak. From the model with the parameters set at the estimated values for 1986 (Table 1), we calculate a reproduction ratio $R_0 = 14$ with an exponential increase of infection of $r = 0.58$ per year. From the UK case data, a growth rate of 0.64 ± 0.14 per year is directly estimated by use of linear regression on the logarithm of the number of BSE cases per week, in the period between the beginning of 1988 (when reporting levels stabilized) and mid-1991 (after which exponential increase slows down due to control measures). Cases in this period are born before the feed ban, and are therefore considered to be representative for the development of the epidemic without the ban. Thus, the model slightly underestimates the growth rate and the reproduction ratio of the infection in the UK in the 1980s, but the model result for the growth rate still lies within the confidence interval of the growth rate estimated from the field data. Ferguson et al. [12] quantify R_0 from a data driven model, and estimate it between 10 and 18 between 1983 and 1988, with some variation over the years. Valleron et al. [23] estimate a growth rate of 0.6 per year. All these estimates lie easily within our confidence interval (see below) and thus support our quantification.

The measured growth rate $r = 0.64$ can be mimicked with the model by calibrating some of the more uncertain parameter estimates, such as k_2. Such a calibrated model gives an estimate of R_0 equal to 19, leading to a good fit of the model to the observed UK data. However, given that it is unclear which parameters should be calibrated, in this paper we restrict ourselves to the original parameter estimates to quantify R_0.

5. Discussion

This paper describes the calculation of R_0 and r for BSE, where all ingredients are based on proposed underlying mechanisms of infection or estimated from data. A problem in modelling BSE (and other TSEs) is that much of the behaviour of the infectious agent is still unknown or uncertain. Some assumptions made in our calculation have a substantial influence. Especially the behaviour of the infectious agent according to the 'single hit theory' and to a lesser extent the exponential increase of the infectious load in an infected individual. Existence of a minimal infectious dose or polymer formation by prions will affect the transmission of BSE, and if proven, should lead to more accurate calculation of R_0. The single hit theory must be seen as a worst case scenario.

The confidence interval of the estimated reproduction ratio R_0 is wide, due to high uncertainty, especially in parameter values estimated from infectious dose quantification by bioassay. The estimate of k_1, the reduction of infectious load by the rendering process, also shows a high standard error. When, in the future, more advanced and precise tests are developed to measure the concentration of the infectious agent, the uncertainty in this factor may be reduced.

In our calculation we ignore spatial aspects of the infection, so local clustering of BSE cases, as observed in the UK data, is not explained in this model. Hagenaars et al. [13] give a nice overview of some spatial features of the BSE epidemic. It remains to be seen in how far ignoring clustering will affect our calculations. We also

ignore infection of other species than cattle, and long-term persistence of infection in the environment. Although these two factors are worrysome for the future, they are unlikely to have a strong influence on the short-term analysis of the effect of BSE control.

We assessed the effect of different control measures and find that there are three major control measures: a feed ban on MBM to cattle, optimization of the rendering process and SRM removal and incineration. In most cases, to reduce R_0 below 1, it suffices to apply two of these measures, but faster reduction of the problem will be obtained by adding further controls. When the compliance to the control measures is difficult to maintain as was the case in the first decade of controlling the infection, extra measures should be taken to ensure fade out of the epidemic.

When these control measures are sufficiently in place, infection routes other than via feed will become the major remaining transmission routes. These remaining transmission routes will be much harder to control, and therefore, the reproduction ratio cannot be reduced to zero. However, the remaining transmission routes are definitely too small to cause a major BSE epidemic, and under some basic control measures a decrease of 50% per year can easily be achieved. The maximum estimate of the reproduction ratio without feed transmission is 0.06, which leads to fast decrease of the number of BSE infections.

We conclude that countries which had a rather inefficient rendering industry with respect to BSE inactivation and where farmers tended to feed large amounts of MBM to cattle are presently at high risk concerning BSE in their cattle herd. Especially when such countries also imported cattle and/or MBM concentrated feeds from the UK. Countries with a rather efficient rendering and with low amounts of MBM in their cattle feed can expect a very low BSE prevalence, if at all.

Countries with a high BSE prevalence should close the feed infection routes as much as possible, thus minimizing the reproduction ratio and the growth rate, leading to a fast decrease of infection and disease. When the prevalence has become very low, these control measures may be relaxed, but these countries must be more careful in this respect than other countries, because of the unknown long-term survival of the infectious agent in the environment.

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