Review of the Evidence for the Occurrence of 'BARB' BSE Cases in Cattle

I was engaged as an independent consultant by the Animal Health and Welfare Directorate General of Defra in November 2004 with the following remit:

1. To examine the relevant data pertaining to cases of BSE born after the reinforced feeding ban in August 1996 in the UK. (BARBs)
2. To discuss with any appropriate party the background and hypotheses that might underlie the BARBs cases.
3. To consider the available evidence and opinion in the context of the scientific evidence based on the aetiology and transmission of TSEs.
4. To provide written recommendations to the Animal Health and Welfare Directorate General of Defra on:

    4.1 The possible explanations for BARB cases and assessment of whether the approaches being used by Defra to investigate BARBs are robust and sensibly prioritised.

    4.2 The existence of further risk factors that should be investigated and if so suggestion of the means by which they might be explored.

To discuss recommendations with the Chief Veterinary Officer or other senior Defra officials as required.

My report follows.

William G. Hill
Edinburgh, 5 July 2005

Summary of Conclusions and of Recommendations (R:)

Background (paras. 1-5)
a) BSE cases in animals born after the reinforced feed ban was applied in the U.K. in 1996 are still being identified, most by active surveillance. The total now exceeds 100.
b) Defra have made frequent and helpful summaries and analyses of the course of the BSE epidemic, and specifically of BARBs cases, and made updated information freely available on their website. Epidemiological analyses of the BARBs cases have been regularly updated.
R: Making data available and regularly updating analyses should be continued.

Biological characteristics of BARBs cases (6-7)
a) There is no strong reason to believe that BSE in BARBs cases is a different disease from that in animals born before the reinforced feed ban.
b) The diagnostic tests used in active surveillance appear to be effective, but only when the animal is in the last few months of incubating the disease.
R: a) Obtaining hard evidence on the crucial hypothesis on the identity of BSE in BARBs and previous cases is highly desirable and the relevance of atypical molecular forms of BSE found by active surveillance in other countries needs to be resolved.
b) The efficacy and interpretation of the tests used in active surveillance of animals for BSE should be kept under review.

Epidemiology of BARBs cases (8-9)
The only major change in the epidemiology of BSE for animals born after 1996, in addition to the fall in incidence, is that the geographical distribution no longer shows the association with density of non-ruminant livestock.
Although incidence has fallen such that no cases have yet been detected in the 2000/1 cohort, affected animals born in 2001/2 have been detected.

Nature of the disease (10-13)
The profile of BSE is that of a classic transmissible spongiform encephalopathy. Although not fully elucidated, prion protein is heavily supported by scientific evidence to have a central role in the aetiology, transmission and diagnosis of the disease. There is no evidence that BARB cases differ from previous cases in this respect. Other hypotheses as to the nature and causes of BSE do not appear tenable; furthermore they do not specifically relate to BARBs cases.
R: Unless there is new evidence on BSE in cattle that lends support to alternative hypotheses underlying the cause of BARB cases there continues to be little justification for Defra to pursue research on them. Monitoring hypotheses and the free exchange of ideas is, however, encouraged.

Spontaneous occurrence (14-16)
The evidence from the absence of BSE in many countries and the surveillance schemes abroad indicates that most BARBs cases cannot have arisen spontaneously, although the possibility cannot be excluded that a very few of them did so. The possibility of a very low frequency of spontaneous occurrence of BSE may be monitored from the output of surveillance in cattle populations elsewhere.
R: A watching brief is kept on surveillance efforts world-wide.

Genetic variation in susceptibility (17-21)

a) Previous statistical and molecular genetic studies indicate there is little genetic variation of cattle associated with susceptibility to BSE.

b) Preliminary information from the GB analysis and detailed information from the NI analyses of DNA sequence data on BARBs cases and controls as yet show no clear associations, with no genotype exclusively associated with BARBs cases whether acquired by infection or arising spontaneously.

R: a) The analysis of the GB data should be completed and arrangements sought to combine them with the NI data.

b) If the results of the current analyses are confirmed as negative, it is unnecessary to continue genotyping all but atypical BARBs cases.

c) Even if genetic differences in susceptibility to infection were revealed, removing the source of infection rather than genetic selection is the route of choice for disease control.

d) Defra should be better positioned to have quick access to DNA sequencing for TSE research.

Maternal and lateral transmission as a route of infection (23-26)

As for BSE infection before the reinforced feed ban, there is no evidence that either maternal or lateral transmission, directly or though a reservoir host, is a significant factor for BARBs cases. Although these routes cannot be excluded in individual cases, they do not appear to be capable of sustaining the epidemic in the face of current controls.

Environmental contamination and other non-feed borne sources of infection (27-30)

a) Whilst limited infection via environmental or other non-food borne contamination cannot be excluded, the likelihood is low given current knowledge on the behaviour of the disease in cattle and in the absence of evidence otherwise.

b) The persistency of the infective agent in the environment (e.g. on the land, in premises) is not known, but in any case is likely to depend on local factors.

c) Animal health products are a most unlikely source, but there are no controls on products not claiming medicinal value.

R: a) If feasible, it might be a useful long shot to consider prior cases in neighbouring farms as a route to checking environmental contamination.

b) In view of the need to eradicate the disease, consideration should be given to decontamination procedures on premises.

c) The analysis of environmental risk factors being undertaken in the FATEPriDe study should be facilitated.

Feed borne infection (31-34)

a) Recent unpublished experiments at the VLA have shown that feeding exceptionally low doses (0.001g) of infected neural tissue can cause BSE.

b) The working hypothesis of Defra that the major cause of BSE in BARBs cases has been through the ingestion of contaminated feed, most likely by young animals, is strongly supported. Thus control of the disease requires, as it has always required, completely eliminating the agent from the cattle feed chain.

c) Understanding causes of variation in infectivity are important in terms of understanding the disease, but do not particularly impinge on the control of BSE, where risks have to be avoided.

R: Defra continues to operate on the basis that BSE transmission via feed is the major route involved in BARB cases.

Control measures on feed (35-37)
The problem, if there is one, is not with the principle of the feed control regulations, but whether they have been and are being broken knowingly or unknowingly, through use of banned material or for example of feed that has remained in plants or on farms, e.g. in corners of feed bins.
R: a) The feed controls currently in place seem adequate but require vigilant enforcement. Defra should continue to review appropriate controls.
b) Efforts to obtain consistent quality of feed testing for animal derived material in all EU member states should be made.
c) In view of the likelihood that breaches of regulations have occurred and enabled BSE contaminated material to enter the cattle feed chain, Defra should help facilitate the recent recommendations of the Advisory Committee on Animal Feedingstuffs to ensure a more coordinated and risk-based programme of over-all animal feed law enforcement.

Potential feed borne sources of BARBs cases (38-43)
a) It may not be possible to attribute BARBs cases to any single source of feed contamination. Some feed may not have been fully cleared out on farms in 1996 in GB in 1996 and in other countries later. Imported feed seems a likely cause, certainly until controls were tightened up throughout Europe, but does not readily explain the random country-wide distribution in GB.
b) Firm conclusions cannot be drawn from the recent case-control study of BARBs.
R: a) No source of feed contamination should be ruled out and detailed investigations should be continued.
b) Some improvements in the design of the case-control study can be effected fairly straightforwardly and should be undertaken. A major effort to obtain feed records on control animals does not seem justified, if indeed it is feasible.

Inferences from changes in incidence (44-46)
a) There has been a fall in the underlying incidence of BSE by birth cohort 1996/97 to 99/00 in GB, but the 2001/2 case leaves doubt subsequently. There has also been a fall in other countries except where feed controls were introduced later.
b) Consequently risks of infection are in general falling and should progressively reduce the risks of contamination and cross contamination.
c) As cohorts of affected animals are tested, numbers of index cases rather than total numbers may give a better indication of progress of the epidemic.

General conclusions
Elimination of feed borne sources seems to be now, as before, the key to elimination of BSE. The incidence of the disease can be greatly reduced but not readily eliminated in any country by adequate imposition of controls, particularly on animal feed. As the level of incidence falls both in the UK and internationally, the risks of contamination through cattle feed, pet food, or indeed through any other source, fall whether or not controls in the UK and abroad are further tightened. With the current expertise in Defra and the VLA, GB is well placed to keep on top of and promote developments.
R: It is essential that appropriate, risk based, controls and monitoring should be maintained on animals and feed until no cases of BSE are found, and controls

tightened up where feasible, both in the UK and elsewhere that the UK can influence. In view of the very long incubation period of BSE in some animals, long-continued vigilance is necessary. It is not evident, however, that specific new measures are needed. Basically it is necessary to ‘keep taking the medicine’. Nevertheless, in view of new discoveries on the nature of the disease and the possibilities of new or changed TSEs arising, relevant research capacity in GB should be maintained.

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Background

1. Bovine Spongiform Encephalopathy (BSE) was first diagnosed in Great Britain (GB) in 1986. Following demographic analysis (Wilesmith *et al.*, 1988) and the report of the Southwood Committee (1989), the use of materials of ruminant origin was banned in feed for ruminants in mid 1988. Whilst a substantial fall in the incidence of new infections followed, it did not decline to zero and animals born well after 1988 were infected, the likely source of infection identified as cross contamination with feed for non-ruminants or incomplete compliance with the Order. Later, following the identification of vCJD in man, further steps were taken leading to the Reinforced Feed Ban in the UK, which came fully into effect from 1 August, 1996, and in which feeding of mammalian meat and bone meal to any farm animal was banned, and such feed already produced was recalled. Various reinforced checks on compliance were put in place. In December 2000 a ban on the feeding of processed animal proteins to farm animals kept for the production of food was implemented across the EU. Controls on feed and animals introduced over the years are summarised for reference in Appendices I and II.

2. Following introduction of the reinforced feed ban the evidence for food-borne transmission indicated that new infections of BSE infection should no longer occur. However cases of BSE born after 1 August 1996 when the reinforced feed ban was in force (BARBs) were found from May 2000 on. As of 31 May, 2005, 106 such cases had been confirmed in GB. The detailed statistics and analyses referred to in this report apply to 93 cases confirmed in GB by March 15, 2005 (Wilesmith *et al.*, 2005a,b). Numbers detected by years, July – June, were:

To 01	01/02	02/03	03/04	04/05	Total
3	14	35	28	13	93

Of these, 27 were detected as clinical cases (passive surveillance) and 66 by active surveillance (identified as casualty or slaughter animals, fallen stock, or in the Over Thirty Months scheme (OTMS)), which commenced in late 2001. The number of cases detected in the earlier years would likely have been higher had active surveillance then been in place; for example, the first 4 of these 93 but only 23 of the remaining 89 were clinical cases. Five cases (subsequent to the 93) have been identified recently by active surveillance in culls of cohorts of affected animals.

3. When classified by year of birth, August – July, the numbers of BARBs cases were:

96/97	97/98	98/99	99/00	00/01	01/02	Total
42	30	15	5	0	1	93

Not all the animals in these cohorts, particularly the most recent, may yet have developed the disease. These figures contrast with the much larger, but declining, number of cases of BSE detected in animals born before August 1996. Statistics on numbers affected by calendar years of birth in GB where known are as follows, with more detail given in Appendix IV:

92	93	94	95	96
3493	2960	2128	1059	62

These and other data are available from the Defra BSE Statistics web page, which is comprehensive and up-to-date.

4. Cases of animals with BSE born after the introduction of the UK feed ban have been found in Northern Ireland and after their respective feed bans in, for example, France (Savey *et al.*, 2000) and Ireland (Sheridan *et al.*, 2005) although, as in GB, a

major reduction in incidence followed the controls introduced. Some statistics are given in Appendix IV.

5. Reports on the epidemiology of BARBs cases in GB, including discussions of the possible causes, have been made regularly, both published (Wilesmith, 2002) and most recently submitted to the SEAC *ad hoc* Epidemiology Subgroup on UK BARB Cases (Wilesmith *et al.*, 2005a). Reports have also been discussed by SEAC, most recently in November 2003 (paper SEAC 80/4). There has also been a series of reports from the EU's Scientific Study Committee (e.g. 2003). Much of the present report retreads old ground, for arguably it is has already been well established that BSE is a transmissible spongiform encephalopathy that is transmitted by feed, and therefore BARBs cases merely represent incomplete feed controls. Relevant new information has become available which informs this conclusion. Various questions are addressed and reviewed here and, where appropriate, recommendations are made: Is the disease in BARBs BSE cases the same as in cases prior to the reinforced ban? What are the epidemiological features of the disease? What is the nature of the disease? How might BARBs cases arise? Might some be spontaneous or arise because of particular genetic susceptibilities? Might transmission of the disease be via maternal, lateral, environmental or other animal sources? Do results accord with transmitted by feed? If so, what are the likely routes of contamination? What other work may be undertaken to assess causes and actions? Finally, what is the impact of changing incidences in BSE and the rest of the world on the likely progress of the disease?

> *a) BSE cases in animals born after the reinforced feed ban was applied in the UK in 1996 are still being identified, most by active surveillance. The total now exceeds 100.*
> *b) Defra have made frequent and helpful summaries and analyses of the course of the BSE epidemic, and specifically of BARBs cases, and made updated information freely available on their website. Epidemiological analyses of the BARBs cases have been regularly updated.*
> *Recommendation: Making data available and regularly updating analyses should be continued.*

Biological characteristics of BARBs cases

6. The clinical symptoms, tissue histopathology, and prion protein immunochemistry of BARBs cases have been reported as being identical to those observed in all other bovine BSE cases in the UK (Matthews, 2005). That BARBs are infected with the same form of the disease is the clear working hypothesis, but hard confirmation in terms of, for example, passage through mice has not been reported. From this one cannot be certain it is exactly the same disease, that indeed there is only one BSE, but in view of other evidence that is reviewed in more detail subsequently, it seems the only sensible working hypothesis. Also all cases reported of animals born after strict feed bans were in place have been in countries where BSE has occurred previously. There are, however, reported cases of atypical forms of BSE found by active surveillance in Japan, Italy or France, these were either very young animals (<2 yrs, Japan) or old animals (8 – 15 yrs) and have not shown clinical symptoms (G. Wells, unpublished), so it is unclear whether these animals actually had BSE. Further cases have been found in other countries, but not GB, all by Western blotting with no

additional criteria. They have been put into two categories according to the molecular mass of the unglycosylated band. It is not possible to disregard post-mortem change or technical artefacts, however (D Matthews, pers. comm.). Thus the available data and circumstantial evidence are nevertheless strong indeed that BSE is uniform and of single origin, but there is an absence of definitive proof (proving identity is de facto difficult in science) and some queries about particular cases in other countries.

7. As noted, more recent statistics include animals detected by active surveillance. This is done using one of the EU approved tests for BSE, all of which have been checked and all conform to the validation criteria applied (European Commission, 2002a). There is no evidence of false positives, all animals testing positive on the ELISA test having been subsequently confirmed by Western blotting. A substantial proportion of animals diagnosed by active surveillance have also been found to show clinical signs of BSE. The false negative rate obviously depends on the stage of the disease at which the tests become positive. Evidence based on VLA data is that there is over 99% probability of detection at 3 months before onset of clinical signs, falling to half 8 months before onset, or, expressed differently, detectable infectivity likely appears at about three-quarters of the incubation time (European Food Standards Agency, 2005). Currently trials at VLA are under way, funded by the Canadian government, to investigate this in more detail by serial slaughter of experimentally challenged animals. In discussions the efficacy of the test was queried, in particular of showing false negatives until very late in the stage of disease due to low amount of PrP^{Sc} relative to PrP^{c} (transformed relative to untransformed prion protein), and inadequate digestion of the latter. In terms of the causes of BARBs, a low level of underreporting would not be important if all animal feed controls were in place unless, and evidence is otherwise (see also later), there is significant infection other than by feed.

> *a) There is no strong reason to believe that BSE in BARBs cases is a different disease from that in animals born before the reinforced feed ban.*
> *b) The diagnostic tests in active surveillance appear to be effective, but only when the animal is in the last few months of incubating the disease.*
> *Recommendation: a) Obtaining hard evidence on the crucial hypothesis of identity of BSE in BARBs and previous cases is highly desirable and the relevance of atypical forms of BSE found by active surveillance in other countries needs to be resolved.*
> *b) The efficacy and interpretation of the tests used in active surveillance of animals for BSE should be kept under review.*

Epidemiology of BARBs cases

8. Comprehensive information on animal, herd and feeding history of BSE including BARBs cases has been collected by Defra as part of the monitoring scheme for the disease. For reference, the following are among the important statistics from the most recent update of the epidemiology based on the 93 BARBs cases to 15 March 2005 (Wilesmith *et al* 2005a), in addition, of course to the very much lower incidence in BSE compared to animals born before 1996:

a. Of these, 27 were born in beef suckler and 66 in dairy herds. Although approximately 64% of GB herds are beef suckler, the higher prevalence in dairy herds was also found in earlier cases of BSE, indicative of different feeding practice.

b. Probably the most striking feature of the epidemiology is that the geographical distribution of BARBs cases is quite different from that for BSE cases in animals born before 1996. Noticeably, there has been a substantial reduction in cases in eastern England, particularly East Anglia, with a distribution across the country now roughly in proportion to the regional cattle density. There was clear evidence of cross contamination of cattle feed with feed for non-ruminants before the reinforced ban was in force (Stevenson *et al*., 2005), which was presumably a major contributor to the previous distribution of BSE cases in regions of high pig density.

c. Of the 93 cases, all were born in different herds except for 5 pairs (more than expected by chance) reared in the same herd (age differences between animals in the pairs were 12, <1, 5, 10, 13 months). Of these cases, 32 were purchased animals from other herds, for 25 of which feed records indicate their exposure was likely in the natal herd and 7 in an intermediate or in the diagnosed herd; the remaining cases were still on their natal herd. There had been no previous evidence of BSE in 16 of the 87 herds reporting BARBs, even though 60% of all herds in GB have had cases of BSE. Herds with a high number of previous BSE cases were not disproportionately represented in BARBs cases.

d. The mean age of detection averaged c.66 months for those born in the 96/97 and 97/98 cohorts. This is almost identical to that for cases born prior to 1996 (although it might have been expected to be lower due to active surveillance).

e. For 77 of the 93 BARBs cases for which information was available, 63 dams survived over 1 year after the birth of the case, and 41 of the 47 dams for which information was available survived to at least 5 years of age. None had other offspring so far affected by BSE or revealed by active surveillance.

f. BARBs cases have been identified in 15 breeds or crosses, with 60 animals being Holstein Friesians. Of those 40 animals for which the sire was known, all but two were by different sires. A widespread distribution of breeds and sires was also characteristic of earlier cases.

9. The 2001 case: During the period this review was in progress, a case of BSE in an animal born on 3 October 2001 was detected in January 2005 in the casualty slaughter scheme, although she was showing clinical symptoms of BSE. (It is included among the 93 in the previous analysis.) No cases have yet been detected in the 2000/01 cohort. Further, the animal's birth postdates the tighter controls on animal feed introduced across Europe in late 2000. The animal's dam and granddam are still alive. The last previous case of BSE in the herd was in 1994. In tests on members of the cohort of this animal, another two cases have already been confirmed, born 28 September 2001 and 1 May 2002. These are the youngest BARB cases, although younger animals (under 2 years) with BSE have been detected previously. In view of the age of these animals and the multiple cases, it is likely that they were exposed to large quantities of infective agent.

The only major change in the epidemiology of BSE for animals born after 1996, in addition to the fall in incidence, is that the geographical distribution no longer shows the association with density of non-ruminant livestock.
Although incidence has fallen such that no cases have yet been detected in the 2000/1 cohort, affected animals born in 2001/2 have been detected.

Nature of the disease

10. BSE is a transmissible spongiform encephalopathy (TSE), with characteristic lesions in the brain and clinical symptoms. Affected brain tissue is infective to cattle when fed orally (Wells *et al.*, 1994), and the disease can be transmitted to mice via injection into the brain (Fraser *et al*., 1992). Characteristic of other TSEs such as scrapie, there is accumulation of an abnormal protein (denoted PrP^{Sc} or PrP^{res}), derived from and different in structure from a protein (PrP^{C}) coded by the PRNP gene in the host genome (Basler *et al*., 1986*).* Transmission can be effected apparently without the presence of DNA or RNA. The putative transmissible agent is termed a prion and, in the most commonly accepted model, is assumed to be solely the protein accumulated in the infected tissue (Prusiner, 1982; reviewed, e.g. Weissmann, 2004). The PrP^{Sc} protein is believed to act as a template for the conversion of PrP^{C} protein to the non-degradable form, leading to further deposit of the abnormal protein (Prusiner, 1982), and the consequent physical and clinical changes. Transgenic mice lacking the PRNP gene cannot develop TSEs (Büeler *et al*., 1993; Manson *et al*., 1994). Recent work has added weight to the hypothesis: Legname *et al.* (2004) reported infection and transmission of a TSE in mice initiated solely from a protein source, and Castilla *et al.* (2005) have mimicked PrP^{c} to PrP^{Sc} conversion in vitro by cyclic amplification to produce indefinite amplification of PrP^{Sc}. Although various alternative hypotheses have been proposed, there is no recent evidence of which I am aware from laboratory experiments or field studies that robustly refutes the protein only prion model. Nevertheless it is not uniformly accepted, one important question being how differences between agents, e.g. strains of scrapie, are determined; in the prion model it is solely the conformation of the PrP^{Sc} protein template.

11. The prion hypothesis for BSE was reviewed in the BSE Inquiry (1988) led by Lord Justice Phillips with Prof. M. Ferguson-Smith as scientific assessor and by the subsequent committee chaired by Sir Gabriel Horn (2001) on the origins of the BSE epidemic. Both concluded that the prion played a central role in the transmission of BSE. Based also on the epidemiological evidence, they agreed with the Southwood Committee (1998) that transmission of BSE largely occurred through feed contaminated with BSE affected material derived from animal sources. In view of the homogeneity of BSE, in terms of the brain lesions and biochemical signature of abnormal PrP and as shown by strain typing experiments in mice (Bruce, 2003), these committees concluded that a single point origin was most likely, and I am aware of no more recent evidence to suggest otherwise.

12. Many other hypotheses have been put forward over the years as to the causes of BSE, including organophosphates (Purdey, 1994), bacterial and fungal toxins from ergot in Italian ryegrass (Stockdale, 2001), an autoimmune disease in response to Acinetobacter (Ebringer *et al*., 1997), and trace element deficiency or excess (e.g. Purdey, 2000; Nishida, 2003). This is not the place for a review of all these, but in most cases evidence is circumstantial and does not explain, for example, either the transmissibility of the disease to mice or by feeding of neural tissue to cattle, or the demography of BSE including the highly, albeit not totally, effective feed bans in the UK and elsewhere. Further the brain lesions, protracted incubation period of infection and evidence of transmissibility to mice, for example, all indicate that BSE is a classical TSE such as scrapie or Kuru. Thus I find no other hypotheses on the nature and transmissibility of the disease convincing; nor do these hypotheses relate specifically to BARBs cases, and I do not consider any are strengthened by their

existence. Factors such as the genotype and health of the animal, or environmental factors such as soil trace elements may, however, influence susceptibility to an infective source or the probability of spontaneous disease through transformation of normal PrP^c protein.

13. Nevertheless BSE has been found in animals born after 1996 despite strenuous regulatory and enforcement steps to prevent infection of cattle via feed. As noted above (para 6) the clinical symptoms and the brain lesions and histochemistry indicate it is the same disease as before the reinforced feed ban was introduced, arising from the same one initial source. Thus most of the features of the epidemiology which applied to cases born before the reinforced feed ban (Anderson *et al.*, 1996) are likely to apply subsequently. As, however, it is possible some cases arose spontaneously or there is some peculiar genetic phenomenon, albeit both are *a priori* unlikely, they will be addressed in the following paragraphs. It has not been resolved as to whether the original infection arose as a consequence of a single spontaneous occurrence of BSE in cattle, such as occurs for CJD in man (the BSE Inquiry view), or whether it was a single occurrence of a modification or selection of a strain of scrapie or other TSE (the Horn Committee view); indeed the origin may not be resolvable. In both models it was considered that infection originated some time before the disease was first recognised in 1986.

> *The profile of BSE is that of a classic transmissible spongiform encephalopathy. Although not fully elucidated, prion protein is heavily supported by scientific evidence to have a central role in the aetiology, transmission and diagnosis of the disease. There is no evidence that BARB cases differ from previous cases in this respect. Other hypotheses as to the nature and causes of BSE do not appear tenable; furthermore they do not specifically relate to BARBs cases.*
> *Recommendation: Unless there is new evidence on BSE in cattle that lends support to alternative hypotheses underlying the cause of BARB cases there continues to be little justification for Defra to pursue research on them. Monitoring hypotheses and the free exchange of ideas is, however, encouraged.*

Spontaneous occurrence

14. Spontaneous cases of classical CJD in humans are found at a rate of about 1/million around the world (Will, 1993), without appreciable racial or geographical variation except in a few specific cases, notably Jews of Libyan origin that have a mutation in the open reading frame of the PRNP gene (Chapman and Korczyn, 1991). Thus it is theoretically possible that spontaneous cases of BSE could occur as a consequence of a germ line mutation, in which case relatives would also have a certain or increased incidence of the disease, or a somatic mutation, which would be unlikely to be detectable unless the appropriate tissue were identified, or after some transformation in the PrP protein in the animal concerned. BSE was unknown prior to its detection in Britain in the mid 1980s, and Index cases found around the world since then can all be explained in terms of export from the UK directly or indirectly of cattle or of feed components. No BSE affected animals have been reported in many developed countries with large cattle populations, including Australia, New Zealand, Norway and Sweden, which have mixed cattle populations; and the only infected

animal detected in the US was of Canadian origin. The disease seems to have a highly homogeneous aetiology (e.g. Bruce, 2003).

15. Data from the USA, where the dairy population in particular is highly related to that in GB provide an upper limit to the spontaneous rate. A programme of testing is in place of a target population of adult cattle exhibiting some clinical sign that might be consistent with BSE (animals reported as having CNS or clinical signs of BSE or were non-ambulatory). In the intensive programme from June 2004 over 375000 animals were tested in the following 12 months. No positive results have yet been obtained in these or previous tests (USDA BSE Testing). This implies a putative upper limit of under 10 per million in this target group. Assuming, as analysis has shown, the relative risk in this group is about 30 times higher than in the population as a whole (European Commission, 2002c), then the incidence in the population as a whole is under 3 per 10 million. This figure could possibly be biased downwards if affected animals are diagnosed and disposed of without being tested. Taking account of testing done and the lack of clinical cases seen in many other countries also, it seems highly unlikely that the spontaneous rate can be as much as 3 per 10 million head. Nor can spontaneous occurrence explain incidences of ca. 30 cases of BARBs per year in 2002/4 in the UK adult cattle population of ca. 4 million. [NOTE ADDED 30 JUNE: The recent confirmation of a previously inconclusive case in the USA affects these calculations. If the animal did not have access to infected feed, the calculations have to be revised: they suggest a sporadic incidence in the population of 1/(375000 x 30) or almost 1 per 10 million, with the upper limit under 5 per 10 million.]

16. Calculation of a maximum rate of possible transformation from scrapie to BSE is less feasible. Nevertheless, BSE appears not to have arisen in the UK until around the early 1980s, despite the presence of scrapie in sheep here for at least 200 years. Although a change in the scrapie prion may have been the cause of the initial cases of BSE, the difference between their properties in mice and the uniformity of the BSE brain lesions suggest it is unlikely that more than one such mutation was the source of BSE. It is most unlikely that the same mutation could be occurring often enough to contribute significantly to BARBs cases. Furthermore BARBs cases do not match the geographical distribution of the sheep population.

> *The evidence from the absence of BSE in many countries and the surveillance schemes abroad indicates that most BARBs cases cannot have arisen spontaneously, although the possibility cannot be excluded that a very few of them did so. The possibility of a very low frequency of spontaneous occurrence of BSE may be monitored from the output of surveillance in cattle populations elsewhere.*
> *Recommendation: A watching brief is kept on surveillance efforts world-wide.*

Genetic variation in susceptibility

17. Transgenic mice experiments show that the PRNP gene that defines the sequence of the PrP^{c} and derived PrP^{Sc} is essential for the development of TSEs (Büeler *et al.*, 1993; Manson *et al.*, 1994; review, Weissmann, 2004). Genetic polymorphisms in the coding region of this locus which lead to differences in amino acid sequence of PrP are strongly associated with variation in susceptibility and/or incubation time as

shown in the mouse to scrapie agent and BSE (Bruce, 2003; Barron *et al.* 2005), in sheep to scrapie (Bossers *et al*., 1996) and in humans to vCJD (Collinge and Palmer, 1994). The situation is different in cattle, however (Heaton *et al*., 2003; Hills *et al.*, 2003; Takasuga *et al*., 2003; Sander *et* al., 2004; Seabury *et al.* 2004). Although two amino acid polymorphisms have been identified, both are rare (14 are known in sheep, some common). There is variation in numbers of copies of an octapeptide repeat region in the open reading frame in cattle (but not sheep). There are also silent polymorphisms (i.e. nucleic acid base but not amino acid change) in the coding region, and polymorphisms both in base sequence and in insertion/deletions in the promoter region.

18. Whilst there is some limited evidence for genetic variation in susceptibility to BSE in cattle, this appears to have much less impact on susceptibility than to scrapie in sheep, for example. BSE has been found in very many dairy and beef breeds and crosses of cattle; within breeds in GB a very large number of sires have had affected progeny; and analysis of data found no significant variance among sires in susceptibility of their offspring (Curnow *et al.*, 1994). A number of studies have been undertaken relating variants in the PRNP gene to BSE susceptibility, but little or no association was found. In a recent study in Germany, however, evidence of some association between the promoter region of the PRNP with susceptibility was obtained (Sander *et al.*, 2004). There is also evidence for quantitative trait loci (QTL) at regions other than PRNP being associated with susceptibility (Hernandez-Sanchez *et al*., 2002). None of these variants has shown complete protection to or certain occurrence of BSE. Only if there were such a strong familial component or specific susceptible genotype would there be a case for practising a genetic selection programme, for eliminating the source of the disease renders that redundant. Elimination of a genotype particularly susceptible to spontaneous BSE would be justified if such existed, but spontaneous cases (para 15) have not been found.

19. The 93 BARBs cases have been identified in 15 breeds or crosses, with 60 animals being Holstein Friesians. Of those 40 animals for which the sire was known, all but two were by different sires (Wilesmith *et al* 2005a). There is thus no important familial component to the BARBs incidence, similar to the finding for BSE cases previously. A hypothesis that a germ-line mutation led to the original BSE case, that this rare PRNP genotype may be particularly susceptible to BSE, and been a major contributing cause of BARBs has, however, been propounded (Ferguson-Smith, 2003); a related hypothesis is that one genotype is contributing spontaneous cases in GB. Genotyping of the PRNP locus has been undertaken for BARBs cases and controls in GB by Defra and NI by DARD. Albeit the genetic study was a long-shot, but it took Defra a long time to get theirs started, and they are still not positioned to get sequencing done on an *ad hoc* basis such as would be the case in many laboratories.

20. In the GB study DNA was taken from 70 BARBs animals and 70 breed, age and sex matched animals from the same herd, under the aegis of Defra. At the time of writing, sequencing of the PrP open reading frame has been done, but not yet fully checked, and the statistical analysis has yet to be completed. The sequencing of the promoter region has still to be undertaken. Preliminary analysis of the PrP open reading frame has revealed no obviously significant differences between the BARBs cases and the controls leading to amino acid sequence differences (Report to the

SEAC *ad hoc* Epidemiology Subgroup on UK BARB Cases meeting by TSE Research Unit, 2005). The work needs to be completed, and arrangements sought with DARD to combine GB and NI data. If the results remain essentially negative, it is unlikely to be beneficial to continue genotyping of all BARBs cases as a matter of course, but to do so on special cases (e.g. the 2001 animal, para 9).

21. In the NI study, genotyping of 13 BARBs cases, 13 BSE cases born prior to August 1996, and 26 approximately age matched negative controls from NI was undertaken (A. Douglas, Veterinary Sciences Division, DARD: Genotype analysis of Northern Ireland BSE BARBs. Unpublished report). The PRNP coding and promoter regions, and the Doppel coding region (a related gene, but of unknown function) were sequenced on all animals. The findings were as follows: a) The only significant difference between pre- and post- 1996 BSE cases was a silent polymorphism in the PRNP coding region. b) Significant differences were found within the PRNP promoter region between all BSE and control cases (also found by Sander *et al.*, 2004). c) Polymorphisms in the PRNP coding region included one indel that is within the octarepeat region and is a common polymorphism in cattle. The only single nucleotide polymorphism (SNP) known to be associated with an amino acid change in the coding region was monomorphic. One SNP showed significant genotypic differences between all cases and all controls, but differences were not observed at the allele level. d) In the promoter region a number of genotype but not gene frequency differences were associated with whether BSE positive or negative. e) In the Doppel coding region one site showed a significant difference in genotype but not allele frequency between pre and post 96 BSE cases. There is substantial linkage disequilibrium in the region, so these statistical tests are highly correlated. Cases and controls in NI were not matched for breed, and some frequency differences could be breed associated. At this stage, however, there is no clear indication of important effects; the absence of one genotype among the positives at many sites in the promoter region is not surprising in view of the low allele frequency and the multi-locus disequilibrium. A full haplotype analysis should, if possible, be undertaken of the NI data.

> *a) Previous statistical and molecular genetic studies indicate there is little genetic variation of cattle associated with susceptibility to BSE.*
> *b) Preliminary information from the GB analysis and detailed information from the NI analyses of DNA sequence data on BARBs cases and controls as yet show no clear associations, with no genotype exclusively associated with BARBs cases whether acquired by infection or arising spontaneously.*
> *Recommendations: a) The analysis of the GB data should be completed and arrangements sought to combine them with the NI data.*
> *b) If the results of the current analyses are confirmed as negative, it is unnecessary to continue genotyping all but atypical BARBs cases*
> *c) Even if genetic differences in susceptibility to infection were revealed, removing the source of infection rather than genetic selection is the route of choice for disease control.*
> *d) Defra should be better positioned to have quick access to DNA sequencing for TSE research.*

Possible routes of transmission - introduction

22. On the basis that BSE is indeed a single disease and that affected animals have arisen from infection by the initial source, however that came about, the source of BARBs must be some form of infection despite the controls introduced on animal feed (Appendix I). Control measures on animals are summarised in Appendix II. The important points are that BSE is a notifiable disease, the clinical condition is basically well known and so should be detected by farmers, and that infected animals are removed from farms and not allowed to enter any feed chain. The proportion of suspect animals tested and subsequently confirmed is falling, presumably because the incidence of BSE is falling relative to that of other neurological conditions. Numbers detected by active surveillance, introduced in 2001, now exceed those detected as clinical cases. Offspring of affected animals are slaughtered and more recently culling of all members of its cohort (born within 1 year and in the same herd) has also been introduced. Known infected animals should have no opportunity for contamination of the cattle (or human) food chain, but could contaminate farm premises prior to culling.

Maternal and lateral transmission as a route of infection

23. There was some evidence of a maternal effect in the trials run by MAFF and initiated in 1987-9 in which offspring of affected and unaffected dams were brought together and compared (Wilesmith *et al.*, 1997). As the initial feed ban was not fully effective, some animals had postnatal exposure to potentially contaminated feed. The trial showed a somewhat higher incidence in offspring of affected dams, suggesting a 10% or so enhanced risk. The probability of infection rose the nearer the dam was to developing the disease when the calf was born. Subsequent epidemiological analyses of field data indicate the risk of transmission is considerably lower, of the order of 1%, for calves born in the last 6 months of incubation of the disease in its dam (Donnelly *et al.*, 2002). Further, the observed incidence of BARBs is significantly lower, even allowing for the offspring cull. Whilst genetic predisposition can also explain a maternal correlation, it cannot explain the association with proximity to onset of disease, and in any case the evidence of genetic association is weak. Nevertheless these low levels of maternal transmission would not be sufficient to maintain the disease. Transfer of BSE via embryos, placenta and semen have been considered and rejected by the EU's SSC (European Commission, 2002b).

24. In relation to BARBs, specifically, as noted in para. 8e, 63 of the 77 dams from which information was available survived over a year following the birth of BARB cases, and 41 of the 47 dams for which information was available survived to at least 5 years of age. None had other offspring so far diagnosed by active or passive surveillance. There was no evidence of BSE previously in 16 of the 87 herds, and the geographical distribution changed between pre 1996 and BARBs cases. Furthermore, the offspring of affected animals are slaughtered.

25. There is no direct evidence on horizontal transmission of BSE between cattle, and from epidemiological analysis it was not found to be a significant factor in the epidemic both before and after the 1988 feed ban (Ferguson *et al.*, 1997). The 93 BARBs cases are distributed over 88 herds, with five herds having two cases in approximately the same age cohort, and differing in date at diagnosis by ca. 6, 8, 11, 11, 19 months. In 16 of these herds no BSE had occurred previously. Transmission

could also, in principle, occur in lorries that have carried infected animals or materials and then been inadequately cleaned. Such has not been detected and is hard to envisage in the absence of evidence for on-farm transmission; further the possibility does not accord with the change in geographical distribution since 1996.

26. There is also a theoretical possibility that BSE is surviving asymptomatically in cattle or some other reservoir host, most plausibly sheep. For example, there is now indication of scrapie PrP^{Sc} accumulation in the gastro-intestinal tract and in peri-anal lymphoid tissue in sheep (Espenes *et al*., 2003), from which infective agent might be excreted by animals prior to showing clinical signs of the disease. There is also some evidence of vertical transmission to their lambs by sheep orally exposed to BSE (D Matthews, pers. comm.). There is no evidence, as far as I know, that there has been any such transfer to cattle. BARBs cases have arisen on some farms that have never had BSE, in others not for many years (albeit some animals may have been purchased.) Further, there is no association between the distribution of BARBs cases and that of sheep within the UK; and many farms with cases do not carry sheep that have been in contact with cattle. As cattle have not, as far as is known, previously been infected with scrapie, attempts to infect cattle with it orally have failed, and the brain lesion in cattle infected with scrapie by parenteral routes is quite different from that of BSE in cattle infected with scrapie by parenteral routes, recent infection via scrapie in sheep seems implausible (albeit the initial source of BSE may have been a scrapie strain). Similarly, it is not clear that a TSE in any other animal would be transferable to cattle, except BSE itself in, for example, sheep or goats (BSE has now been confirmed in a single French goat and suspected from analysis of a historical sample of a single UK goat). As vCJD, not classical CJD, has similar aetiology to BSE, it is feasible there could be contamination from human sources as a contribution to BARBs, but the limited number of vCJD cases and the absence of route of infection render this completely implausible, and there is no epidemiological association of BARBs cases with the use of human-derived effluents.

> *As for BSE infection before the reinforced feed ban, there is no evidence that either maternal or lateral transmission, directly or though a reservoir host, is a significant factor for BARBs cases. Although these routes cannot be excluded in individual cases, they do not appear capable of sustaining the epidemic in the face of current controls.*

Environmental contamination and other non-feed borne sources of infection

27. Environmental sources of infection could apply to individual farms, for example contaminated soil or premises resulting from previous cases of BSE. The pattern would be similar to that for lateral transmission in view of the variable incubation period of the disease and so there is also no evidence for on farm environmental contamination. While the level of survival of the agent in non-feed sources is little known, it seems reasonable to assume that despite weathering or dilution or diminution of activity, environmental sources would show themselves through association with recent cases. The geographical redistribution of BARBs cases relative to that prior to 1996 cohorts argues against residual environmental sources. Even so, in view of the need to eradicate the disease completely, a review of decontamination procedures on premises may be useful. Whilst information on persistency of the agent in the environment would be useful, it is not clear that any

definitive experiments are feasible in view of the long time scale needed, heterogeneity of the environment and poor definition of the factors to be studied.

28. Other environmental routes could lead to infection on farms nearby or in the same region, such as cross infection from adjacent farms, transported slurry, ground water, sewage sludge, abattoir waste, incinerator ash, or cattle lorries. If so, local variation in incidence, associated with previous cases on the same or nearby farms or proximity (esp. downwind) to e.g. incinerators, might be expected. The substantial change in the geographical distribution between pre-1996 and BARBs cases implies any carry-over is unimportant. The BARBs cases show much less pronounced regional distribution than previously, albeit the case-control study indicates some regional differences (Wilesmith *et al.*, 2005b). No evidence has been found of association of BSE cases with downwind proximity to incinerators or abattoirs, and studies with test incinerators and mouse TSE have shown that a properly functioning incinerator for disposal of BSE infected materials does not pose a risk of environmental transmission (Brown, P. *et al*., 2000). A further possible source of infection could be via veterinary instruments, i.e. infection at operation. Even if this is plausible, the widespread distribution of BARBs cases in the UK, the absence of local concentration and the change in distribution from 1996 indicate otherwise,

29. A major EU funded study, FATEPriDE, is underway to examine environmental risk factors that affect the development of prion diseases such as BSE and scrapie (FATEPriDE Web site). The study is focussing on manganese and copper in soils, since replacement of Cu by Mn affects prion protein structure (Brown, D.R. *et al*., 2000), and on organophosphate pesticides that may influence Cu absorption. The group has brought it to my attention that they are unable to associate these environmental variables with BSE (including BARBs) incidence as they have not obtained the necessary data on location of cases from Defra. It has been suggested (Purdey, 2000) that susceptibility to spontaneous TSEs is affected by mineral imbalance in the ecosystem, but a source of infection remains necessary for BSE to occur, assuming it has a single source.

30. Animal health products: The possibility that drugs either directly cause the disease as a reaction or that the bases used for vaccines are contaminated with BSE infected material, e.g. serum, cannot be substantiated by the BSE epidemiology, e.g. localisation to the UK and time dependent change. The possible role of drugs in BSE transfer were examined by the Veterinary Products Committee from 1988 (see evidence of J. Armour to BSE Inquiry). Manufactures of animal health products were alerted early in the BSE epidemic to source all animal derived components of products from sources known to be free of BSE. In view of regulatory controls and the tight controls by producers, such a route seems implausible, and would not conform with the sporadic distribution of BARBs cases. Certain other health products, such as udder creams, that do not claim medicinal activity may be used without regulatory screening, albeit manufacturers are likely to have considered BSE risks. Whilst these could be used locally, there is no evidence of transmission by this route.

> *a) Whilst limited infection via environmental or other non-food borne contamination cannot be excluded, the likelihood is low given current knowledge on the behaviour of the disease in cattle and in the absence of evidence otherwise.*

b) The persistency of the infective agent in the environment (e.g. on the land, in premises) is not known, but in any case is likely to depend on local factors.
c) Animal health products are a most unlikely source, but there are no controls on products not claiming medicinal value.
Recommendations: a) If feasible, it might be a useful long shot to consider prior cases in neighbouring farms as a route to checking environmental contamination.
b) In view of the need to eradicate the disease, consideration should be given to decontamination procedures on premises
c) The analysis of environmental risk factors being undertaken in the FATEPriDe study should be facilitated.

Feed borne infection

31. It has been well established that cattle feed was the major, if not exclusive, route of infection with BSE of animals born prior to 1996. Evidence comes from *inter alia* the original and subsequent demographic studies of the disease in GB, the partial effectiveness of the original feed ban in 1988, with cases subsequently being concentrated in areas of high non-ruminant density subject to possible feed contamination, the greater and more even effectiveness of the reinforced feed ban from 1996, and the effectiveness of feed bans introduced elsewhere in Europe (e.g. Ireland and France). Thus notwithstanding the controls in place, infection through feed is *a priori* the most likely cause of most BARBs cases.

32. The epidemiological studies of BSE cases (born prior to the reinforced feed ban) indicate that cattle are generally infected at a young age. The peak infectivity was estimated to be about one year of age (Ferguson *et al.*, 1997), but a more recent estimate is of a peak at about 6 months (Arnold and Wilesmith, 2004), although the higher estimate could not be excluded (as age of infection and time to onset of the disease are confounded) and most infectivity in the range 6-12 months (Supervie and Costaglia, 2004, French data). Both studies indicate that adult cattle have a low risk of infection. There was a strong dependence of season of birth in the study: autumn born calves have a high risk up to 6 months of age, a negligible risk in the following 6 months and a small risk from 13-18 months; spring born animals have a lower risk than autumn born animals up to 6 months of age, but a higher risk from 6-12 months. These differences presumably reflect feeding practices. Modelling of the infection process indicates it is highest in the range 6-12 months (Supervie and Costaglia, 2004, French data).

33. Previous experiments had shown that brain tissue from infected animals fed as an oral inoculum to calves was consistently infective at a dose of 100g and partially infective down to doses of 1g. Further, there was found to be an almost log-linear feed dose – response relationship in terms of incubation time, predicting a mean incubation period of ca. 40 months for a dose of 300g increasing to ca. 70 months for a dose of 1g. Experiments in progress show some infection occurring orally down to doses of 0.01g and 0.001g, with 1 out of 15 affected for both dose levels (unpublished data from VLA experiments SE1821, SE1918 and SE1930, Wilesmith, pers. comm..). Why some animals are infected while others are not remains largely unclear: genetic influences, for example (see above), are negligible or small, but presumably the health of the animal at the time the feed was ingested (e.g. mouth lesions) is a factor. For

example, recently Heikenwalder *et al*. (2005) have shown that mice with chronic inflammation in kidney, pancreas or liver accumulated PrP^{Sc} on inoculation. It may be mainly chance as to whether the infective agent penetrates the gut, influenced by the volume of gut contents, and in trials using low doses whether the agent is actually ingested.

34. In view of the exceedingly low doses of brain material required to infect young cattle, the reductions in incidence consequent on the feed bans in the UK and elsewhere and the lack of evidence that other causes are responsible, the strongest hypothesis for BARBs is infection of animals via ingestion of BSE contaminated material. The similarity of the lesion and similar incubation period in BARBs and previous cases give no indication that BSE associated with BARBs is any different from previously (but see para. 6). Further, the epidemiology of BARBs cases (para 5), in which there is significant, but limited, clustering of cases (five pairs in approximately the same cohort in the first 93 cases, in addition to those in the cohort of the 2001 case) with little association to previous cases, similarly indicate some infective source such as feed. Recent detailed investigation into the 2001 and multiple cases by Defra has been particularly enlightening. In particular it seems that the 2001 case and its cohorts may have had access to old feed that had been stored on farm. Thus while direct evidence of feed transmission as the source of BARBs cases is lacking, there seems no reason not to assume this as the working hypothesis, as Defra have done. It has to be assumed there have been breakdowns in controls and infected bovine material, e.g. as meat and bone meal, has entered the feed chain.

> *a) Recent unpublished experiments at the VLA have shown that feeding exceptionally low doses (0.001g) of infected neural tissue can cause BSE.*
> *b) The working hypothesis of Defra that the major cause of BSE in BARBs cases has been through the ingestion of contaminated feed, most likely by young animals, is strongly supported. Thus control of the disease requires, as it has always required, completely eliminating the agent from the cattle feed chain.*
> *c) Understanding causes of variation in infectivity are important in terms of understanding the disease, but do not particularly impinge on the control of BSE, where risks have to be avoided.*
> *Recommendation: Defra continues to operate on the basis that BSE transmission via feed is the major route in BARB cases.*

Control measures on feed

35. Control measures that have been put in place on feed for ruminants are summarised in Appendix I. Important points are: the increasing stringency of controls in the UK up to the introduction of the reinforced feed ban in 1996, the lower overall level of stringency in the EU until 2001, and later introduction of controls in new member states; the active checking of premises and equipment; the lower levels of controls applying to pet food; and the difficulty of assessing very low levels of animal protein. Whilst attention has been paid largely to meat and bone meal (MBM) as a route for transfer, recent experiments have shown that the heat and chemical treatments applied in the production of tallow may not have been fully effective. (unpublished data from VLA project SE0229). Albeit a small risk factor, tallow is not permitted in animal feed.

36. Animal feeds are tested on a regular basis by the VLA's Luddington laboratory, with 30000 samples tested per year as part of the National Feed Audit. Tests are made of feed ingredients and the feeds themselves on compounder and farm premises and of imports. About 30000 samples of feed are tested per year, and some containing animal products have been detected. In view of the heterogeneity and nature of the materials being tested, the ELISA test for animal protein in feeds is reliable only down to 0.1% contamination with MBM. Microscopic analysis for bone fragments is also undertaken, and is regarded as being more sensitive with a trained operator, but again to a level of 0.1%. Hence, whilst examination of feed cannot ensure no contamination has occurred, it is essential as a way of avoiding abuse and minimising carelessness. There is considered to be some heterogeneity in the quality of feed testing for presence of animal products in the EU due to different level of training of operators, not least because controls have been introduced recently in some new member states.

37. The Advisory Committee on Animal Feedingstuffs has recently undertaken a review of feed law enforcement in the UK and made a number of recommendations (Unpublished report). Among these it concluded that the current situation is unacceptable and that measures need to be introduced to ensure a more consistent and effective programme of animal feed law enforcement, and that further coordination of feed law activities is required. The Committee also expressed concern about the level of checks of imported feed, and proposes a risk-based enforcement programme and statutory prior notification of imports of animal feed.

> *The problem, if there is one, is not with the principle of the feed control regulations, but whether they have been and are being broken knowingly or unknowingly, through use of banned material or for example of feed that has remained in plants or on farms, e.g. in corners of feed bins.*
> *Recommendations: a) The feed controls currently in place seem adequate but require vigilant enforcement. Defra should continue to review appropriate controls, including consideration of regulations on composition of and access of livestock to pet food.*
> *b) Efforts to obtain consistent quality of feed testing for animal derived material in all EU member states should be made.*
> *c) In view of the likelihood that breaches of regulations have occurred and enabled BSE contaminated material to enter the cattle feed chain, Defra should help facilitate the recent recommendations of the Advisory Committee on Animal Feedingstuffs to ensure a more coordinated and risk-based programme of over-all animal feed law enforcement.*

Potential feed borne sources of BARBs cases

38. Imported feed. Importation of contaminated feed constituents into the UK through European ports has been suggested as a route for infection of the BARBs cases (e.g. Wilesmith *et al.*, 2002), as animals elsewhere have been infected with BSE (Appendix IV) and controls were brought in later than in the UK (Appendix I). Possible unintentional sources could be inadequately cleaned out storage or transport facilities, with contamination of ruminant with non-ruminant feed. Although from June 1994 the feeding of mammalian protein to ruminants was prohibited in the EU (to those countries then members of the EU), not until early 2001 were harmonised EU–wide

controls for BSE and other TSEs introduced (feed implications?), and in September 2001 a ban was introduced on the feeding of processed animal protein to farm animals. Thus one hypothesis is that infection was 'imported'. Particular risks are of fish meal contaminated with MBM, as ELISA tests for mammalian protein are not sufficiently sensitive. The concern is that mixing of feed raw materials with other products could be occurring in European ports for example, via residual material in previous shipments or bins, or via unclean equipment such as augers or conveyers that successively move different batches.

39. The importation route might have been expected to lead to more regional variation in distribution associated with port of entry of ingredients. Initially there appeared to be some concentration in the south east of England but not subsequently (Wilesmith 2005a). Also it does not explain the 2001 case born after full controls were in place in the EU (if these controls were fully enforced). Further, cases of BSE in recent cohorts born after their respective feed bans are found in countries that have had previous BSE cases. In many countries there are still no cases of BSE, and the Netherlands, with the biggest EU port, has less BSE cases of animals born after 1996 than in GB.

40. Locally derived feed. Other potential sources of infection are illegal (but possibly unintentional) farm stored food, pet food, and unintentional contamination of mixing and other equipment through poor mixing in bins and lorries. The persistency of the prion infective agent in feed is not known, but there is no reason to assume that BSE activity could not persist for many years in dry feed stored under good conditions. It would be optimistic to assume that all old feed really was cleared out of all farms in the recall scheme in 1996, and that none remained in odd corners of feed bins, for example. Further, it cannot be assumed that all such feed was cleared out in other parts of Europe when tighter controls were introduced there more recently. There is evidence that the 2001 case was infected by feed that had been on the farm in an out of use bin since the late 1990's (albeit post 1996). Such storage does not, however, explain the change in geographical distribution pre-and post-1996, although one explanation suggested to me is that on farms also containing pigs, as in East Anglia for example, old feed might have been fed to pigs rather than leaving it in store for subsequent use by cattle.

41. Pet food. Controls on incorporation of animal derived products in feed are different for pets (see Appendix I) and it seems feasible that contamination of pet food with BSE infected material could still occur, with the possible risk that cattle then get access to it in some way. The 2001 BARBs case (see para. 9) shared housing with dogs when young, and although fed separately might have had access to dog food (directly or e.g. by vomit), although feed at the relevant time is not believed to contain mammalian protein. Although such a source of infection could account for the random distribution of BARBs cases, records indicate that not all BARBs cases had contact with pets and potentially their food and so pet food is unlikely to have been a major source of BARBs cases.

42. Case-control study. Results of a case-control study (Wilesmith *et al*., 2005b) were recently reported to the SEAC *ad hoc* Epidemiology Subgroup on UK BARB Cases. The cases were the 93 BARBs animals born to mid-March 2005 (27 detected as clinical cases and 66 by active surveillance). To obtain information on feeding history, the 367 controls were defined as suspect cases, born after 31 July 1996,

detected on the natal herd and for which the diagnostic confirmatory tests were negative. There was insufficient information on many factors, and many others were non-significant or marginally significant. Those otherwise of interest were: a) Use of purchased proprietary concentrate feed up to 6 months lowered risk compared to other feed types (home-mixed or home mixed & proprietary). b) Risks were lower if the farm had no previous case of BSE. c) The average age of onset of cases (5.4 years) was higher than that of controls (4.0), although the BARBs cases were of similar age to BSE positives born prior to 1996. d) A spatial analysis indicated three small widely dispersed regions where the density of cases was higher than controls.

43. This case-control study was criticised by the SEAC *ad hoc* Epidemiology Subgroup on UK BARB Cases for ascertainment bias, the cases including animals detected by active surveillance and the controls all having some clinical symptoms. (The younger age of controls and low association with previous cases might, for example, be due to a farmer's inexperience in diagnosis.) The committee suggested alternative procedures for removing bias. a) Omit active surveillance cases; but this would reduce the sample size to 27. b) Demographic matching of controls to cases; whilst likely the most productive study, there are only about 100 cases, ranging widely in, for example, herd type, breed of cattle and previous BSE history, with most herds have only one BARBs case. c) A further study using age matched random samples; but although lifetime feeding histories may be obtainable, a pilot study has shown it is often not possible to trace the source of ingredients because of takeovers, etc in the feed industry and consequent loss of old records.

> *a) It may not be possible to attribute BARBs cases to any single source of feed contamination. Some feed may not have been fully cleared out on farms in 1996 in GB in 1996 and in other countries later. Imported feed seems a likely cause, certainly until controls were tightened up throughout Europe, but does not readily explain the random country-wide distribution in GB.*
> *b) Firm conclusions cannot be drawn from the recent case-control study of BARBs.*
> *Recommendations: a) No source of feed contamination should be ruled out and detailed investigations should be continued.*
> b) *Some improvements in the design of the case-control study can be effected fairly straightforwardly and should be undertaken. A major effort to obtain feed records on control animals does not seem justified, if indeed it is feasible.*

Inferences from changes in incidence

44. The number of clinical cases in GB in animals born before the reinforced feed ban has fallen rapidly in recent years, in part because of the drop in age cohort risk rate prior to 1996 and in part due to the fact that no animals born after 1996 are included (see Appendix table III). For example, in calendar years 1999-2004, inclusive, the number of clinical cases approximately halved each year, from 2256 to 82. The introduction and increase in level of active surveillance have influenced total numbers of cases found. Active surveillance was fully in operation only from 2002, with the numbers so diagnosed falling from 594 in 2002 to 227 in 2004. Of the total number of BSE cases in 2004, 21 of the 309 were BARBs. In view of the potentially very long incubation of the disease, a case has occurred in an 18 year old animal and the predicted distribution of the incubation period approaches zero only after about 10

years (Ferguson *et al*. 1997; Arnold and Wilesmith, 2004), it may be very many years before the last case of BSE is diagnosed in GB, even if no new infection occurred after 2002 (when an infected animal in the cohort of the 2001 case was born).

45. As is illustrated by the drop in number of BARBs cases after 2002/03, there has been a fall in the incidence classified by birth cohorts. This has been confirmed more formally. Wilesmith et al. (2005a) have used back calculation and maximum likelihood, allowing thereby for time to detection, active and passive surveillance and other factors, to estimate the prevalence in birth cohorts:

Cohort	Prevalence/million	(95% CI)
96/97	117	(78 - 167)
97/98	66	(44 - 94)
98/99	34	(19 - 54)
99/00	29	(12 - 57)

There are no affected animals as yet in the 2000/01 cohort, and the one case in 2001/2 available at the time of analysis implies a lower 95% CI of 22/million. The declines from 1996/7 are statistically significant, and such that, although more animals are potential BARBs cases in 2003/04 than in 2002/03 because more birth cohorts are eligible, the number of cases dropped. Ascertainment of individuals through the cohort cull, as has happened for the 2001 case, may distort figures if these are assumed to be independent; consequently data and calculations based on one infective event per cohort may become a more useful indicator of the progress of the epidemic.

46. The number of cases detected outside GB (almost entirely in the rest of the UK and Europe) peaked in 2002 at 1107, and was 871 in 2003 and 554 in 2004 (Appendix Table IV, Defra BSE Statistics). There has been a steady decline in numbers of cases in almost all countries that have had appreciable numbers of cases, except Spain and Portugal, which introduced controls later than the UK, and Germany. In Switzerland there was a peak of incidence in 1995 and, despite a previously imposed feed ban, a second peak in 1999; this was associated with an age cohort of a few months, and can almost certainly be attributed to a batch of contaminated feed (Cohen-Sabas *et al.*, 2004). Just as it would be optimistic to assume that no old feed was retained knowingly or unknowingly in the UK in 1996, so it would be optimistic to assume that elsewhere when schemes were adopted. Numbers in countries outside Western Europe, including new member states of the EU, have not yet declined. The birth cohort statistics contrast the large reductions in UK, Ireland and France with rising infection rates by birth cohorts in the Iberian Peninsula. These figures are no more than a guide as they depend on levels of ascertainment and active surveillance and on reliable information on birth dates.

a) There has been a fall in the underlying incidence of BSE by birth cohort 1996/97 to 99/00 in GB, but the 2001/2 case leaves doubt subsequently. There has also been a fall in other countries except where feed controls were introduced later.

b) Consequently risks of infection are in general falling and should progressively reduce the risks of contamination and cross contamination.

c) As cohorts of affected animals are tested, numbers of index cases rather than total numbers may give a better indication of progress of the epidemic.

General conclusions

Elimination of feed borne sources is now, as before, the key to elimination of BSE. The incidence of the disease can be greatly reduced but not readily eliminated in any country by adequate imposition of controls, particularly on animal feed. As the level of incidence falls both in the UK and internationally, the risks of contamination through feed, or indeed through any other source, fall whether or not controls in the UK and abroad are further tightened. With the current expertise in Defra and the VLA, GB is well placed to keep on top of and promote developments.

Recommendations: It is essential that appropriate, risk based, controls and monitoring should be maintained on animals and feed until no cases of BSE are found, and controls tightened up where feasible, both in the UK and elsewhere that the UK can influence. In view of the very long incubation period of BSE in some animals, long-continued vigilance is necessary. It is not evident, however, that specific new measures are needed. Basically it is necessary to 'keep taking the medicine'. Nevertheless, in view of new discoveries on the nature of the disease and the possibilities of new or changed TSEs arising, relevant research capacity in GB should be maintained.

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Oral consultation (+ correspondence)
Dr Peter Barrowman, Defra
Prof Sheila Bird, MRC Biostatistics Unit, Cambridge
Dr Yvonne Boyd, Defra
Dr David Brown, University of Bath
Prof Moira Bruce, Institute of Animal Health, Edinburgh
Prof Neil Ferguson, Imperial College London
Prof Malcolm Ferguson-Smith, University of Cambridge
Prof Noel Gill, Health Protection Agency (Chair, SEAC *ad hoc* Epidemiology Subgroup on UK BARB Cases)
Prof Chris Higgins, Imperial College (Chair, SEAC)
Sir Gabriel Horn, University of Cambridge
Dr Nora Hunter, Institute of Animal Health, Edinburgh
Ms Anastasia Kesisoglou, Defra
Dr Danny Matthews, VLA
Prof Ian McConnell, University of Cambridge
Mr Keith Meldrum, MAFF, Retired
Mr Tony Navid, Defra, Retired
Mr Michael Prince, Defra, Retired
Mr Scott Reaney, VLA
Dr Ginny Saunders, VLA
Dr Marion Simmons, VLA
Mr Roy Smith, Defra
Ms Emma Soto, Defra
Mr Peter Soul, Defra
Dr George Venters, retired epidemiologist
Dr Gerald Wells, VLA, retired
Prof John Wilesmith, Defra
Dr John Williams, Roslin Institute
Mr Duncan Young, formerly Defra

Correspondence
Mr George Bannister, Farmer, Staffordshire
Dr Michael Crawford, London Metropolitan University
Mr Philip Hill, Farmer, Staffordshire
Dr Anna Korre, Imperial College London
Prof Vala Ragnarsdottir, University of Bristol
Mr Tom Stockdale, Farmer, Dumfries and Galloway

Appendices

Appendix I. Summary of feed control measures

In July 1988 the initial ban on use on materials of ruminant origin in feedstuffs for ruminants was introduced. In September 1990, specified bovine offals were banned in any animal fed. A ban was imposed on export of feed to EU and in July 1991 worldwide. In November 1994 the ban was extended to feeding any mammalian protein to ruminants. In the EU, all mammalian protein was banned in animal feed, but restriction removed in March 1995 on e.g. milk and gelatin and blood products. In April 1996 the feeding of meat and bone meal (MBM) to any farm animal (including horses and farmed fish) was banned, in June a feed recall scheme started to remove any such feed from merchants or farms, and from 1 August 1996 the reinforced ban was started, including the prohibition of MBM on premises where livestock feeding stuffs were kept and disinfection of lorries etc. where MBM had been produced or stored. (This is the Reinforced Ban).

Fishmeal, animal derived dicalcium phosphate and hydrolysed protein may be fed to non-ruminant farmed livestock. Restrictions on feeding animal protein do not apply to non-ruminant pets (e.g. dogs and cats) that are not farmed (e.g. horses). The UK is largely self sufficient in production of pet foods, although materials are imported.

Control in the UK to 2001 was mainly by feed sampling and testing, with inspection of plants, vehicles and practices where positives were found, and identification of potential points of cross-contamination. Since 2001 plant and vehicles have been tested: of 2800 tests, 3 may have represented some risk to ruminant feeds.

In October 2000 use of material from condemned animals was stopped in the EU; and from December 2000 temporary, from May 2001, permanent harmonised EU–wide controls for BSE and other TSEs were introduced, and in August 2001 a ban was introduced on the feeding of processed animal protein to farm animals, from when EU regulations can be considered effective. Feedstuffs entering Europe from third countries should be tested for mammalian protein before movement in Europe. Measures in other countries now in the EU have been introduced at different times, and only standardised as of 2002 and when new members joined.

Appendix II. Summary of animal control measures

Those in charge of animals, including veterinary surgeons or others responsible for examining or inspecting animals, must notify the Divisional Veterinary Manager of any animal suspected of being affected with BSE. On receipt of notification the DVM arranges an enquiry by a Veterinary officer (VO). If a VO suspects BSE, the animal is restricted by notice. If a VO believes the suspect animal is affected with BSE the animal is compulsorily slaughtered. Diagnostic samples are removed from the carcase and the remainder incinerated. These clinically diagnosed cases are termed as identified by *passive surveillance*. From July 2001 *active surveillance* by EU approved rapid testing methods on brain tissue *post mortem* has been introduced in all EU member states. Cattle tested are: over 30 months and for human consumption (a limited number in the UK); all fallen stock and all casualties over 24 months; all cattle born after 31 July 1996 and aged over 42 months; and a random sample of 10000 OTMS animals born before August 1996. All offspring and, introduced more recently, all members of the farm–age (within 1 year) cohort of a BSE case are compulsorily slaughtered and tested. There are also measures in relation to human consumption.

Appendix III. Pattern of the epidemic from 1996 in Great Britain

Total number of BSE cases confirmed per year in GB since 1996 has been (Table 1a, Defra February 2005 report), including BARBs (Table 6)

	Slaughtered	Other	Total	BARBs confirmed
1996	8013	3	8016	0
1997	4310	3	4313	0
1998	3179	1	3180	0
1999	2256	20	2276	0
2000	1311	44	1355	1
2001	781	332	1113	5
2002	445	594	1039	23
2003	173	374	547	41
2004	82	227	309	21

Appendix IV. Pattern of the epidemic from 2002 world wide

BSE cases world-wide for the years 2002, 2003 and 2004, for countries (or regions) which had cases in at least one of those years (Defra web page)

	Year of detection			Year of birth		
	2002	2003	2004	1995	1996	1997
GB	1039	547	309	1059	62	39
NI	98	63	34	123	28	5
Channel Isles	2	0	0	0	0	0
Belgium+Lux.	39	14	11	25	35	15
Denmark	2	2	1	7	2	1
France	239	137	54	355	95	39
Germany	106	54	65	83	141	42
Ireland	308	215	115	393	139	10
Italy	36	29	7	22	42	20
Netherlands	24	19	6	8	32	11
Portugal	86	133	92	84	70	58
Spain	127	167	137	83	100	126
Switzerland	24	21	3	40	12	15
East^{n} Europe	13	11	23	4	3	2
Non Europe	3	6	6	2	5	1