These are electronic appendices to the paper by Ferguson & Donnelly 2003 Assessment of risk from BSE in GB cattle and the impact of potential changes to current control measures. *Proc. R. Soc. Lond.* B **270**, 1579–1584. (DOI 10.1098/rspb.2003.2484.)

Electronic appendices are refereed with the text. However, no attempt has been made to impose a uniform editorial style on the electronic appendices.

Address: Department of Infectious Disease Epidemiology, Faculty of Medicine, Imperial College London, St Mary's Campus, Norfolk Place, London W2 1PG

e-mail: neil.ferguson@imperial.ac.uk

Electronic Appendix A: Data sources

Clinical case data

Data regarding each confirmed case of BSE arising in GB have been entered into a database maintained at the Veterinary Laboratories Agency (see Donnelly *et al.* (1997) and Donnelly & Ferguson (2000) for additional details). The variables considered in the following analyses include date of birth and date of onset of the clinical signs of disease as well as the estimated age of the animal at clinical onset, used if the dates of birth and/or onset are unknown. As noted previously (Ferguson *et al.* 1997), until late 1990 when the farmer estimated the age at clinical onset, it was biased towards whole years of age. This bias was corrected for by resampling the ages of *ca*. 5000 cases by randomizing uniformly the month of the reported age at onset, as in earlier analyses (Ferguson *et al.* 1997).

The cohort-specific age distributions of clinical cases (conditional on onset by age *a*) reveal an unexpected increase in cases in 2001 (Figure 5). The reason for this is not clear but could have been due to the foot and mouth disease epidemic, either through the resulting demographic changes or perhaps simply the heightened surveillance of cattle. An alternative possibility is increased case recognition induced by compulsory screening of casualty and fallen stock animals, such that animals that would have previously been registered as casualties are now being classified as clinical cases. More work is being undertaken examining this issue.

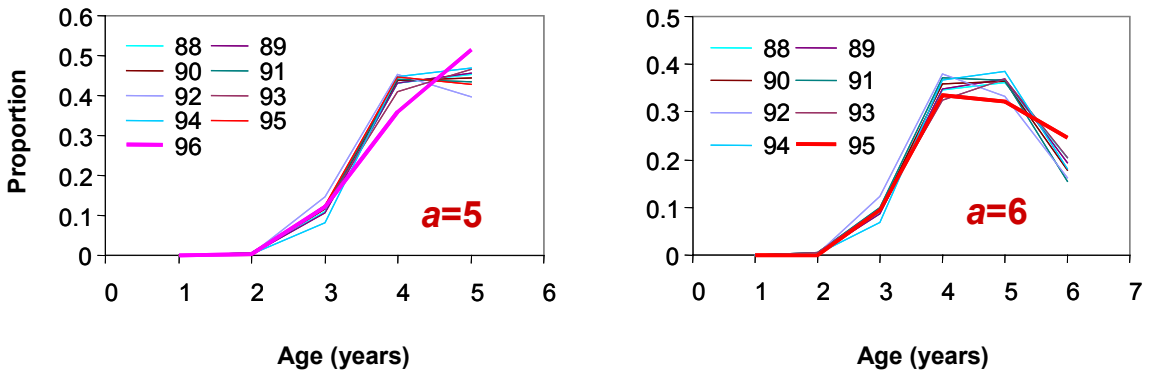

Figure 5 Proportion of cases by age at clinical onset and birth cohort demonstrating the unexpected increase in incidence observed in 2001 (when animals in birth cohorts 1996 and 1995 were 5 and 6 years of age, respectively.

BSE screening data

The first two surveys of apparently healthy cattle in GB were mainly targeted at animals over 5 years of age (Figure 6). One survey, conducted between January and March 1999, detected 18 positives in 3945 cattle with test results. The second, conducted between May and December 2000, detected 42 positives in 10,032 cattle with test results. These were the only screening data analyzed in the previous integrated backcalculation analysis (Donnelly *et al*. 2002).

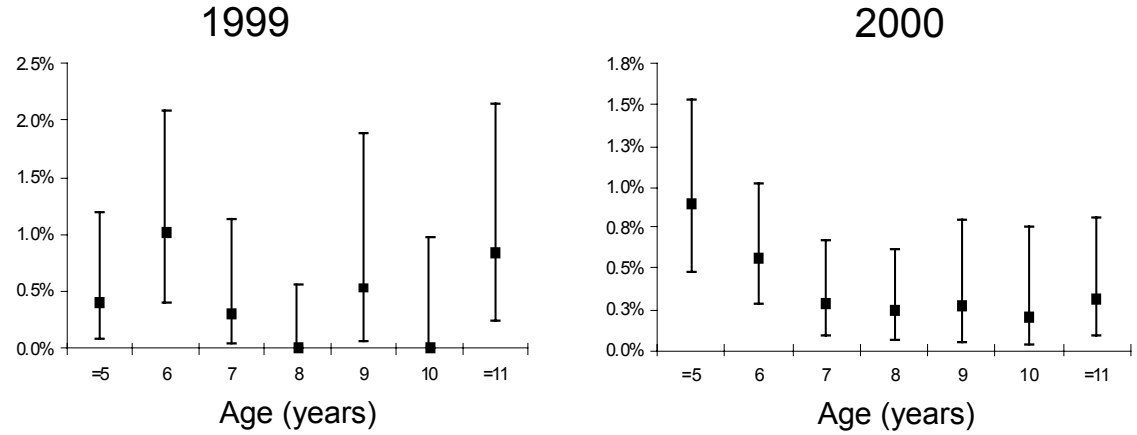

Figure 6 Detected prevalence by age group in apparently healthy cattle slaughtered by year tested (with exact 95% CI) [assuming the cattle without age data (all negative) had the same age distribution as the cattle with only year of age data, *i.e*. no date of birth available].

The current analysis incorporates additional screening data collected in 2001 and 2002 on 70,739 apparently healthy cattle, 108,533 casualty cattle and 67,889 fallen stock cattle. Table 2 below lists this data.

Table 2. Screening data collected in 2001 and 2002 (up to October 2002), stratified by risk group and animal age. Number of test positives and total number screened in each category is listed. This data is plotted in Figure 1 of the main text.

	Apparently healthy animals		Casualty animals		Fallen stock	
Age (years)	***Positive***	***Total***	***Positive***	***Total***	***Positive***	***Total***
1	0	0	0	9	0	1
2	0	6440	0	7872	0	9629
3	0	10306	2	14369	0	7960
4	1	21978	5	13347	1	7086
5	0	26354	10	12654	2	6723
6	2	867	87	12365	23	6838
7	0	875	123	12493	40	6718
8	1	824	82	12087	25	5993
9	0	713	50	6955	18	4106
10	0	514	56	6686	14	4793
11	0	364	22	3223	10	2317
12	2	237	21	2694	13	2318
13	1	142	13	1259	2	1066
14	0	87	6	979	9	773
15	0	73	5	712	4	699
16	0	20	3	330	2	286
17	0	14	0	161	2	169
18	0	3	1	133	0	132
Not known	0	928	2	205	0	282

Cattle Tracing System (CTS) database

The CTS database had up to 16% more births recorded per year (since mid 1996) than did the first annual agricultural census. However, at second census (18 months of age on average) the numbers recorded as surviving in the two databases were within 5%. The survival function estimated from the longitudinal data for cattle in the 1997, 1998, 1990 and 2000 birth cohorts in the CTS database was consistent with that previously estimated beyond 18 months of age. However, no such data were available relating to survival beyond 4 years of age. CTS data on the age distribution of slaughters in 2001/2 were used to estimate a non-time varying survival curve beyond 2.5 years and it was found to be very similar to that previously estimated (Donnelly *et al*. 1997) (Figure 7).

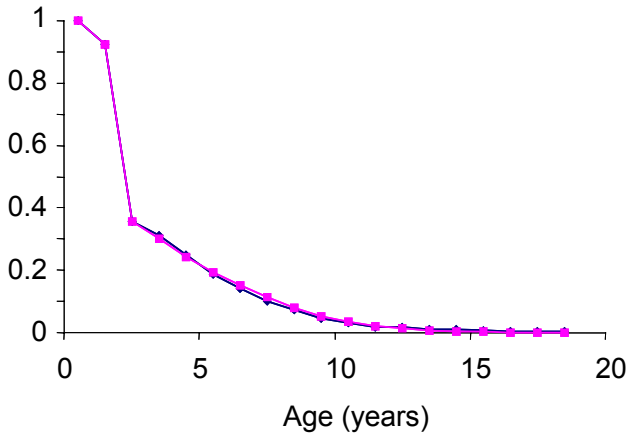

Figure 7 The probability of survival as a function of age previously estimated (pink, Donnelly *et al*. 1997) and estimated from the age distribution of cattle slaughtered in 2001/2 as recorded in the CTS database (blue).

The CTS database was also used to estimate the proportion of cattle mortality resulting from fallen stock or the on-farm slaughter of casualty animals. Although the CTS does not record specifically which cattle deaths are fallen stock or casualties, the number of animals (older than 12 months of age) reported as dying on farm was similar to number of high-risk animals screened in 2001/2. Roughly 24% of all cattle deaths, among cattle 3 years of age and older, were recorded to have died on farm and were assumed to have been fallen stock and casualties. This proportion was remarkably consistent over the age range of adult cattle (Figure 8).

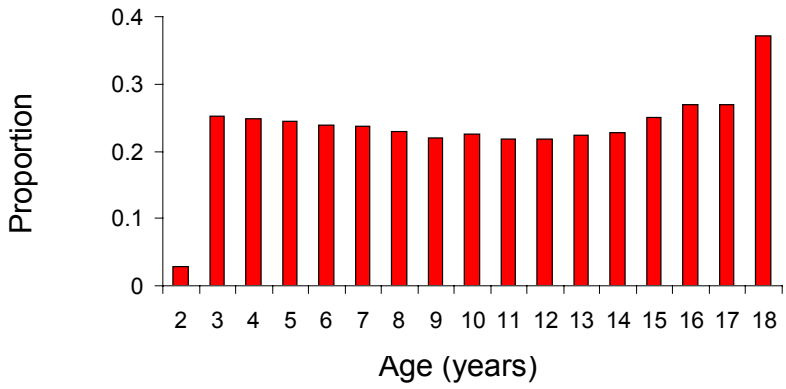

Figure 8 The proportion cattle deaths (recorded since mid-2001), by age, recorded to have been on-farm deaths.

The clinical case, screening and CTS databases were cross-linked to evaluate how representative the cattle screened for BSE infection were of the cattle population as a whole. No systematic bias in the apparently healthy OTM animals screened for infection was detected on the basis of the proportion of animals coming from a herd in which confirmed clinical cases of BSE onset. However, in female cattle, the proportion coming from BSE-positive holdings was greatest in casualty animals, followed by

slaughterhouse-killed animals, followed by fallen stock. Similarly, in male cattle, the proportion in casualty animals was greater than in slaughterhouse-killed animals and fallen stock.

EU screening data

Data from apparently healthy animals screened in other EU countries (between January 2001 and August 2002) were analyzed to obtain independent estimates of the lower limit on sensitivity of the diagnostic tests used for rapid screening. These results, presented in Table 3, demonstrate that the specificity of the screening tests, though possibly less than 100%, is unlikely to be less than 99.9984% (the highest lower limit was obtained from Austria). Thus, very few if any of the animals detected as positive in the screening programmes are likely to have been false positives.

Table 3 The lower confidence limit on diagnostic test specificity was obtained for each EU country based on the sample size and number of positive test results obtained in apparently healthy animals tested by August 2002.

	Number tested by Aug 2002	Number positive	Prevalence (per 100,000 cattle)	Lower limit on specificity
Belgium	626,979	37	5.9	99.9919%
Denmark	397,160	4	1.0	99.9974%
Germany	4,427,415	65	1.5	99.9981%
Greece	28,311	1	3.5	99.9803%
Spain	615,649	58	9.4	99.9778%
France	4,320,910	133	3.1	99.9964%
Ireland	964,164	58	6.0	99.9922%
Italy	775,169	39	5.0	99.9931%
Luxemburg	30,313	0	0.0	99.9878%
Netherlands	774,351	16	2.1	99.9967%
Austria	346,667	1	0.3	99.9984%
Portugal	73,310	49	66.8	99.9116%
Finland	81,489	0	0.0	99.9955%
Sweden	11,883	0	0.0	99.9690%

Electronic Appendix B: Model extensions

The mathematical framework required to incorporate differential mortality and underreporting into a backcalculation model has been previously published (Donnelly *et al*. 2002). For this study, we extended this past work to separately model infection risk in animals which die on farm (casualty/fallen stock) or at abattoirs, The excess risk in infected animals of ('differential') mortality prior to clinical onset were allowed to have an increased risk of dying on farm, as casualty or fallen stock animals, than those dying on farm. This risk ratio function, φ(*w*), of the time to clinical onset, *w*, allowed the screening data from casualty/fallen stock to be fitted separately from screening data from apparently healthy animals slaughtered at abattoirs using the probabilities of infection in casualty/fallen stock and apparently healthy animals.

In previous work $Z_I(a|t)$ is the age-specific rate at which clinically unaffected infected animals are slaughtered; $Z_{ID}(a|t)$ is the age-specific rate at which clinically unaffected infected animals are slaughtered and detected as test positive; $Z_U(a|t)$ is the age-specific rate at which uninfected animals are slaughtered; and ξ is the specificity of the test (or combination of tests) used (see Donnelly et al. 2002). These slaughter rates are further distinguished by the addition of the subscript *R* for high risk animals or *A* for apparently healthy animals, noting that the high risk slaughter rate actually includes a small amount of on-farm mortality in which the animal died rather than was slaughtered.

The rates for animals being slaughtered as risk animals are then given by:

$$Z_{UR}(a\,|\,t) = R(a)Z_U(a\,|\,t) = R(a)\left(B(t_0)\mu(a)\exp\left(-\int_0^a \mu(a'')da'' \right)\left[1 - p_I(a\,|\,t_0)\right] \right)$$

$$Z_{IR}(a\,|\,t_0) = R(a)\left(B(t_0)\int_0^\infty\int_0^a \left[\mu(a) + \phi(w)\kappa(w)\right]\exp\left(-\int_0^a \mu(a'')da'' - \int_w^{w+a-a'} \kappa(w')dw' \right)\rho_C(a', a+w\,|\,t_0)da'dw \right)$$

$$Z_{IDR}(a\,|\,t_0) = R(a)\left(B(t_0)\int_0^\infty\int_0^a \psi(w)\left[\mu(a) + \phi(w)\kappa(w)\right]\exp\left(-\int_0^a \mu(a'')da'' - \int_w^{w+a-a'} \kappa(w')dw' \right)\rho_C(a', a+w\,|\,t_0)da'dw \right)$$

and the rates for animals being slaughtered as apparently healthy are then given by:

$$Z_{UA}(a\,|\,t) = (1 - R(a))Z_U(a\,|\,t) = (1 - R(a))\left(B(t_0)\mu(a)\exp\left(-\int_0^a \mu(a'')da'' \right)\left[1 - p_I(a\,|\,t_0)\right] \right)$$

$$Z_{IA}(a\,|\,t_0) = \left(B(t_0)\int_0^\infty\int_0^a \left[\left(1 - R(a)\right)\mu(a) + \left(1 - \phi(w)R(a)\right)\kappa(w)\right]\exp\left(-\int_0^a \mu(a'')da'' - \int_w^{w+a-a'} \kappa(w')dw' \right)\rho_C(a', a+w\,|\,t_0)da'dw \right)$$

$$Z_{IDA}(a\,|\,t_0) = \left(B(t_0)\int_0^\infty\int_0^a \psi(w)\left[\left(1 - R(a)\right)\mu(a) + \left(1 - \phi(w)R(a)\right)\kappa(w)\right]\exp\left(-\int_0^a \mu(a'')da'' - \int_w^{w+a-a'} \kappa(w')dw' \right)\rho_C(a', a+w\,|\,t_0)da'dw \right)$$

where $R(a)$ is the age-specific proportion of uninfected animals that die or are slaughtered on farm, estimated matching the age distribution of proportion deaths occurring on-farm observed in the CTS data. As in previous work, $\psi(w)$ denotes the sensitivity of the diagnostic test for a time w from disease onset; $\mu(a)$ represents the hazard of death for an uninfected animal at age a; $\kappa(w)$ represents the additional mortality hazard experienced by BSE-infected cattle, prior to the onset of overt clinical

signs of disease, as a function of the time until clinical onset of disease, w; $\rho_C(a,u|t_0)$ represents the probability density function (PDF) that an individual born at time t_0 becomes infected at age a and a case at age u in the absence of mortality,

The probability that an animal slaughtered at age a between time t_0 and $t_0+\Delta$ is tested positive is

$$\frac{\int_{t_0}^{t_0+\Delta}(1-\xi)Z_{UR}(a\mid t)+Z_{IDR}(a\mid t)dt}{\int_{t_0}^{t_0+\Delta}Z_{UR}(a\mid t)+Z_{IR}(a\mid t)dt}$$

for a risk animal and

$$\frac{\int_{t_0}^{t_0+\Delta}(1-\xi)Z_{UA}(a\mid t)+Z_{IDA}(a\mid t)dt}{\int_{t_0}^{t_0+\Delta}Z_{UA}(a\mid t)+Z_{IA}(a\mid t)dt}$$

for an apparently healthy animal. The parameters are estimated by maximum likelihood using log likelihood contributions from the clinical incidence and screening data as detained in Donnelly et al (2002).

In addition, the computational efficiency and numerical accuracy of the model were enhanced.

Electronic Appendix C. Quantifying exposure: animals slaughtered in last year of incubation period

A simple summary statistic of the level of human exposure to infected tissue is the number of animals in the last year of incubation entering the food supply per year. While this measure is not as directly correlated with risk as the infectivity measures used in the main text, it has the advantage of not depending on relatively uncertain estimates of the relative infectivity of different bovine tissues entering food (DNV 2003), and how that infectivity develops through the incubation period in an infected bovine. Figure 9 presents estimates of the number of animals in the last year of incubation entering the food supply per year for age- and birth-date-based policies (all assumed to be introduced at the start of 2004) for the baseline scenario discussed in the main text (*i.e.* differential mortality occurring in the last 3 months of the incubation period and sensitivity profile 1). As discussed in the next section, while exposure estimates increase as one assumes a longer period of differential mortality, so do the estimates of historical exposure (and total epidemic size), meaning there is much less variation between these scenarios in terms of relative changes in exposure levels over time than might first be thought.

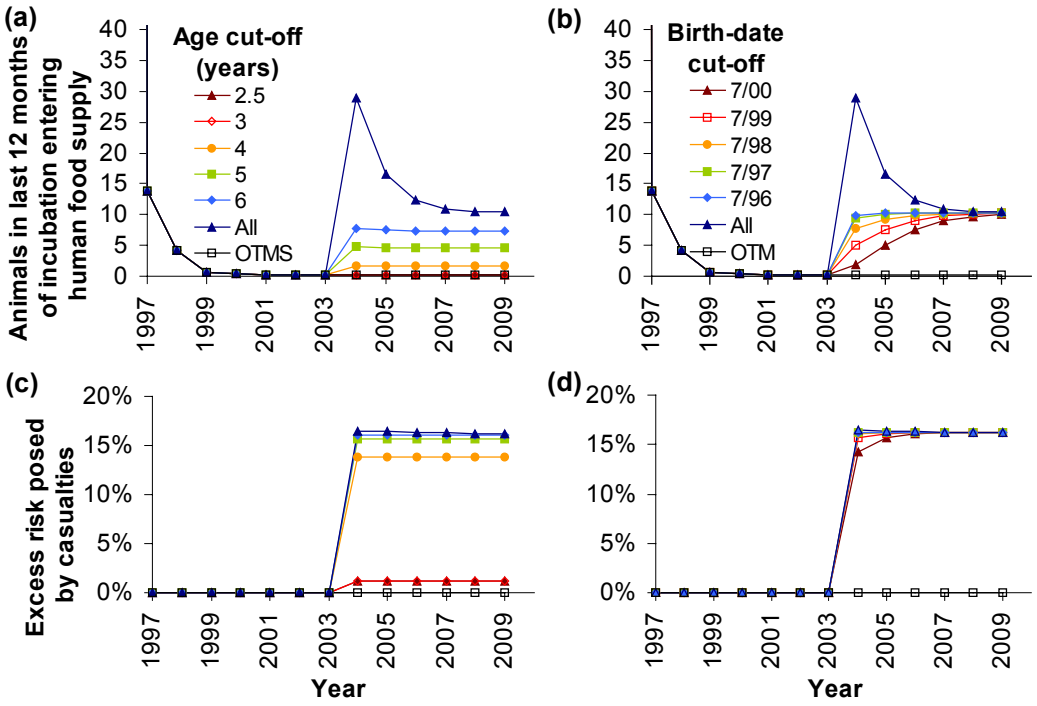

Figure 9 Exposure estimates as a function of risk reduction policy (OTMS or age- or birth-date-based alternatives) for the baseline scenario. (a) Exposure due from infected non-casualty animals for age-cutoff based policies. (b) As (a) but for birth-date-based policies. (c) Increase in exposure that would occur over (a) if casualty animals were allowed to enter the food supply. (d) As (c) but for birth-date-based policies.

As might be expected, the effect of assuming the screening test is sensitive to detecting infection earlier in the incubation period is to reduce estimates of ongoing exposure

under all the policy options explored. Exposure estimates approximately halve moving from sensitivity profile 1 to profile 3, though the effect of making more optimistic assumptions about test sensitivity is substantially greater for the more pessimistic scenarios regarding differential mortality. A 5-fold drop in exposure is seen assuming sensitivity profile 3 and differential mortality over the last 12 months, due to the much larger proportion of infected animals dying via differential mortality being detected in testing under the more optimistic screening scenario.

The above results assume ongoing BSE infection incidence in cattle remains constant from 1999 for the indefinite future, which is why the model outputs shown in Figures 9-10 tend to constant levels towards the end of this decade. One can also make the more optimistic that introduction of common controls on use of mammalian protein in mammalian feed in the EU in 2001 would have steadily reduced any residual risk of infection of GB cattle through cross-contamination after that time. We therefore examine the effect of assuming that the infection risk in cattle declines from 2001 onwards (halving each year). Figure 10 presents results for the baseline scenario, showing that exposure peaks in 2004 but declines thereafter. Similar trends are seen for all the scenarios of test sensitivity and differential mortality shown previously, and when risk is represented in terms of bovine ID50 infectivity units entering the food supply.

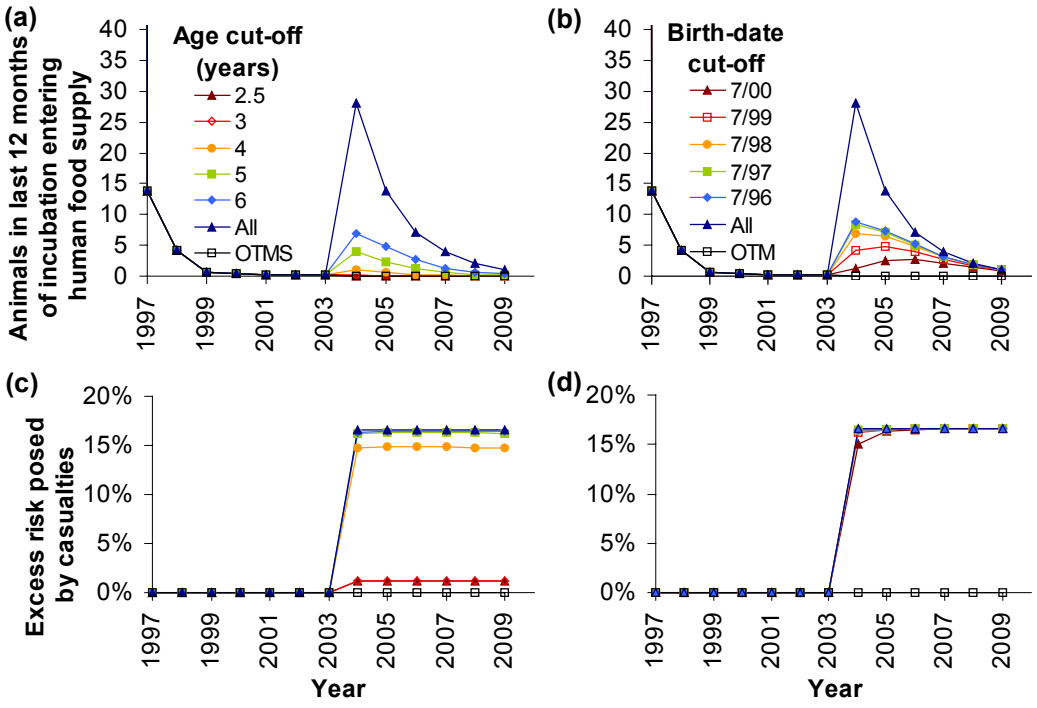

Figure 10 As Figure 9, but assuming BSE infection risk in cattle halves each year from 2001 onwards.

Electronic Appendix D. Size of the epidemic and model fit

Previous analyses of clinical case incidence data and data on apparently healthy animals screened in 1999 and 2000 yielded estimates, from the better fitting differential mortality models, of between 2 and 2.5 million infections over the course of the epidemic (Donnelly *et al*. 2002). This was a substantial increase over the 1 million infections estimated assuming no differential mortality and complete case reporting since 1988 (Anderson *et al*. 1996; Ferguson *et al*. 1997; Donnelly *et al*. 2002). Assuming that all differential mortality occurs in the last 3 months of the incubation period and sensitivity profile 1 (the baseline scenario), the estimated number of infections increased to 4 million (Figure 11). This is due to the high prevalence of infection in casualty and fallen stock animals and the assumption that the patterns of differential mortality were constant throughout the epidemic.

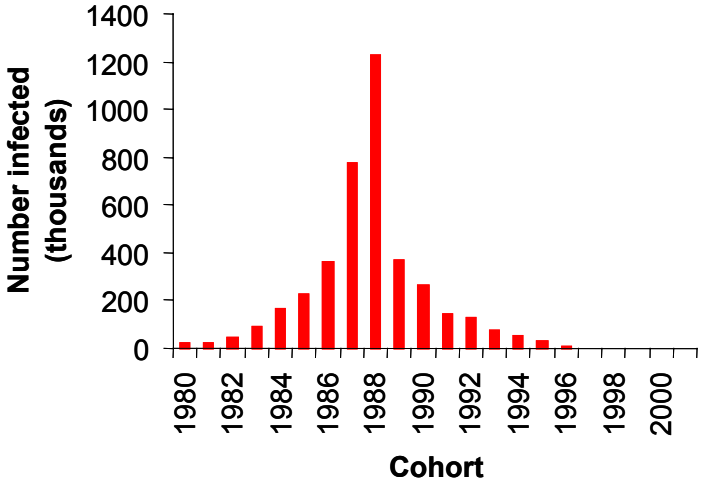

Figure 11 Infection incidence by birth cohort for the baseline scenario.

For the scenarios assuming differential mortality is distributed over a longer period than the last 3 month of the incubation period, estimates of the total scale of the BSE epidemic are correspondingly larger. Unfortunately, it is unlikely that any additional historical data will ever become available to show whether patterns of case-reporting and/or differential mortality varied through time.

For completeness, Table 4 shows estimates of BSE epidemic size, underascertainment parameters and predicted number of detected test positives in apparently healthy animals in 2004 assuming complete removal of the OTM rule, for the 12 parameter scenarios explored in Table 1 in the main text.

Table 4. Estimated under ascertainment and differential mortality risk parameters, total BSE epidemic size and expected number of test positives in apparently healthy cattle in 2004. 95% confidence bounds are shown in parentheses. Results are shown for the 12 scenarios used in Table 1 of the main text.

Sensitivity profile [see Figure 3(a) of main text]	Months of differential mortality	*Level of clinical case under-ascertainment (= proportion of infected animals subject to excess mortality prior to onset of disease symptoms)*	*Factor increase in risk of dying on farm for infected animals suffering excess mortality*	*Total number of animals infected during BSE epidemic (millions)*	*Expected number of test positives in apparently healthy animals in 2004, assuming complete removal of OTM rule and testing of all OTM animals slaughtered*
1	3	71% (70%,72%)	2.6 (1.8,3.4)	4.0 (3.6, 4.5)	55 (40,200)
1	6	82% (81%, 85%)	2.6 (1.8, 3.4)	6.5 (6.0, 7.3)	51 (38, 200)
1	9	87% (86%, 89%)	2.7 (1.9, 3.5)	8.6 (7.8, 9.7)	46 (35, 180)
1	12	89% (88%, 90%)	2.8 (2.0, 3.6)	10.4 (9.5, 11.6)	40 (30, 160)
2	3	70% (68%, 70%)	2.7 (1.9, 3.5)	3.7 (3.4, 4.2)	56 (42, 210)
2	6	72% (71%, 73%)	2.7 (1.9, 3.5)	4.1 (3.8, 4.6)	54 (42, 190)
2	9	79% (78%, 80%)	2.7 (1.9, 3.6)	5.4 (5.0, 6.0)	52 (41, 180)
2	12	83% (82%, 84%)	2.7 (19., 3.6)	6.8 (6.2, 7.5)	50 (40, 170)
3	3	68% (67%, 69%)	2.8 (2.0, 3.8)	3.6 (3.3, 4.0)	57 (44, 210)
3	6	70% (69%, 71%)	2.8 (2.0, 3.7)	3.8 (3.5, 4.2)	54 (42, 180)
3	9	74% (73%, 75%)	2.8 (2.0, 3.7)	4.4 (4.0, 4.9)	52 (41, 170)
3	12	78% (77%, 79%)	2.8 (2.0, 3.7)	5.3 (4.8, 5.9)	51 (40, 160)

The fit of the model to the screening data is quite good allowing for expected Poisson variability in the number of positives that would be detected (see Figure 12 for fit assuming that all differential mortality occurs in the last 3 months of the incubation period and sensitivity profile 1). The quality of fit of the model to clinical case data is nearly identical to that shown in Donnelly *et al*. 2002, though the model under-estimates clinical case numbers in 2001 and 2002 by 10-20% (see comments in section A1 of this electronic supplement for possible explanations for this).

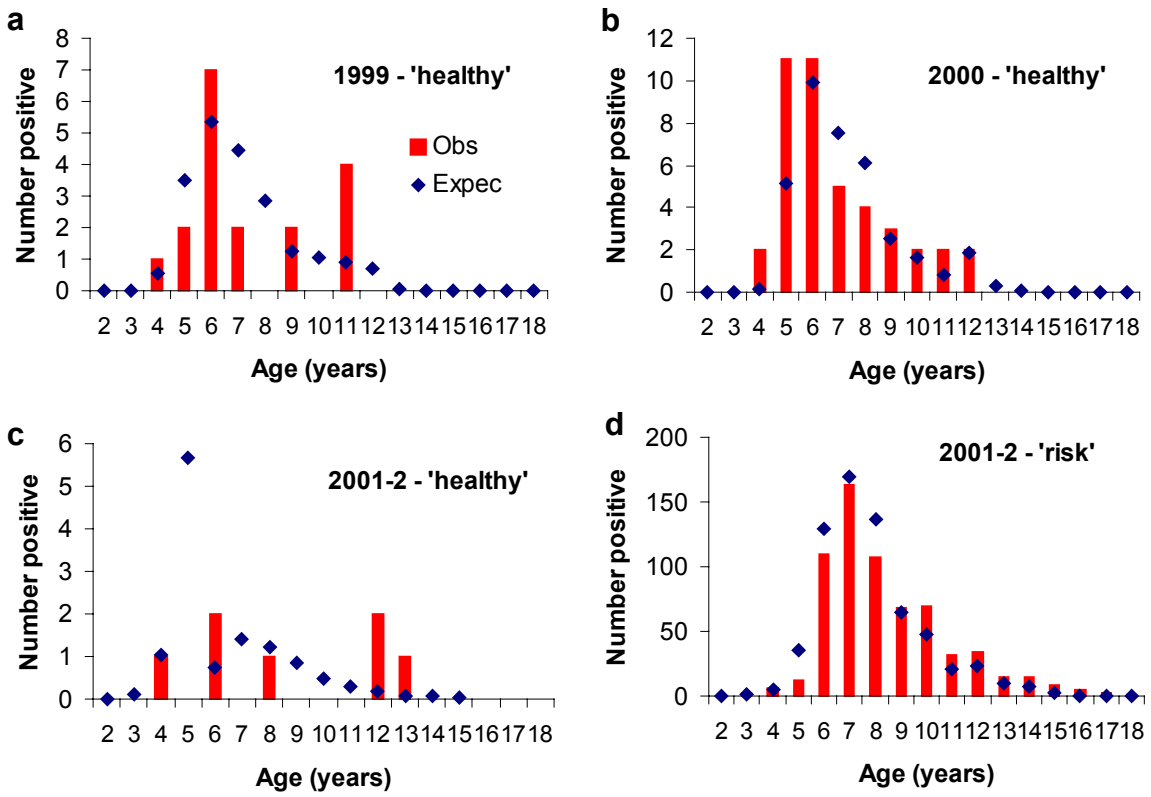

Figure 12 The observed (red) and expected (blue) number of detected positive animals by year of testing and risk category (apparently healthy or risk animals which includes both casual and fallen stock animals): (a) 1999, healthy animals; (b) 2000, healthy animals; (c) 2001-2, healthy animals; (d) 2001-2, risk animals. Expected values were obtained assuming that all differential mortality occurs in the last 3 months of the incubation period and sensitivity profile 1.

Bibliography

Citations in this document refer to the bibliography in the main text.