Analysis of dam–calf pairs of BSE cases: confirmation of a maternal risk enhancement

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SUMMARY

We investigate whether a calf born to a dam that develops bovine spongiform encephalopathy (BSE) (prior or subsequent to the birth) is itself at an enhanced risk of developing BSE. Analyses utilize the main database on reported BSE cases in the British cattle herd maintained by the Central Veterinary Laboratory in Weybridge to trace the dams of BSE-affected animals born following the ruminant feed ban in July 1988. The data reveal a significantly enhanced risk of disease in calves born to BSE-affected dams, with the risk being greatest when birth occurs after the onset of clinical signs of disease in the dam. The dependence of the maternally enhanced risk on the maternal incubation stage at birth argues for a significant component of direct maternal transmission of the aetiological agent of BSE, and offers little support for the hypothesis of genetic predisposition. Using a statistical likelihood model, we obtain estimates of the rate of direct maternal transmission by maternal incubation stage; however, biases in the available data make these values minimum estimates.

1. INTRODUCTION

Interim results from a long-term cohort study of maternal transmission of the aetiological agent of BSE, conducted by Ministry of Agriculture, Fisheries and Food (MAFF) scientific and veterinary staff at the Central Veterinary Laboratory (CVL), were reported by the Spongiform Encephalopathy Advisory Committee (SEAC) in July 1996. They revealed an enhanced risk of developing BSE in calves born to BSE-affected dams either before or after the onset of clinical signs in the dam. This maternal risk enhancement could be due to direct maternal transmission of the aetiological agent, genetic predisposition to infection, given that calves born to both infected and uninfected dams were exposed to contaminated feed, or some combination of these two processes. Study follow-up was completed in December 1996 and the full results, plus analyses of their significance, are reported in a series of recent papers (Wilesmith *et al.* 1997; Donnelly *et al.* 1997*b*,*c*; Curnow *et al.* 1997; Gore *et al.* 1997). These studies confirm the presence of a maternally enhanced risk and, furthermore, reveal an association between the magnitude of this risk and the stage of the incubation period of BSE in the dam at the time of calving, with the risk of offspring developing BSE rising as calving occurs closer to (or following) the time at which BSE is diagnosed in the dam. This trend is highly suggestive of an element of direct maternal transmission of an aetiological agent, but does not exclude a component of genetic predisposition contributing to the overall risk enhancement.

Further information on the epidemiological signature of the maternally enhanced risk of infection in calves born to BSE-affected dams is contained within the large database of BSE case reports in Great Britain compiled at the CVL, which records the course of the epidemic since 1986. This paper reports results and analyses arising from the tracing of the dams of BSE cases born following the introduction of the ban on the use of ruminant material in cattle feed in July 1988 (BSE Order 1988), paying particular attention to the influence of the maternal incubation stage at the point of calving on the maternal risk enhancement. Tracing such animals has identified 1346 BSE-affected dam–calf pairs. The analysis of the data is complicated (as in the maternal cohort study (Donnelly *et al.* 1997*b*)) by the continued use of feed contaminated with the BSE agent after the introduction of the feed ban in July 1988. As such, it is again not possible to dissect completely the contributions made by direct maternal transmission and genetic predisposition to any enhancement of risk detected in the epidemiological database. Genotype information would be required to assess precisely the relative contribution of the two possible causes of a maternally enhanced risk.

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2. THE CVL DATABASE OF CONFIRMED BSE CASES

All confirmed BSE cases arising in Great Britain have been entered into a database maintained at the CVL. The information recorded is obtained from the questionnaire completed by a veterinary officer called to investigate a suspect case of BSE. Post-mortem diagnosis of BSE is based on brain pathology. The variables considered in the following analyses include herd and holding of diagnosis, natal holding, date of birth, date of onset of the clinical signs of BSE, and adult herd size for herd of diagnosis. From 21 June 1988, all BSE suspects were required to be reported to the CVL; prior to this time, reporting was not compulsory (BSE Order 1988).

Investigation of the CVL database was based on the determination of the BSE status of the dams of 31 192 confirmed BSE cases in cattle born following the introduction of the ruminant feed ban in July 1988 ('Born After the Ban' cases, or BABs). Of these BABs, 29 709 could be linked to the main database of confirmed BSE cases (40 animals could not be linked; the remainder experienced disease onset after June 1996). Since the occurrence of the first BAB case, an additional effort has been made to identify and trace dams of these cases where the dam or the BAB had been sold from the natal herd. For each dam identified, the BSE status of the dam could be determined from the main epidemiological database of confirmed BSE cases.

In our analyses, data were excluded if (i) the dam was under 560 days old at the birth of a calf, (ii) the dam was recorded with male sex, (iii) the natal holding of the BAB could not be identified, (iv) the BAB was born after 30 June 1992, or (v) the BAB experienced the onset of clinical signs of BSE after June 1996. The data set contained information from 29 076 BABs after these exclusions were made. The main database contained an additional 1775 BABs with onset of clinical signs of BSE prior to July 1996 which were not included in the dam identification database. We treated these cases of missing dam identification data in the same manner as those animals included in the dam identification database with unidentified dams. The total data set therefore contained 30 851 BABs.

Overall, a high proportion of dams (85.9%) of BAB cases were identified. This proportion varied as a function of the time of onset of clinical signs of BSE in the BABs (figure 1), with a drop in the proportion identified occurring between August and November 1994. After exclusions, of the 26 502 BABs with identified dams, 1212 of the dams of the BABs were recorded as confirmed BSE cases.

To make the data from different birth cohorts of BABs comparable, we censored the data with respect to the time of onset of clinical signs of BSE both in the BABs and their dams. As in Anderson *et al.* (1996), annual birth cohorts were defined so that, for example, the 1989 cohort consists of cattle born between 1 July 1988 and 30 June 1989. For each cohort, we therefore considered only confirmed BSE cases in BABs and dams with onset of clinical signs within 48 months from the end of the cohort birth year. A contingency

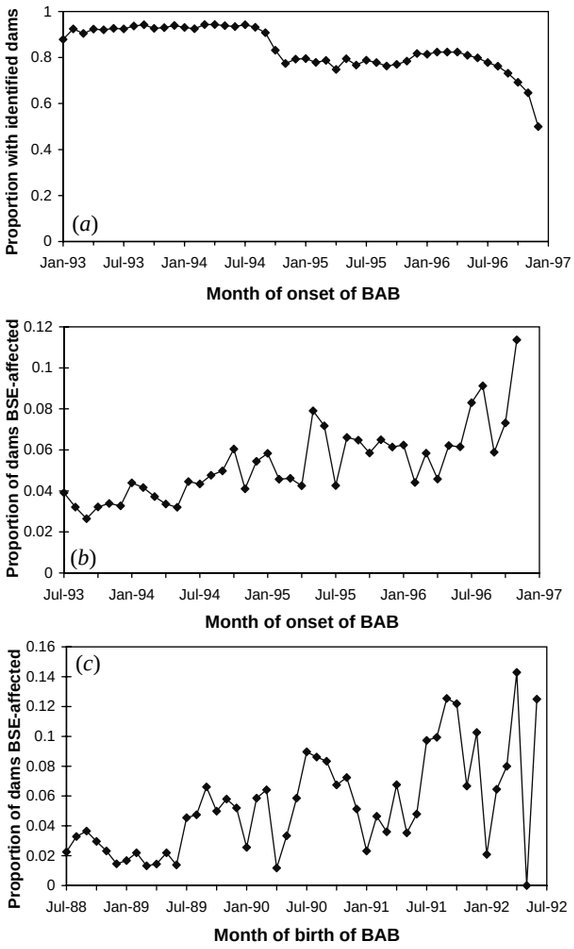

Figure 1. (*a*) The proportion of dams identified by month of disease onset in the BAB case. The proportion of identified dams confirmed as BSE cases by month of disease onset in the BAB (*b*), and by month of birth of the BAB case (*c*).

table of positive pairs by the time of BSE onset in the dam until the birth of the calf for the censored data, by birth cohort and by year of onset of clinical signs of BSE in the BABs, is given in table 1.

To compare the observed number of dam–calf positive pairs with that expected by chance, we require estimates of the number of calves born to dams that experience the onset of clinical signs of BSE as a function of time from birth of the calf to onset in the dam. To obtain these estimates, we utilize data recorded on the pregnancy status of confirmed BSE cases in the main database. These data were available for 88.4% of BSE cases. The variable indicating stage of pregnancy at the onset of clinical signs of BSE was recorded as the number of months pregnant (1–9), a code denoting 1–4 weeks post-calving, a code denoting 5–8 weeks post-calving, or a code denoting that the animal was not pregnant. The frequency distribution of pregnancy status data is given in figure 2.

Cows recorded 1–4 weeks and 5–8 weeks post calving were estimated to have calved one month and two months earlier, respectively. The date of last calving for pregnant animals was estimated by

Table 1. *Observed number of dam–calf positive pairs by maternal incubation period and (*a*) cohort and (*b*) year of the onset of the clinical signs of BSE in the BAB*

(*a*)

birth cohort	birth after onset of BSE in dam	months from birth of BAB until the onset of BSE in dam 0–6	6–12	12–24	24–36	36–48
1989	2	8	15	46	39	16
1990	4	18	19	55	45	31
1991	4	12	17	38	33	11
1992	5	28	22	45	21	12
1989–1992	15	66	73	184	138	70

(*b*)

year of onset of BSE in the BAB	birth after onset of BSE in dam	months from birth of BAB until the onset of BSE in dam 0–6	6–12	12–24	24–36	36–48
1992	0	3	8	23	24	12
1993	4	15	18	51	44	17
1994	3	19	18	50	35	21
1995	7	15	20	35	27	13
1996	1	13	9	25	8	6
1992–1996	15	65*	73	184	138	69†

*There was an additional onset of BSE in 1990. †There was an additional onset of BSE in 1991.

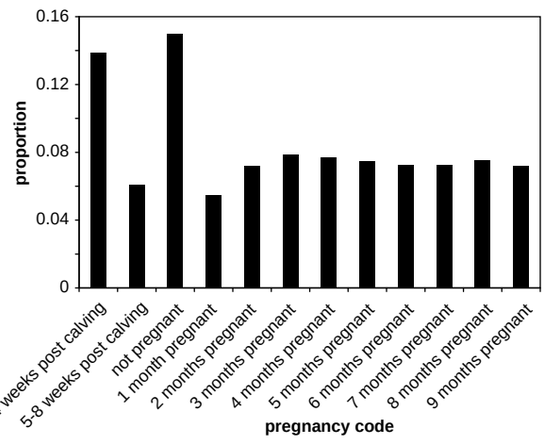

Figure 2. Frequency distribution of the recorded pregnancy stage data for female confirmed BSE cases.

assuming that the last calving prior to onset was three months prior to the last conception. For each female with missing pregnancy data, a calving date was generated in the 12 months prior to disease onset using the birth seasonality probability distribution observed in the BABs. Figure 2 indicates an excess of animals recorded as not pregnant. *A priori*, we would have expected those animals coded as not pregnant to have calved three months prior to onset; however, the observed excess suggests that some animals remain not pregnant for longer than expected. For animals coded as not pregnant we assign half (randomly chosen) to have calved three months prior to disease onset, the remainder being randomly assigned calving dates between 3 and 12 months prior to disease onset according to the calving seasonality observed in the birth dates of the BABs. This redistribution produces approximately equal numbers of animals in each pregnancy stage. It was assumed that pregnant animals which survived sufficiently long after the date of onset to have calved did so.

Experience in the collection of the epidemiological data and attempts to validate the various items indicate there are inaccuracies in pregnancy status data in the absence of on-farm records. For example, comparison of the pregnancy data with the birth dates of BABs born to BSE-affected dams revealed inconsistencies. To avoid any biases that may be introduced by using these data, we therefore also considered an alternative method for the estimation of calving dates. In this method, the calving dates of all female-confirmed BSE cases which were not dams of BABs were randomly assigned to a month within the 12 months prior to disease onset with the birth seasonality probability distribution observed in the BABs.

Cows were assumed to calve annually, and not before the age of 560 days. Whilst the mean inter-calving interval of British cows is approximately 380 days (Esslemont 1992), the assumption of annual calving considerably simplifies the calculations and maintains calving seasonality. It should be noted that neither pregnancy record-based estimates nor randomly assigned calving dates were used for the BSE-confirmed dams of BABs, since it was assumed that they calved annually on the same day of the year as the BABs were born.

Data on calving intervals (Esslemont 1992) give the average age of first lactation at 26 months. To avoid over-estimating the number of dams that calve at a young age, estimated calving dates between the ages of 560 and 730 days were given a weight equal to the frequency distribution of ages at first calving in the

intervals 561–594, 595–628, 629–662, 663–696 and 697–730 days, obtained from the 99 BSE-confirmed dams of BABs which were recorded as having calved between the ages of 560 and 730 days. All estimated calving dates after the age of 730 days were given the full weight of 1.

Herds are organized into holdings, a unit which most often corresponds to the farm. Holding sizes are estimated by summing the sizes of herds within holdings. As not all herds within a holding will generate at least one BSE case, these are clearly minimum estimates. For animals which experienced BSE onset in a holding other than their natal holding, the CVL database recorded this natal holding rather than the natal herd. The size of natal holdings could be estimated for those holdings which also appeared in the database as BSE-onset holdings.

3. ESTIMATORS FOR THE MAGNITUDE OF THE MATERNAL RISK ENHANCEMENT

The probability of direct maternal transmission of the aetiological agent of BSE may be a function of the maternal incubation stage at the time of calving, but it would be naive to expect that this effect would be apparent in table 1. To determine the significance of these results, we need to calculate the expected number of dam–calf positive pairs under the null hypothesis that within a holding, the BSE cases in the dams are independent of BSE cases in their offspring. Under this hypothesis, the probability that a BAB would have a BSE-confirmed dam depends on the number of dams calving in that birth cohort which are eventually confirmed as BSE cases. Due to the observed clustering of cases within holdings (Donnelly *et al.* 1997*a*), it is necessary to test the hypothesis that within holdings the observed cases in dams and offspring are independent. Thus, the calculation of the expected number of positive pairs is based on calculations for each natal holding of a given BAB.

Let D^{+}_{ijk} be the number of dams in holding i giving birth to animals in birth cohort j in BSE incubation stage k and D_{ij} be the total number of dams in holding i giving birth to animals in birth cohort j. Similarly, let B_{ij} denote the number of BABs in holding i and birth cohort j, and B^{I}_{ij} denote the number of such animals with identified dams. Finally, let O_{ijk} denote the number of BABs with identified BSE-confirmed dams in holding i born in birth cohort j with dams in BSE incubation stage k. The value of O_{ijk} is bounded above by the minimum of B^{I}_{ij} and D^{+}_{ijk} (i.e. the number of observed dam–calf positive pairs cannot be greater than the number of BABs or the number of BSE-confirmed dams in incubation stage k).

Under the hypothesis of independence of BSE occurring in either the dams or the calves within a holding, the expected number of dam-positive–calf-positive pairs (P_k) for dam incubation stage k is estimated by the number of BABs with identified dams weighted by the proportion of dams confirmed as BSE cases:

$$\widehat{P}_k = \sum_{i,j} \frac{D^{+}_{ijk}}{D_{ij}} B^{I}_{ij}.$$

Thus, the indirect association between BSE in the dam and the calf caused by the clustering of BSE cases in holdings is adjusted for by calculating the expected value within each holding and then summing over all holdings.

An alternative method of estimating the expected number of dam-positive–calf-positive pairs is given for dam incubation stage k as:

$$\widetilde{P}_k = \frac{\sum_{i,j} B^{I}_{ij}}{\sum_{i,j} B_{ij}} \sum_{i,j} \frac{D^{+}_{ijk}}{D_{ij}} B_{ij}.$$

These estimates will be similar to $\widehat{P}$ if the probability that a dam was identified is independent of the disease prevalence in the dams. Substantial differences between these two sets of estimates suggest biased identification rates of dams.

The ratio of the observed and expected numbers of BABs with identified BSE-confirmed dams can be obtained by maternal incubation stage k. Thus, we define the ratios:

$$\widehat{R}_k = \frac{\sum_{i,j} O_{ijk}}{\widehat{P}_k}.$$

and

$$\widetilde{R}_k = \frac{\sum_{i,j} O_{ijk}}{\widetilde{P}_k}.$$

Although a standard Pearson χ^2 statistic could be calculated for each pair of observed and expected values, the ratio and its 95% confidence interval yield more information about the underlying infection processes. A ratio significantly greater than 1 indicates a significant enhanced risk of BSE occurring in calves born to dams that subsequently developed BSE.

Alternatively, the ratio of observed to expected numbers of positive pairs can be calculated as a within-holding variable so that

$$r_{ijk} = \frac{O_{ijk}}{(D^{+}_{ijk}/D_{ij})B^{I}_{ij}}.$$

The mean of this variable by dam incubation stage k, denoted $\bar{r}_k$, is an alternative estimator of the maternal risk enhancement. The distribution of the variable r_{ijk} is highly skewed with most values equal to zero, and therefore it cannot be assumed that the sample mean is normally distributed.

One hundred realizations of the data set were generated (i) using the pregnancy data but using the sampling methods described in § 2 for animals with missing pregnancy data and animals recorded as not pregnant and (ii) randomly allocating calving dates for all animals according to the birth seasonality pattern observed in BABs. Confidence intervals in each case need to account for sampling variability in the calving dates in addition to variability in the outcome variable. For this reason we utilise a two-stage procedure obtaining bootstrap samples while allowing for sampling variability in the calving dates.

For each stage of the maternal incubation period, the ratios of the observed to the expected number of positive pairs are given in figure 3, with their corresponding 95% bootstrap confidence intervals.

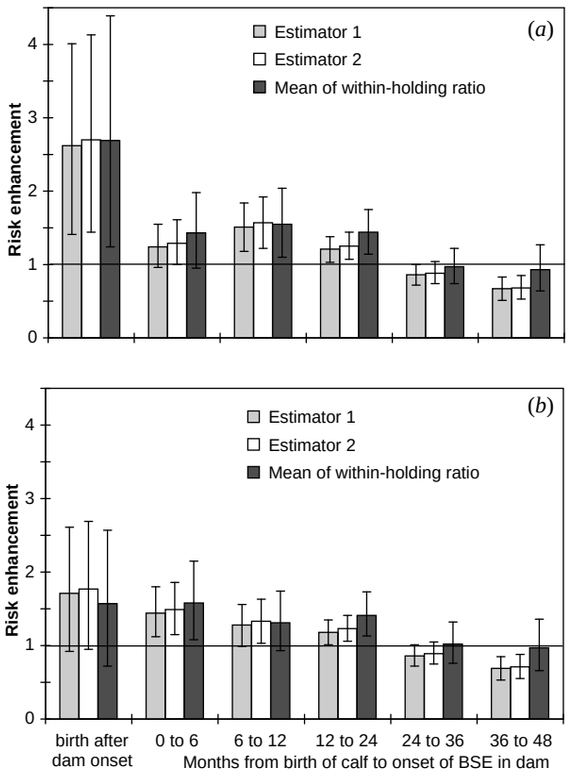

Figure 3. The ratios of observed to expected number of dam–calf positive pairs, $\widehat{R}$ and $\widetilde{R}$ denoted estimators 1 and 2, respectively, and the mean of the within-holding ratios, $\bar{r}$, for BAB cohorts between 1989 and 1992 by dam incubation stage (*a*) using pregnancy data and resampling, and (*b*) randomly assigning calving dates. Corresponding 95% bootstrap confidence intervals are presented.

The results show a statistically significant enhanced risk in calves born to affected dams within 24 months of the onset of clinical signs in the dam. In general, as the period between birth of the calf and onset of clinical signs in the dam increases, the maternally enhanced risk decreases. Such a decrease is inconsistent with the hypothesis that the maternally enhanced risk can be explained solely in terms of a genetically enhanced susceptibility, which would generate a risk enhancement independent of maternal incubation stage, and therefore provides some evidence for direct maternal transmission of the aetiological agent.

The random allocation of calving dates does not allow for the observed correlation between recent calving and disease onset as shown in figure 2 (the excess of cattle which experience disease onset in the month following calving). This results (figure 3*b*) in a less pronounced trend of increasing risk for calves born close to or after disease onset in the dam than seen when the pregnancy data are used (figure 3*a*). The random allocation of calving dates results in fewer calves being allocated to the 0–6 months category, with correspondingly more being allocated to the 6–12 months category, and thus the after-onset category. However, random allocation has virtually no effect on the annual intervals. Two factors possibly contributing to the correlation between recent calving and disease onset are the increased clinical observation of animals at the time of calving and the exposure of animals to environmental stimuli which enhance the manifestation of clinical signs.

Our results using the first two population estimators, $\widehat{R}$ and $\widetilde{R}$, give remarkably similar results. This similarity is consistent with the hypothesis that the identification of dams is independent of the disease status of the dams. However, the within-holding estimator, $\bar{r}$, gives slightly greater estimates for the maternally enhanced risk in those animals born between 36 and 48 months before the onset of clinical signs of disease in the dam. In particular, whilst the first two estimators show a significantly decreased risk of BSE in the offspring of BSE-affected dams in this category (a result that is difficult to explain under either the hypothesis of direct maternal transmission or that of genetically enhanced susceptibility), the within-holding estimator gives a non-significant risk enhancement. It should be noted, however, that as the natal holding size is likely to be underestimated, the ratio estimates presented above should be interpreted as minimum bounds.

4. MODELS TO ASSESS THE CONTRIBUTION OF DIRECT MATERNAL TRANSMISSION AND GENETIC SUSCEPTIBILITY TO THE OBSERVED RISK ENHANCEMENT

To distinguish direct maternal transmission from genetic predisposition to infection and associated disease requires the use of a full likelihood model. Such a model would need to include variable feed-risk within holdings and the survival distributions for each calf born to an affected dam. Given the quantity of data, such a model, whilst straightforward to specify, would be highly computationally intensive to fit. For this reason a full likelihood model has not been attempted. Instead, we consider a simplified approach which fits the data within holdings to retain the variable feed-risk (thereby controlling for clustering of dam–calf positive pairs by holding), but uses a simplified survival distribution.

We allow calves born to BSE-infected dams to experience risks of maternal transmission that depend on the stage of the maternal incubation period in which they were born. Let the maternal incubation period be divided into six stages (following the onset of clinical symptoms, 0–6 months, 6–12 months, 12–24 months, 24–36 months and 36–48 months prior to the onset of clinical signs). Let ϵ_k denote the probability of direct maternal transmission of the aetiological agent of BSE to calves born in stage k of the maternal incubation period.

Let y_{ij} be the feed infection hazard for an animal born to an unaffected dam, and sy_{ij} be the feed infection hazard for an animal born to a BSE-affected dam in holding i and cohort j, where s is a measure of genetic susceptibility. The baseline feed infection hazard y_{ij} is estimated as

$$y_{ij} = \alpha \frac{\sum_j B_{ij}}{\sum_j D_{ij}} \frac{\sum_i B_{ij}}{\sum_{i,j} B_{ij}},$$

where α is the probability that an infected animal will be observed to become a case. Thus, the probability that an animal born to a BSE-affected dam in incubation stage k is infected is

$$p^+_{ijk} = \epsilon_k + (1-\epsilon_k)(1-e^{-y_{ij}s/\gamma}),$$

and the probability that an animal born to an unaffected dam is infected is

$$p^-_{ij} = 1 - e^{-y_{ij}/\gamma},$$

where $1/\gamma$ is the factor by which the holding sizes are underestimated.

We model the probability that the identified dam of a BAB was at incubation stage k (where $k = 0, \ldots, K$) at the time of calving. This probability depends on the number of eligible dams at each incubation stage at calving. Let

$$D^-_{ij}(r) = \mathrm{int}[\gamma D_{ij}(r)] - \sum_k D^+_{ijk}(r),$$

where the function $\mathrm{int}(z)$ rounds z to the nearest integer. Then the probability is given by

$$\zeta\left(x_r|s, \epsilon_k, y_{ij}, \gamma, D_{ij}(r), D^+_{ijk}(r)\ \forall k\right) = \begin{cases} \dfrac{S^+_{ij} D^+_{ijk}(r) p^+_{ijk}}{S^-_{ij} D^-_{ij}(r) p^-_{ij} + S^+_{ij} \sum_k D^+_{ijk}(r) p^+_{ijk}} \\ \quad \text{for } x_r = 0, \ldots, K \\ 1 - \sum_k \dfrac{S^+_{ij} D^+_{ijk}(r) p^+_{ijk}}{S^-_{ij} D^-_{ij}(r) p^-_{ij} + S^+_{ij} \sum_k D^+_{ijk}(r) p^+_{ijk}} \\ \quad \text{for } x_r = -1, \end{cases} \quad (1)$$

where S^+_{ij} and S^-_{ij} are the probabilities that an animal in holding i and cohort j born to a BSE-affected dam and to an unaffected dam survives until disease onset, respectively. For calf r in holding i and cohort j, the variable x_r equals the dam incubation stage at calving for incubation stages $0, \ldots, K$ and takes the value -1 for the calves of unaffected dams. For $r = 1$, the stratified numbers of eligible dams, $D^+_{ijk}(r) = D^+_{ijk}$, whereas for $r > 1$, the numbers are conditional so that $D^+_{ijk}(r) = D^+_{ijk} - \Sigma^{r-1}_{q=1} I_k(x_q)$ where $I_k(x_q) = 1$ when $x_q = k$ and 0 otherwise. Similarly, $D_{ij}(1) = D_{ij}$, and $D_{ij}(r) = D_{ij} - \Sigma_k \Sigma^{r-1}_{q=1} I_k(x_q)$ for $r > 1$. These conditions ensure that once a dam in incubation stage k has been allocated to a BAB, she is removed from the set of eligible dams. The joint likelihood for all BABs with identified dams in holding i and cohort j (L_{ij}) therefore depends on the order in which dams are allocated to BABs, and thus is the sum over all possible orderings ($\mathbf{X}$) of the product of the conditional probabilities:

$$L_{ij} = \sum_{x \in \mathbf{X}} \prod_{r=1}^{B^I_{ij}} \zeta(x_r|x_1, \ldots, x_{r-1}, s, \epsilon_k, y_{ij}, \gamma, D_{ij}, D^+_{ijk}\ \forall k). \quad (2)$$

The assumption that the calves of BSE-affected and unaffected dams have the same survival probability can be explored univariately by letting $S^+_{ij} = \lambda S^-_{ij}$. For example, for $B^I_{ij.} = 2$ the likelihood can be written as:

$$\begin{aligned} L_{ij} &= \sum_{x \in \mathbf{X}} \prod_{r=1}^{2} \zeta(x_r|x_1, \ldots, x_{r-1}, s, \epsilon_k, y_{ij}, \gamma, \\ &\quad D_{ij}, D^+_{ijk}\ \forall k) \\ &= \sum_{x \in \mathbf{X}} \zeta(x_2|x_1, s, \epsilon_k, y_{ij}, \gamma, D_{ij}, D^+_{ijk}\ \forall k) \times \\ &\quad \zeta(x_1|s, \epsilon_k y_{ij}, \gamma, D_{ij}, D^+_{ijk}\ \forall k) \\ &= \sum_{x \in \mathbf{X}} \zeta(x_2|s, \epsilon_k, y_{ij}, \gamma, D_{ij}(2), D^+_{ijk}(2)\ \forall k) \times \\ &\quad \zeta(x_1|s, \epsilon_k, y_{ij}, \gamma, D_{ij}, D^+_{ijk}\ \forall k), \end{aligned}$$

where the set of orderings $\mathbf{X}$ is $\{(x_a, x_b), (x_b, x_a)\}$. Thus, the conditional nature of the likelihood arises from the dependence of D_{ij} (r) and D^+_{ijk} (r) on x_1 to x_{r-1}.

Maximum likelihood estimates can be obtained for the parameters s and ϵ_k using direction set techniques (Press *et al.* 1992; Jacobs 1977). The simultaneous likelihood ratio confidence region contains all combinations of parameters which provide a similar goodness-of-fit to the observed data, as measured by the likelihood ratio χ^2 statistic. The goodness-of-fit of the model is measured by the comparison of the difference between the maximized model likelihood with the saturated data likelihood with the distribution of the analogous differences obtained from bootstrap samples from the model. The saturated likelihood is obtained using equations like equations (1) and (2) where p^+_{ijk} and p^-_{ij} are functions of r, such that

$$p^+_{ijk}(r) = \frac{O_{ijk}(r)}{B^I_{ij}(r)}$$

and

$$p^-_{ij}(r) = 1 - \sum_k p^+_{ijk}(r).$$

For $r = 1$, $O_{ijk}(r) = O_{ijk}$ and $B^I_{ij}(r) = B^I_{ij}$ whereas for $r > 1$ the numbers are conditional so that $O_{ijk}(r) = O_{ijk} - \Sigma^{r-1}_{q=1} I_k(x_q)$ and $B^I_{ij}(r) = B^I_{ij} - \Sigma_k \Sigma^{r-1}_{q=1} I_k(x_q)$.

The parameters of key interest are the rates of maternal transmission by incubation stage, ϵ_k, and the genetic susceptibility ratio, s. However, values of α and λ are required to obtain estimates. To estimate α, the ratios of cases observed by ages 3, 4, 5 and 6 years to infections were obtained from the back-calculation model presented in Ferguson *et al.* (1997*a*) (figure 4). We analyse the number of BABs arising within four years of the end of their birth cohort interval. If calvings were uniformly distributed throughout the year, then on average the BABs are censored at 4.5 years of age. However, calving is more likely in the autumn months (i.e. early in the birth cohort interval) and so, on average, the BABs are censored between 4.5 and 5 years. Interpolating between the backcalculation ratios for cases arising by four years of age and by five years of age gives an estimated value of α in the range 18–25 for cases arising by 4.75 years of age.

Results were obtained from two sets of 100 data realizations. The first set was obtained using the available pregnancy data, redistributing the excess of animals recorded as non-pregnant and randomly

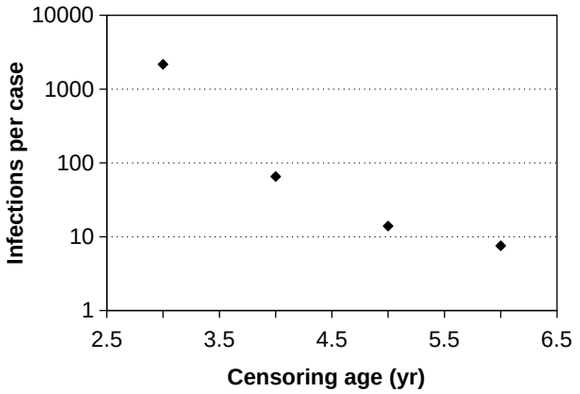

Figure 4. The infections per case observed by the censoring age as calculated from the backcalculation model presented in Ferguson *et al.* (1997*a*).

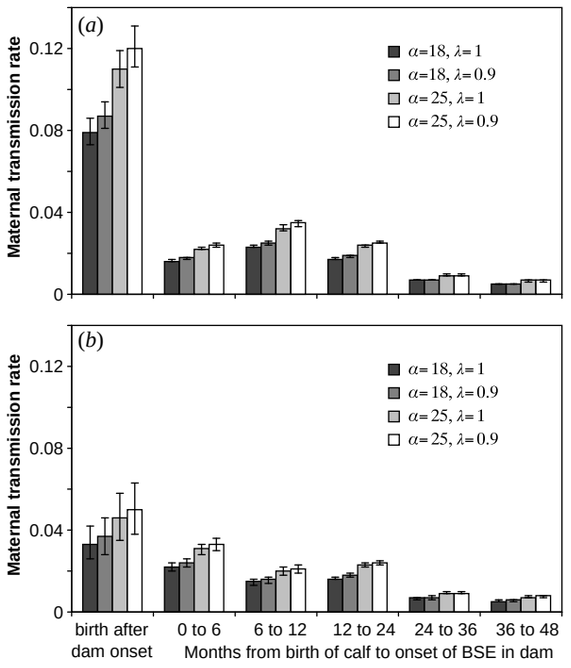

Figure 5. The mean (and range) of the maximum likelihood estimates of the maternal transmission rate, ϵ_k, by maternal incubation stage for α values of 18 and 25 and λ values of 1 and 0.9 (*a*) using pregnancy data and resampling and (*b*) randomly assigning calving dates.

assigning the calving dates for those animals with missing data. A second set was obtained randomly, assigning the calving dates for all animals.

Initially, estimates of s and ϵ_k for all k were obtained using two values of α (18 and 25) and $\gamma = 1$, and assuming no difference in the survival probability of calves born to BSE-affected and unaffected dams (i.e. $\lambda = 1$) (table 2, figure 5). Significant maternal transmission is found in both cases, although the decreasing trend with increasing time from calving to disease onset observed when pregnancy data are used is not seen when all calving dates are assigned randomly. The unexpected result, that the estimated rate of maternal transmission for animals born between 0 and 6 months prior to disease onset in the dam is less than the estimated rate for animals born between 6 and 12 months, could result from differential survival or success in tracing, with animals born closer to disease onset in the dam being less likely to survive or more likely to be sold from the natal herd in calfhood. Although this has not been confirmed, such a pattern would also result in underestimates of the maternal transmission rate for animals born after disease onset in the dam.

The estimate of s is significantly less than unity for both cases. Such a result is biologically implausible. The result is due to the fact that the observed to expected ratios are less than 1 for the calves born early in the dam-incubation stage. One possible explanation for this is differential survival probabilities or dam identification rates for BABs born to BSE-affected dams compared with those born to unaffected dams, suggesting that $\lambda < 1$.

We therefore examined estimates resulting from a range of λ values and two values of α (18 and 25). Similar patterns are seen for both α values (results for $\alpha = 18$ are shown in figure 6). In both cases, the estimates of s and the maternal transmission rate for calves born after onset of BSE in the dam increase dramatically with decreasing λ. The estimates of the maternal transmission rates for animals born 0–6 months, 6–12 months and 12–24 months before onset of BSE in the dam remain relatively constant for $\lambda > 0.1$. Whilst the estimates of the maternal transmission rates for animals born more than 24 months before dam onset decrease for $\lambda < 0.4$, they do not differ significantly from 0 for any values of λ examined.

The effect of underestimating holding size can be examined by increasing the value of γ. Figure 7 illustrates the positive linear relationship between the maximum likelihood estimate of s and γ. This relationship can be seen by taking a first order approximation to $e^{y_{ij}s/\gamma}$, so

$$\zeta(x_r|s, \epsilon_k, y_{ij}, \gamma, D_{ij}(r), D^+_{ijk}(r)\forall k)$$

$$\simeq \begin{cases} \dfrac{S^+_{ij} D^+_{ijk}(r)(\epsilon_k + y_{ij}s/\gamma)}{S^-_{ij} D^-_{ij}(r)(y_{ij}/\gamma) + S^+_{ij}\sum_k D^+_{ijk}(r)(\epsilon_k + y_{ij}s/\gamma)} \\ \quad \text{for } x_r = 0, \ldots, K \\ 1 - \sum_k \dfrac{S^+_{ij} D^+_{ijk}(r)(\epsilon_k + y_{ij}s/\gamma)}{S^-_{ij} D^-_{ij}(r)(y_{ij}/\gamma) + S^+_{ij}\sum_k D^+_{ijk}(r)(\epsilon_k + y_{ij}s/\gamma)} \\ \quad \text{for } x_r = -1 \end{cases}$$

$$\simeq \begin{cases} \dfrac{S^+_{ij} D^+_{ijk} s}{S^-_{ij} \gamma D_{ij}} & \text{for } x_r = 0, \ldots, K \\ 1 - \sum \dfrac{S^+_{ij} D^+_{ijk} s}{S^-_{ij} \gamma D_{ij}} & \text{for } x_r = -1 \end{cases} \quad (3)$$

where equation (3) holds for $\gamma D_{ij} \gg D^+_{ijk}$ for all i, j, and k and $\epsilon_k \ll (y_{ij}s/\gamma)$. For most animals, the latter condition will hold for plausible values of γ since the majority of dams are in an early incubation stage. Whilst increasing γ has a strong effect on the estimate of s, it has a weaker effect on the values of ϵ_k. Thus, the relative contributions of direct maternal transmission and

Table 2. *The mean (and range) of the maximum likelihood estimates for* s *and* ϵ_k *obtained from 100 data sets (assuming* $\lambda = 1$*) with the results of the likelihood ratio tests of the null hypotheses* H_0*:* $s = 1$, H_0*:* $\epsilon_k = \epsilon$ *for all k and* H_0*:* $\epsilon_k = 0$ *for all k and the goodness-of-fit test for a representative realization of the data (a) using pregnancy data and resampling and (b) randomly assigning calving dates*

	$\alpha = 18$		$\alpha = 25$	
	$\lambda = 1$	$\lambda = 0.9$	$\lambda = 1$	$\lambda = 0.9$
(*a*)				
s	0.822	0.931	0.823	0.941
	(0.818,0.826)	(0.927,0.937)	(0.819,0.828)	(0.936,0.946)
H_0: s = 1	0.012	0.365	0.018	0.454
ϵ_k birth after onset of BSE in dam	0.079	0.087	0.110	0.120
	(0.073,0.086)	(0.081,0.094)	(0.101,0.119)	(0.111,0.131)
0–6 months before onset	0.016	0.018	0.022	0.024
	(0.016,0.017)	(0.017,0.018)	(0.022,0.023)	(0.023,0.025)
6–12 months before onset	0.023	0.025	0.032	0.035
	(0.023,0.024)	(0.024,0.026)	(0.031,0.034)	(0.033,0.036)
12–24 months before onset	0.017	0.019	0.024	0.025
	(0.017,0.018)	(0.018,0.019)	(0.023,0.024)	(0.025,0.026)
24–36 months before onset	0.007	0.007	0.009	0.009
	(0.007,0.007)	(0.007,0.007)	(0.009,0.010)	(0.009,0.010)
36–48 months before onset	0.005	0.005	0.007	0.007
	(0.005,0.005)	(0.005,0.005)	(0.006,0.007)	(0.006,0.007)
H_0: $\epsilon_k = \epsilon \; \forall k$ *p* value	0.011	0.015	0.013	0.013
H_0: $\epsilon_k = 0$ *p* value	<0.001	<0.001	<0.001	<0.001
goodness-of-fit *p* value	0.021	0.023	0.021	0.025
(*b*)				
s	0.829	0.941	0.833	0.952
	(0.822,0.838)	(0.932,0.950)	(0.825,0.842)	(0.943,0.962)
H_0: $s = 1$	0.021	0.484	0.032	0.603
ϵ_k birth after onset of BSE in dam	0.033	0.037	0.046	0.050
	(0.026,0.042)	(0.028,0.046)	(0.035,0.058)	(0.038,0.063)
0–6 months before onset	0.022	0.024	0.031	0.033
	0.020,0.024)	(0.022,0.026)	(0.028,0.033)	(0.030,0.036)
6–12 months before onset	0.015	0.016	0.020	0.021
	(0.013,0.016)	(0.014,0.017)	(0.018,0.022)	(0.019,0.023)
12–24 months before onset	0.016	0.018	0.023	0.024
	(0.016,0.017)	(0.017,0.019)	(0.022,0.024)	(0.023,0.025)
24–36 months before onset	0.007	0.007	0.009	0.009
	(0.006,0.007)	(0.006,0.008)	(0.009,0.010)	(0.009,0.010)
36–48 months before onset	0.005	0.006	0.007	0.008
	(0.005,0.006)	(0.005,0.006)	(0.007,0.008)	(0.007,0.008)
H_0: $\epsilon_k = \epsilon \; \forall k$ *p* value	0.157	0.177	0.165	0.165
H_0: $\epsilon_k = 0$ *k p* value	<0.001	<0.001	<0.001	<0.001
goodness-of-fit *p* value	0.025	0.021	0.027	0.028

enhanced genetic susceptibility to the observed enhanced risk are changed by assuming underestimation of holding sizes. These results indicate that genetically determined predisposition to BSE is only likely to be important if natal holding sizes have been substantially underestimated in the above analyses.

5. CONCLUSIONS

The results of the dam identification study confirm the presence of an enhanced risk of BSE in calves born to dams that had either already developed BSE or did so soon after the birth of the offspring. The magnitude of the risk enhancement is related to the maternal incubation stage of the dam when calving. This suggests that there is direct maternal transmission of the aetiological agent of BSE. If susceptibility to feed-borne infection were entirely genetically determined, any enhanced risk in calves born to diseased dams would be independent of the maternal incubation stage. When pregnancy data were used, the three estimators of the magnitude of the maternal risk enhancement consistently indicated an increased risk enhancement in calves born close to or after BSE onset in the dam.

Given the available data, a likelihood model was utilized to assess the relative contribution of direct maternal transmission and genetically determined susceptibility to the observed maternally enhanced risk of BSE. All models examined consistently indicate direct maternal transmission of the aetiological agent of BSE in the late stage of BSE incubation (for calves born after or less than 24 months before the onset of

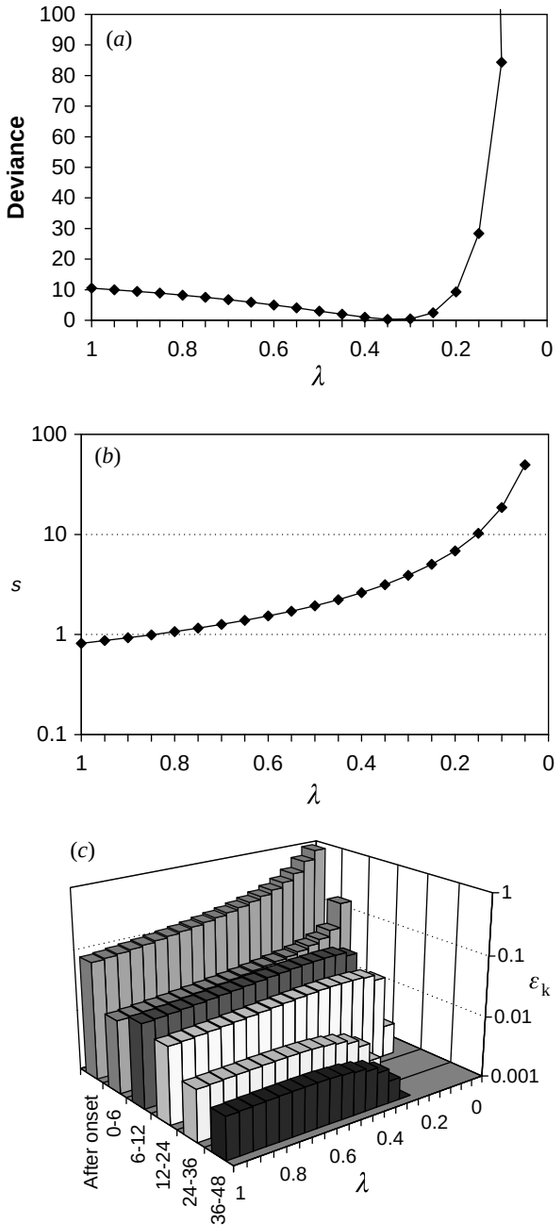

Figure 6. The deviance (*a*), estimated values of s (*b*) and ϵ_k (*c*) for a realization of the data set using the pregnancy data.

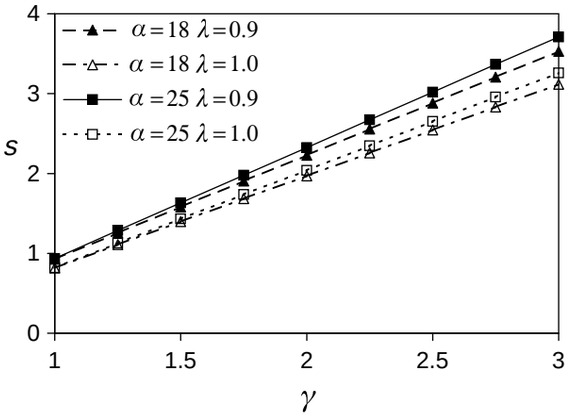

Figure 7. The maximum likelihood estimate of s as a function of γ for α values of 18 and 25 and λ values of 1 and 0.9, for a realization of the data set using the pregnancy data.

clinical signs of BSE in the dam), with the risk being greatest for those calves born after the onset of BSE. This evidence confirms the findings of the maternal cohort study which indicated significant maternal transmission in animals born near or after the onset of clinical signs of BSE in the dam. This model does not provide any evidence of genetically enhanced susceptibility to BSE infection. Indeed, the current lack of any evidence for a significantly enhanced risk for animals born more than two years before the onset of BSE in the dam argues strongly against a genetic component. However, in the absence of pedigree and genotype data we cannot completely exclude this possibility (Ferguson *et al.* 1997*b*). Furthermore, the estimates of maternally enhanced risk presented here should be treated as minimum bounds; the incorporation of better estimates of natal holding size, differential survivorship or dam identification rates, would all lead to higher estimates of risk enhancement that may allow a role for genetically determined susceptibility.

Clearly, there remain unresolved issues in the analysis of dam–calf BSE pairs. The power of the analyses would be increased if the identification of dams were more complete for animals showing clinical signs of BSE since August 1994. In order to take our current analyses further, data would ideally be collected on all calves of dams eventually confirmed with BSE. These calves would have their dates of birth, death, and the onset of the clinical signs of BSE (for confirmed cases) recorded, and living animals would be monitored until the onset of BSE or death. However, analyses do not require that all calves have been followed until death, as the living animals can be treated as censored observations. Such an analysis requires a great deal of data to be collected, but it would allow for better specification of variable survival distributions, thereby enabling better estimation of the holding cohort-specific feed risks. The Bovine Animal Registration, Identification and Moment Order (BARIMO) legislation introduced in 1995, and the Cattle Traceability System (CTS), currently being developed, will aid future investigations.

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