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Assessment of the prevalence of vCJD through testing tonsils and appendices for abnormal prion protein

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The objective of this study was to determine the age group or groups which will provide the most information on the potential size of the vCJD epidemic in Great Britain via the sampling of tonsil and appendix material to detect the presence of abnormal prion protein (PrP^{Sc}). A subsidiary aim was to determine the degree to which such an anonymous age-stratified testing programme will reduce current uncertainties in the size of the epidemic in future years. A cohort- and time-stratified model was used to generate epidemic scenarios consistent with the observed vCJD case incidence. These scenarios, together with data on the age distribution of tonsillectomies and appendectomies, were used to evaluate the optimal age group and calendar time for undertaking testing and to calculate the range of epidemic sizes consistent with different outcomes. The analyses suggested that the optimal five-year age group to test is 25–29 years, although a random sample of appendix tissue from all age groups is nearly as informative. A random sample of tonsil tissue from all age groups is less informative, but the information content is improved if sampling is restricted to tissues removed from those over ten years of age. Based on the assumption that the test is able to detect infection in the last 75% of the incubation period, zero detected infections in an initial random sample of 1000 tissues would suggest that the epidemic will be less than 870 000 cases. If infections are detected, then the model prediction suggests that both relatively small epidemics (800+ cases if one is detected or 8300+ if two are detected) and larger epidemics (21 000+ cases if three or more are detected) are possible. It was concluded that testing will be most informative if undertaken using appendix tissues or tonsil tissues removed from those over ten years of age. Large epidemics can only be excluded if a small number of infections are detected and the test is able to detect infection early in the incubation period.

Keywords: vCJD; tonsils; appendices; prion protein

1. INTRODUCTION

By the end of August 1999, 46 deaths from new variant Creutzfeldt–Jakob disease (vCJD) had been reported. Infection and the associated disease are believed to have arisen via the consumption of tissues from cattle infected with the aetiological agent of bovine spongiform encephalopathy (BSE) (Collinge *et al*. 1996; Will *et al*. 1996; Bruce *et al*. 1997; Hill *et al*. 1997). Predicting the size of any future epidemic of vCJD remains difficult because of the many gaps in our knowledge of the key biological and epidemiological processes determining the typical course of infection and transmission. In particular, uncertainty about the incubation period distribution, which could conceivably have a median length of 15–25 years or longer, means that the current time-series of cases could be consistent with epidemics ranging from less than 100 to several million cases (Ghani *et al*. 1998*a*).

To improve the understanding of the potential scale of the epidemic, the Department of Health and the Medical Research Council have recently supported two projects assessing the prevalence of infection with the aetiological agent of vCJD in Great Britain. The projects are based on plans to test tonsil and appendix tissue anonymously for the presence of the abnormal form of the prion protein (PrP^{Sc}) which is believed to be the aetiological agent of vCJD. Recent research suggests that the abnormal form can be detected in certain tissues, such as tonsils and appendices, prior to the onset of clinical symptoms (Hilton *et al*. 1998; Schreuder *et al*. 1998; Hill *et al*. 1999). Such information on the prevalence of incubating disease can then be used to reduce the current uncertainty in future epidemic size and provide a basis for health-care planning in a manner akin to the unlinked anonymous testing programme for HIV-1 which was initiated in the UK in the 1980s.

The interpretation of the results from these studies will be complicated by two factors. First, it is not known at what stage of the incubation period infection will be detectable by current immunochemical tests. The detection of PrP^{Sc} in the appendix of one vCJD patient three years before death demonstrated its presence late in the incubation period (Hilton *et al*. 1998) whilst experimental studies on scrapie in sheep have demonstrated its presence much earlier in the incubation period (Schreuder *et al*. 1998). Second, most cases have been confirmed in 20–40 year olds (figure 1*a*) and, hence, the detectable prevalence may be clustered in certain age groups. The majority of both tonsillectomies and appendectomies are undertaken on children and young adults (figure 1*b*). Interpretation of results must ideally be based on the age-specific prevalences of infection with the aetiological agent of vCJD and not simply rely on a single point estimate of prevalence in the population as a whole.

We employed a time- and cohort-stratified model of the transmission dynamics of vCJD to simulate possible

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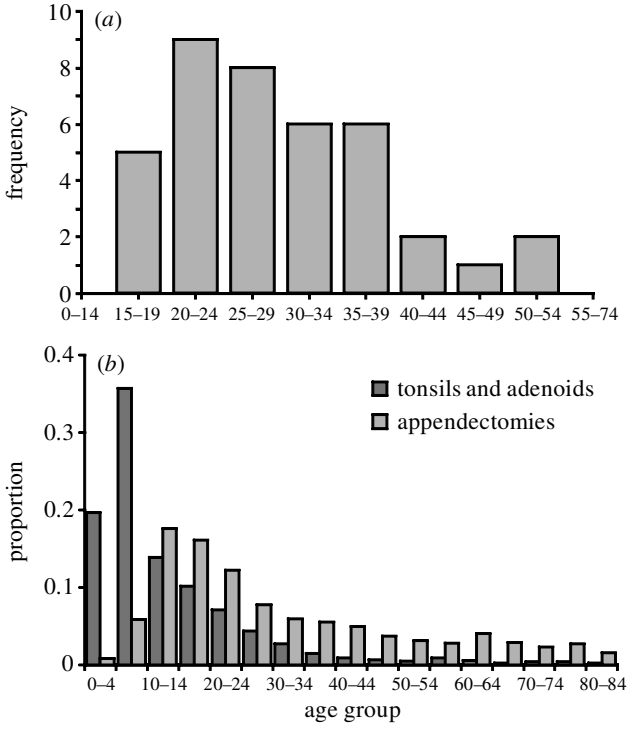

Figure 1. (*a*) The age at death of the 39 cases of vCJD reported to the end of 1998 (R. Will, personal communication). (*b*) The number of tonsillectomies ($n = 7096$) and appendectomies ($n = 4049$) in each age group as a proportion of all undertaken in 1993 in the Oxford region (taken from the Oxford Record Linkage Study; Newton *et al.* 1994). A steady decline in the number of tonsillectomies has occurred over the past three decades with this decrease being most notable in young children (five to nine years). Similarly, a significant decline in operation rates for acute and incidental appendectomies has occurred over time with the greatest decline in those aged under ten years (Primatesta & Goldacre 1994).

future epidemic scenarios, ensuring that they were consistent with the time-series and age distribution of vCJD cases seen to date. We used these scenarios to assess the optimal age group to test for asymptomatic infection and compared this to testing a random sample of tissues, taking into account the present age distribution of tonsillectomies and appendectomies in Great Britain. These results provide a statistical template for the design of unlinked anonymous testing programmes and also provide estimates of the degree to which the results obtained from these testing programmes will reduce the current uncertainty in epidemic size.

2. METHODS

A time- and cohort-stratified mathematical model of the transmission dynamics of vCJD was used to generate epidemic scenarios. The model relates the risk from the consumption of tissues from BSE-infected animals to the observed time-series and age distribution of the 39 cases to the end of 1998 (see Appendix A; Ghani *et al.* 1998*a*). A wide range of assumptions regarding the infectivity of the animals at different stages of their incubation period, the effectiveness of control measures at preventing infectious material entering the human food supply, the effect of the species barrier and the vCJD incubation period distribution were explored. Approximately 100 000 simulations were performed in sensitivity analyses designed to address these uncertainties. As the cases to date have only been in individuals who are methionine homozygous at codon 129 of the prion gene—*ca.* 40% of the population (Owen *et al.* 1990; Collinge *et al.* 1991)—we scaled our analysis to this section of the population. Those who are not methionine homozygous at this codon may also be susceptible to the disease (perhaps with longer incubation periods), but we did not consider this possibility in the present study. Two mechanisms which could give rise to the observed age distribution of cases were explored. Age-dependent susceptibility exposure to infection was examined, assuming either that only individuals within a given age range were susceptible–exposed (a uniform susceptibility–exposure function) or that age-dependent susceptibility–exposure followed a normal distribution (left-truncated at zero age). Age dependency in the length of the incubation period was also explored separately by using an inverse logistic function to lengthen the incubation period in older individuals (see Appendix A).

The detectable prevalence of infection in five-year age groups (zero to four years, five to nine years, ten to 14 years, etc.) was calculated at different points in calendar time for each epidemic scenario. In these calculations it was assumed that (i) the tests are able to detect infection throughout the full incubation period, (ii) they are only able to detect infection in the last 75% of the incubation period and (iii) they are only able to detect infection in the last 50% of the incubation period. We assumed that the tests in tonsil and appendix tissue are 100% sensitive and specific in these periods.

Stepwise linear regression (on log-transformed data) was used to identify the age groups in which the prevalence is most accurately able to predict epidemic size. This regression was repeated for different calendar times.

We assumed that the samples tested were a random selection of the tonsil or appendix tissues available in a given age group. The sample sizes were scaled by 40% to reflect the restriction of the model to the methionine homozygous population. The exact binomial 95% confidence interval for the prevalence of infection was calculated for each possible outcome (zero, one, two, etc. infections detected) and sample size. Model scenarios were selected if the prevalence in the tested age group at the calendar time of testing was within these bounds. The age distribution of tonsillectomies or appendectomies in the Oxford region (figure 1*b*) was used to weight the prevalence of infection in model scenarios in which more than one five-year age group was tested. Data were available up to 1993 and, thus, the prevalences in later years were weighted using the 1993 distributions.

3. RESULTS

(a) *Uncertainty in future epidemic size without unlinked anonymous testing*

At present it is still not possible to predict the future size of the vCJD epidemic with any degree of certainty—possible epidemic sizes range from around 50 cases to several million. Table 1 shows the upper and lower bounds of epidemic size stratified by the number of cases in 1999 and 1999–2000. Table 1 also includes information on what is assumed concerning the average number of humans infected by one maximally infectious bovine (a composite parameter which incorporates the relative infectiousness of different tissues, their infectivity to humans and the

Table 1. *Range of vCJD epidemic sizes stratified by the number of deaths in 1999 and in 1999–2000 and by the mean number of individuals infected by one maximally infectious bovine* (r)

(Scenarios were accepted if the mean number of cases was consistent at the 95% level with the marginal age and time distributions of the 39 vCJD cases to the end of 1998 (see Appendix A). The results shown are based on 40 stochastic realizations for each parameter combination. The ranges were obtained assuming that infectiousness peaked in the last six months of the bovine incubation period.)

	cases in 1999			cases 1999–2000		
r	4–14	15–29	30–49	10–29	30–49	50–69
0–0.01	52–3300	56–4300	70–4400	52–1700	69–3300	89–4100
0.01–0.1	720–26000	690–27000	960–38000	690–6700	710–22000	880–26000
0.1–1.0	4500–62000	4300–323000	4500–323000	6000–14000	4300–87000	4300–322000
1–10	18000–247000	18000–2.0×10^6	23000–2.0×10^6	—	18000–247000	18000–381000
10–100	82000–496000	82000–2.9×10^6	150000–6.1×10^6	—	82000–497000	82000–2.1×10^6
100+	—	325000–4.4×10^6	324000–6.1×10^6	—	—	235000–2.1×10^6

average number of susceptible individuals who will consume one carcass). By the beginning of 2000, the possibility of very large epidemics will be eliminated if a small number of cases (less than 15) are observed in the current year. If less than 30 cases arise in the next two years then the epidemic will almost certainly be small (less than 14 000 cases).

(b) *Age-dependent incidence of vCJD*

The age distribution of the 39 vCJD cases reported to the end of 1998 peaked in those between 20 and 40 years of age (figure 1*a*) and could not have arisen without some form of age dependency in the transmission process or incubation period. Figure 2*a*,*b* shows the range of susceptibility–exposure functions which are able to produce this distribution, with young children and teenagers being either more susceptible and/or exposed to a greater extent than adults. Figure 2*c* shows the scaling of the incubation period with age required to match the current reported age distribution of vCJD cases for an age-dependent incubation period. Relatively small increases in the length of the incubation period in those aged *ca.* 35 years or more at the time of infection are able to reproduce the age distribution of cases observed to date accurately. Equally, a more dramatic increase in the incubation period in those aged over 35 years (with the length of the incubation period increasing up to four times that in children and young adults) is also consistent with the observed age distribution.

(c) *Age-targeted testing*

Under the scenario of age-dependent susceptibility–exposure, if the test is able to detect infection in the last 75% of the incubation period, the optimal age group to test is always tissues taken from 25–29 year olds (figure 3*a*). If the test is only able to detect infection in the last 50% of the incubation period, then an older age group (30–34 or 35–39 years) may be optimal (figure 3*b*). Under the scenario of an age-dependent incubation period, the optimal age group is predicted to be slightly older (35–39 years).

A random sample of appendix tissues is predicted to be as good as restricting testing to the single optimal five-year age group of 25–29 years of age (figure 3). This is due to the spread of the material removed in Great Britain at present across the most informative age groups (those aged *ca.* ten to 50 years) (figure 1*b*). A random sample of tonsil tissue is always slightly less informative, since *ca.* 50% of tonsillectomies are undertaken in children under ten years of age. These children will have been less exposed to infection. Restricting the tonsil sample to those over ten years of age does improve the correlation between the sample prevalence and epidemic size, but is still less informative than a random sample of appendix tissue. This is because the distribution of tonsillectomies is more strongly peaked in the young, with less than 10% of tonsillectomies undertaken in those over 40 years of age.

Independent of the age group chosen, the optimal tissues to test are those removed as close to the present time as possible. Prospective testing will provide a similar picture to testing tissues removed recently.

(d) *Ranges of epidemics consistent with the observed prevalence*

If no infections are detected in an initial sample of 1000 randomly selected tissues, then the most conservative approach is to assume that the test is only able to detect infection late in the incubation period. For either tonsil or appendix tissues, this result is then consistent with mean epidemic sizes of between 53 and approximately seven million cases. If one or more infections are detected in the sample, then the most conservative approach is to assume that the test is able to detect infection throughout the incubation period. This will provide an estimate of the lower bound on epidemic size. To estimate the upper bound it is necessary to assume that the test is only able to detect infection late in the incubation period. The range of total epidemic sizes is shown in table 2 for a random sample of the available tonsil and appendix tissues.

The difference between the results for tonsil and appendix tissues illustrates the importance of the age distribution of the sampled tissues in determining estimated epidemic sizes consistent with the outcome from unlinked anonymous testing programmes. Appendix tissues are predicted to be more informative (and, hence, the range of predicted epidemic sizes narrower) than

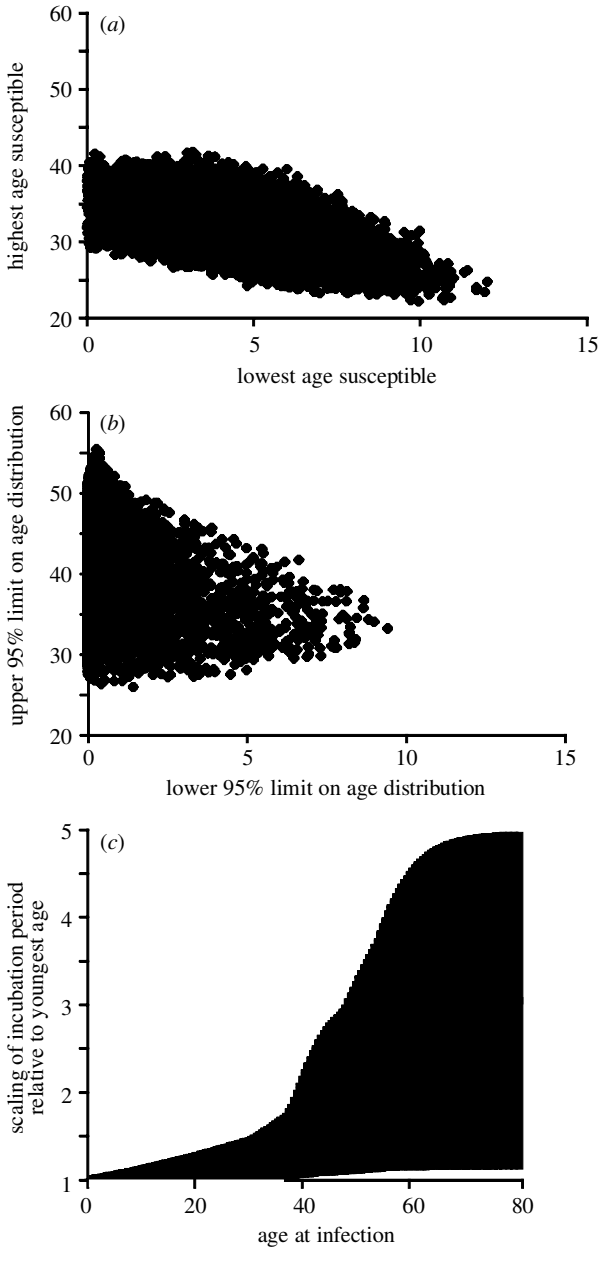

Figure 2. The range of age-dependent susceptibility–exposure distributions consistent with the 39 cases to the end of 1998. (*a*) Lowest and highest ages susceptible if susceptibility–exposure is limited to this age range. Each point represents a scenario consistent with the current case data. (*b*) Ages of the lower and upper 95% limits of an age-truncated normal distribution for age-dependent susceptibility–exposure. Each point represents a scenario consistent with the current case data. (*c*) The range of increases in the incubation period length by age at infection compared to the youngest age group if the age distribution of cases arose from an age-dependent incubation period. A scaling of one indicates that the incubation period distribution is the same at that age as in young children. A scaling greater than one indicates that the incubation period is lengthened compared to young children. The shaded area shows the range of different scalings which are consistent with the case data.

tonsil tissues because of the age distribution of the individuals from whom they have been removed. The predicted epidemic sizes for a given number of infections detected are also smaller than those based on tonsil material, reflecting the wider age distribution of the appendix samples. A key requirement in interpreting the results from the testing programmes will therefore be knowledge of the precise age of the patients from whom tissues have been sampled.

Table 2. *The range of epidemic sizes consistent with detecting x infections in a random sample of tonsil tissues and a random sample of appendix tissues*

no. of infections detected in a random sample of 1000 tissues from all age groups	range of epidemic sizes assuming infection detectable in the last 75% of the incubation period	conservative range of epidemic sizes
tonsils		
0	58–870000	$58–3.1\times10^7$
1	$1200–1.4\times10^6$	$1000–3.1\times10^7$
2	$11000–1.4\times10^6$	$9200–3.1\times10^7$
3	$37000–1.7\times10^6$	$25000–3.1\times10^7$
4	$84000–1.9\times10^6$	$42000–3.1\times10^7$
5	$111000–1.9\times10^6$	$54000–3.1\times10^7$
10	$350000–3.5\times10^6$	$177000–3.1\times10^7$
20	$868000–5.7\times10^6$	$477000–3.1\times10^7$
appendices		
0	58–349000	$58–3.1\times10^7$
1	810–600000	$810–3.1\times10^7$
2	8300–706000	$6900–3.1\times10^7$
3	$21000–1.0\times10^6$	$17000–3.1\times10^7$
4	$44000–1.4\times10^6$	$32000–3.1\times10^7$
5	$70000–1.4\times10^6$	$44000–3.1\times10^7$
10	$198000–1.6\times10^6$	$131000–3.1\times10^7$
20	$494000–2.9\times10^6$	$330000–3.1\times10^7$

Large sample sizes are required to estimate a small prevalence accurately. If the sample sizes are too small then no infections will be detected, but this result may still be consistent with relatively large epidemics (Ghani *et al.* 1998*b*). Table 3 indicates the range of epidemic sizes consistent with different results by increasing the sample size, given that random samples were taken from tonsil and appendix tissues removed in 1998. Clearly, the larger the sample size the less uncertain the future epidemic size will be. However, studies will inevitably be limited by the number of samples available (*ca.* 800 000 tonsil and 45 000 appendix tissues are removed each year), the resources available and the costs of large-scale testing programmes. Thus, whilst restricting samples to the most informative age groups is optimal in terms of the information gained per sample tested, the design of large-scale studies must also take into consideration the availability of samples.

4. DISCUSSION

Assuming no differences in the ability of the currently available tests to detect infection in either tonsil or appendix tissues, unlinked anonymous testing will be most informative if undertaken on random samples of

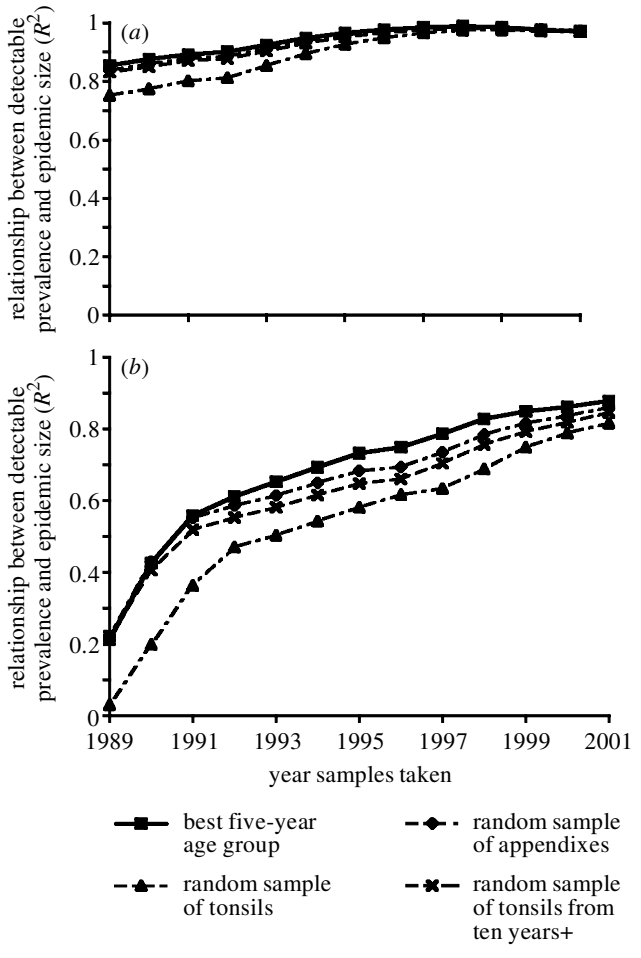

Figure 3. The relationship between detectable prevalence and epidemic size (summarized by least-squares R^2) for the optimal five-year age group, a random sample of tonsils, a random sample of tonsils from those aged ten years or more and a random sample of appendix tissue. It is assumed (*a*) that infection is detectable in the last 75% of the incubation period, and (*b*) that infection is detectable in the last 50% of the incubation period. The results for age-dependent susceptibility–exposure are shown—similar patterns are observed for an age-dependent incubation period.

appendix tissues removed recently from individuals. This is because the age distribution of appendectomies is such that *ca*. 75% of these tissues will have been removed from those aged between ten and 50 years. The strongly peaked age distribution observed in the cases seen to date can only have arisen if young individuals were either more susceptible to infection, were exposed to a greater extent or have shorter incubation periods. In the latter case, even if the prevalence in the population as a whole is uniform, younger individuals are more likely to be in the later stages of the incubation period and, hence, more likely to have detectable abnormal prion protein. In the former cases, we can expect a higher prevalence of infection in the young. However, because the risk of infection decreased in the early 1990s with the introduction of the specified bovine offal ban and the decline in the BSE epidemic in cattle, the prevalence of infection in young children (under ten years of age) can be expected to be somewhat lower. Thus, testing a random sample of tonsil tissue, whilst still informative, is less so than for appendices because nearly 50% of tonsil samples are removed in children aged under ten years.

If we assume that the tests are able to detect abnormal prion protein for the last 75% of the incubation period and are 100% sensitive in this period, then the results from an initial sample of 1000 tissues can be informative about future epidemic size. However, it is important to note that even a relatively high prevalence (one or two infections detected) may be consistent with relatively small epidemics (minimums of 810 and 8300 cases, respectively). Small epidemics could arise if the nature of the age dependency is such that only a narrow range of age groups were susceptible and/or exposed, so that the lower 95% confidence bound on the estimated prevalence relates only to a small cohort of individuals. Particular caution must be exercised in interpreting a null result as this may simply indicate that the tests are unable to detect infection early in the incubation period. Thus, the results from unlinked anonymous testing will only be able to exclude the possibility of large epidemics if a very large number of tissue samples are tested or if we can be certain that the tests are highly sensitive early in the incubation period.

Information on the ages of the patients from which samples are taken is essential in interpreting the findings and, hence, narrowing the range of possible future epidemic sizes. Furthermore, if incubating infections are detected, such information may help in understanding the age-dependent mechanisms which have resulted in the observed age distribution of the 46 vCJD cases confirmed by August 1999. However, in undertaking testing for an invariably fatal disease, we must also be certain that the testing programmes are truly unlinked and anonymous, such that the presentation of the results does not cause concern amongst those individuals who may have been tested. Thus, a delicate balance must be reached, whereby sufficient information (e.g. accurate age) is available for interpreting the results, whilst ensuring everything possible is done to protect anonymity. It may be sensible to record five-year age bands to ensure some information on age is acquired whilst not threatening the requirement of anonymity, although this is less informative than information on the precise age of the participants.

Even if unlinked anonymous testing is not implemented, it should be possible to narrow the range of possible epidemic sizes over the coming two-year period on the basis of the reported vCJD cases. If less than 15 cases are confirmed in 1999 then, assuming that vCJD affects only methionine homozygotes (*ca*. 40% of the population), the maximum epidemic size is reduced to *ca*. 500 000 cases. If less than 30 cases are confirmed in the next two years, then the maximum epidemic size is reduced to approximately 14 000 cases.

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Table 3. *The range of epidemic sizes consistent with detecting x infections in various sample sizes of tonsil tissue and appendix tissue*

(It is assumed that tissues are randomly sampled and that the test is able to detect infection in the last 75% of the incubation period. Substantially wider bounds are obtained if the latter assumption is relaxed.)

	sample size			
infections detected	2000	5000	10 000	20 000
tonsils				
0	58–350 000	58–163 000	58–90 000	58–45 000
1	620–600 000	290–295 000	180–136 000	110–69 000
2	5100–730 000	1960–320 000	1100–154 000	620–90 000
3	18 000–1.0×10^6	5100–342 000	2500–217 000	1400–104 000
4	33 000–1.4×10^6	11 000–526 000	4800–220 000	2300–136 000
5	46 000–1.4×10^6	17 800–600 000	7500–321 000	3100–154 000
10	144 000–1.7×10^6	46 000–870 000	26 000–349 000	11 000–220 000
20	520 000–2.9×10^6	144 000–1.4×10^6	86 000–600 000	37 000–333 000
appendices				
0	58–217 000	58–70 000	58–32 000	58–23 000
1	440–321 000	210–125 000	130–56 000	91–26 000
2	3500–348 000	1500–154 000	813–70 000	444–32 000
3	10 000–464 000	3800–192 000	1900–97 000	978–45 000
4	18 000–600 000	7300–217 000	3400–110 000	1600–56 000
5	28 000–600 000	11 000–220 000	5100–136 000	2500–56 000
10	86 000–1.0×10^6	37 000–348 000	18 000–217 000	7500–97 000
20	275 000–1.6×10^6	96 000–600 000	46 000–333 000	21 000–157 000

APPENDIX A

The probability that an individual develops clinical disease at time u and age a is

$$p(u,a) = S(u,a)\int_{u-a}^{u} f(u-t,a-u+t)I(t,a-u+t) \times \exp\left[-\int_0^t I(t',a-u+t')\mathrm{d}t'\right]\mathrm{d}t, \quad \text{(A1)}$$

where $S(u,a)$ is the survival probability, $f(u,a)$ is the incubation period distribution, and

$$I(t,a') = \nu(t)\beta g(a')\int \Omega(z)w(z,t)\mathrm{d}z \quad \text{(A2)}$$

is the infection hazard. $\nu(t)$ is the effectiveness of control measures, β is the transmission coefficient, $g(a)$ is the age-dependent susceptibility–exposure, $\Omega(z)$ is the relative infectiousness of bovines at time z prior to disease onset and $w(z,t)$ is the proportion of cattle slaughtered at time t and time z away from disease onset.

Estimates of the proportion of cattle slaughtered by time and disease onset ($w(z,t)$) were obtained from back-calculation models of BSE (Anderson *et al*. 1996). The infectivity of bovine tissues ($\Omega(z)$) was assumed to rise exponentially during the course of the incubation period from some baseline level, consistent with currently available data on transmissible spongiform encephalopathies (TSE) pathogenesis (Fraser *et al*. 1992; Ministry of Agriculture, Fisheries and Food 1996; Wells *et al*. 1998). By varying the two parameters (the baseline level and exponential rate of increase) a wide variety of distributions were explored. The effectiveness of the specified bovine offal ban in July 1988 ($\nu(t)$) was assumed to be anywhere between 0 and 100% effective, with $\nu(t) = 1$ prior to this date.

A modified form of the four-parameter generalized lambda distribution (Ramberg *et al*. 1979), which encompasses the frequently used survival distributions (gamma, Weibull and lognormal), was used to explore incubation period distributions, with the mean length ranging from nine to 90 years (Ghani *et al*. 1998*a*). Since many of the incubation period distributions for other TSEs (including BSE) have minimum lengths (Ghani *et al*. 1998*a*), the possibility of an initial delay was included as an extra parameter in the distribution.

Two forms were explored for the age-dependent susceptibility–exposure—a uniform distribution and a normal distribution truncated at zero. The age-dependent incubation period was modelled using the scalings $f(u,a) = h(u)/s(a)$, where $h(u)$ is an age-independent incubation period and $s(a) = \{a_2\exp(-a_3 a) + a_1\}/\{\exp(-a_3 a) + a_1\}$, where a_1, a_2 and a_3 are parameters. As the age-dependent susceptibility–exposure cannot at present be distinguished from an age-dependent incubation period, the two forms were explored separately so that any differences in the results could be assessed.

The parameter values were explored using extensive Latin hypercube sampling of the multidimensional parameter space (McKay *et al*. 1979; Stein 1987), with the parameters sampled on either a linear or log scale and with the range of parameters determined by the acceptancy criteria. Scenarios were accepted if both the age- and time-structured marginal case distributions were consistent at the 95% level with the observed marginal case distributions. Consistency was judged by the distribution of Poisson likelihood deviances (G^2) obtained from parametric bootstrap sampling of the case data (Efron & Tibshirani 1993). Approximately 100 000 parameter sets were explored using multiple Latin hypercubes, with 15 000 accepted scenarios used in the analysis. To ensure that the results were not dependent on sampling of the

parameter space, additional runs using different random number seeds were performed. Further details of the parameter ranges accepted are given in Ghani *et al.* (1998*a*).

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As this paper exceeds the maximum length normally permitted, the authors have agreed to contribute to production costs.